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A SERIES OF MONOGRAPHS ON THE CHEMISTRY, PHYSICS, AND TECHNOLOGY OF HIGH POLYMERIC SUBSTANCES

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VOLUME IV

NATURAL AND SYNTHETIC HIGH POLYMERS

A TEXTBOOK AND REFERENCE BOOK
FOR CHEMISTS AND BIOLOGISTS

Second Completely Revised
and Augmented Edition

KURT H. MEYER
Professor of Chemistry
University of Geneva, Switzerland

1950
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PREFACES

to the First Edition

The great and ever-increasing importance of the study of high polymers for the chemist, the technologist, and the biologist justifies the attempt, made here for the first time, to give a systematic account of the entire field of natural and synthetic inorganic and organic high polymers.

At the present time, studies of high polymeric compounds reveal a marked attempt to replace the purely descriptive approach by a more profound analysis of the general principles involved and their theoretical foundations. We have proceeded from the standpoint that all those phenomena frequently regarded as peculiar to high polymers are to be referred, in the last analysis, to the interplay of atomic forces of attraction and thermal agitation, as in the behavior of compounds of low molecular weight. This type of exposition has necessitated the critical consideration of all the facts available and of many theories, much discussed and widely accepted at the present time.

The chemist and biologist will not find in these pages a detailed practical guide to investigations in their specialized fields. It is hoped, however, that their attention will be drawn to near and remote parts of the field of high polymer chemistry, and that the resulting extension in outlook may provide a stimulus to new experimental and theoretical investigations on the part of both. The theoretical treatment adopted here should make it possible to view individual problems in a clearer light than that which illumines the empiric approach, thus facilitating the task of even the specialist of experience.

Lest the biologist should at first feel a little surprised at the dedication of this book to his use, the translator may perhaps be permitted to address these remarks to the more biologically minded reader. It is no new experience for the biologist to be faced with the necessity of trespassing on the less familiar ground of physics and chemistry in search of more exact methods of measurement. During the last ten years, however, a branch of chemistry has been developing which is destined to provide biology with much more than methods. It is this branch of chemistry which forms the subject of this book.

The common meeting ground of chemists and biologists in high polymer chemistry is that of morphology. Since the day when, as a result of development of x-ray analysis, chemical formulas ceased to be symbols on paper and became models in three dimensions, the chemist has become more and more accustomed to think in terms of what may be called morphologic chemistry. To the biologist, who observes that both shapes and properties of cells and cell components reflect their molecular organization, it seems likely that morphologic chemistry may prove to be the chemistry of morphology. It is for him now to consider biological structure and organization in their high polymer guise and to formulate for himself the questions which high polymer chemistry will go far toward answering.

KURT H. MEYER
L. E. R. PICKEN

Geneva, 1942
Cambridge, 1942

PREFACE

to the Second Edition

Twenty years have passed since the appearance of Meyer and Mark's *Der Aufbau der hochpolymeren organischen Naturstoffe*, and ten years since that of their book *Hochpolymere Chemie*, whose translation formed Volumes II and IV of this series. It is the latter volume which is presented here in a new form.

During the first decade, macromolecular chemistry, called polymer chemistry in the United States, became a science in its own right, and in the second decade it developed at an ever-increasing rate. Practically the whole book therefore had to be rewritten, although the fundamental concept discussed in the first edition has remained unchanged. The question arose as to whether it is desirable to continue the presentation of all aspects of polymer chemistry in one volume– proteins as well as plastics and clay minerals, thermodynamics as well as physical and chemical methods of structural analysis. We think that our attempt is justified by the features common to all high polymers, regardless of constitution and origin, and by the information the specialized worker may obtain from other chapters of polymer science.

As in the first edition, the author has tried to review the whole of the available material from a critical standpoint. However, while at that time the value of theoretical consideration had often to be emphasized, on this occasion warning must be given against too confident use of so-called quantitative theories that are based upon more or less inadequate models.

The author feels deeply indebted to Dr. A. J. A. van der Wyk, who collaborated on many chapters, to Dr. P. Bernfeld (enzymes), Dr. C. Weissenberg (rheological phenomena), and Dr. K. O. Pedersen, who compiled the molecular constants of proteins. He wishes also to express his gratitude to Drs. L. E. R. Picken, M. G. M. Pryor, and G. C. Gibbons, and to Mrs. M. M. T. Georg (née Plant), who shared the considerable work of translation.

KURT H. MEYER

Geneva, 1950

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INTRODUCTION

1. Definition and Classification of High Polymers

High polymers may be defined as chemical compounds in which a large number of identical or similar atoms or groups of atoms are united by homopolar primary valences. All multivalent elements forming homopolar compounds, and hence all multivalent metalloids, are able to participate in the formation of this type of compound, which is encountered in the fields of both inorganic and organic chemistry. The structural features common to all high polymeric compounds often lead to similarities in physical behavior between substances which are not chemically related. As examples we may mention the fibrillar structure of asbestos and cellulose, the elastic properties of polyphosphonitrilic chloride and rubber, and the optical properties of inorganic and organic glasses. In spite of these common features, however, it is convenient to consider inorganic high polymers separately, before organic polymers. A polymer may be united with a low molecular substance or with another polymer by chemical linkages; it is then termed a conjugated polymer.

Both polymer and conjugated polymer molecules are also referred to as "macromolecules." In practice, there is no difference in meaning between a high polymer and a macromolecular substance; the former expression is more frequently used in the Anglo-Saxon countries, whereas the latter is employed on the European continent. The term "giant molecule" is only used in popular expositions.

The fact that the atoms or groups of atoms (structural units or residues) from which high polymers are built up may be united with one another either at two or at several points provides a possible basis for the classification of the material to be presented. We distinguish accordingly: (*1*) *chain polymers*, in which each group of atoms (with the exception of the terminal residues) is united with two others by primary valences, so that the assemblage of atoms forms a primary valence chain; (*2*) *two-dimensional net polymers*, in which each residue (with the exception of the terminal residues) is united with three or more others in such a way that plate-like, giant molecules are formed, which in turn are stacked together to

form laminated solids; and (*3*) *three-dimensional polymers*, in which primary valence linkages occur in all directions.

This classification will be used for the treatment of inorganic polymers but it is unsuitable as a basis for the discussion of organic polymers. Many synthetic and natural polymers in the latter category consist of long primary valence chains, united with one another by primary valences at relatively few points, so that a loose, three-dimensional net is formed. Such compounds will be considered with the organic chain polymers, from which they may be derived by a process of cross linking. We shall classify organic polymers according to chemical composition, thereby ignoring the division between natural and synthetic compounds, a division which is no longer made in organic chemistry. Rubber, for example, will be included among the high polymeric hydrocarbons and cellulose is treated as a polyglucosan. This point of view links up directly with the classical ideas about the structure of such natural products; these ideas were a logical extension of organic structural theory; they were supported by the comparison of natural products with known compounds of low, medium, and high molecular weight, and remained unchallenged until about twenty-five years ago. At that time, substances such as cellulose and proteins were classed with synthetic compounds of high molecular weight and were supposed to consist of enormous molecules. The mode of linkage of the residues was fairly well understood, as in the case of the peptide linkage of amino-acid residues in proteins, and the continuous glucoside linkages in polysaccharides.

Although this point of view was both plausible and consistent, it did not remain unquestioned. In the years between 1920 and 1934 a number of workers expressed the opinion that natural products, such as cellulose and starch, consist of small molecules possessing unique powers of association; this concept was the subject of much discussion during the following years. In spite of the arguments in favor of small, highly associating structural units, many authorities upheld the classical view. Gradually it became clear that none of the arguments in favor of small structural units was valid; many observations which had been reported were not confirmed; others, such as the abnormally high osmotic effects of rubber and other polymers, which suggested the presence of small particles in solution, were not necessarily incompatible with the assumption of very high molecular weights. Of decisive importance for the solution of the problem of whether primary valence linkages or van der Waals' forces unite the residues in cellulose or protein was the systematic study, in all types of compounds, of the forces uniting atoms. As a result of such studies, linkages of great

strength, which require considerable activation energy, and a considerable amount of energy for their rupture, may now be described without hesitation as homopolar primary valences.

2. Homologous Polymeric Series and Mixtures of Polymeric Homologues

From compounds of low molecular weight to high polymers there is a continuous transition through compounds of intermediate molecular weight. An example of such a series was described by Lourenço[1] as early as 1863. He obtained from ethylene oxide, by polymerization in the presence of a trace of water, a series of polyethers, all with terminal hydroxyl groups and differing only in the number of —O—CH_2—CH_2—O— groups united in the molecule. He gave the following formula to this series:

$$HO \cdot C_2H_4 \cdot O \cdot C_2H_4 \cdot O \ldots C_2H_4 \cdot O \cdot C_2H_4 \cdot OH$$

and showed that both boiling point and viscosity increased with increasing molecular weight. Such a series is known as a homologous polymeric series. Another example of a series of this type, $R \cdot NH \cdot CH_2\ CO.NH \ldots CH_2.CO_2H$, derived from glycine, was described by Curtius[2] who also showed the regular change in the various physical properties with molecular weight.

Finally, Emil Fischer[3] systematically prepared numerous polypeptides and by this means obtained compounds with a molecular weight of over 1000. He was able to link up results obtained using the synthetic method with studies of natural proteins, since it was possible to identify intermediate breakdown products of these (of silk fibroin, for example) with synthetically prepared polypeptides.

At the present time a large number of homologous polymeric series are known. In the preparation of synthetic high polymers it is possible, according to the conditions, to obtain products of medium, high, or very high molecular weight; conversely, during the breakdown of natural high polymers, it is possible to prepare, step by step, products of lower molecular weight.

The higher the molecular weight, the more do the properties of adjacent members of a homologous polymeric series resemble one another; it is evident that the addition of one more residue to a chain consisting of many residues will change the properties of the chain relatively little. If, in the

[1] A. V. Lourenço, *Ann. chim. phys.*, (3), **67**, 275 (1863).
[2] T. Curtius, *Ber.*, **29**, 759 (1896); *J. prakt. Chem.*, **70**, 57 (1904).
[3] E. Fischer, *Ber.*, **39**, 530 (1906); **46**, 3253 (1913); **52**, 809 (1919).

preparation of high polymeric compounds, the long and tiresome method of adding systematically one group after another (as Fischer did in the synthesis of peptides) is abandoned and, instead, the shorter method (involving polymerization or condensation of reactive compounds) is followed, the product obtained is always a mixture of homologous polymers. Many natural high polymers are themselves mixtures of molecules differing in molecular weight; in the realm of the proteins, however, several homogeneous high polymers have been found.

The preparation of "chemically pure" substances (that is, substances composed of identical molecules) from such mixtures of large chain molecules is not as yet possible in the present state of our technique. "Purification" of preparations is limited to obtaining mixtures of molecules of similar chain length by fractionation; where fractionation is impossible, purification is confined to the removal of chemically different impurities, as an example of which the removal of the substances associated with native cellulose may be mentioned.

Since, in the field of high polymers we are not dealing as a rule with "chemically pure" preparations, the idea of chemical purity should not enter into the formulation of concepts, into the statement of problems for solution or into the experimental treatment: the mixtures themselves are the objects of investigation. In this we depart consciously from the procedure adopted in the synthetic and analytic chemistry of compounds of lower molecular weight.

At the present time, the expression "chemically identical" should be avoided in speaking of two high polymeric products, if the products are not homogeneous. It is, of course, permissible to speak of great similarity or practical identity between two preparations, if they agree in their main properties. Perhaps it would be advisable to make use of concepts similar to those used in biology for the characterization of two individuals of the same species, or in mineralogy for two specimens of the same mineral. In these cases the expression "identical" is also avoided, and it is sufficient to say that a particular individual belongs to a given class defined by the principal characteristics of the individuals it includes.

Of considerably greater importance than the question of terminology, however, is that of the individual character of the objects under investigation. It is essential not to lose sight of the individual character of a particular specimen. This is a complication unknown in the chemistry of substances of low molecular weight. No two preparations of a high polymeric natural product, such as starch or cellulose, are identical, and the technologist is continually reminded of the difficulty of preparing high polymers of constant composition and properties on an industrial scale.

From what has been said, it is clear that great caution must be exercised in the interpretation of elementary analyses; it is impossible to draw rigid conclusions such as may be drawn from the analyses of chemically homogeneous substances. Stoichiometrical analytical results cannot be used directly for the determination of constitution, as in the case of substances of low molecular weight, and conclusions about the molecular weight drawn from such analyses are not always binding.

3. Molecule and Molecular Weight

The term *molecule* originally denoted the group of atoms which persisted as a group in the gaseous state or in solution and whose size could be determined in these states by the usual methods adopted for the determination of molecular weight. In the case of compounds of low molecular weight, such molecules are for the most part identical with the groups of atoms united by ordinary valences; such groups of atoms are the "chemical molecules" of the organic chemist. The two concepts are not, however, coextensive in those cases in which chemical molecules, in solution or as vapor, associate by means of residual valences to form larger units. Such associated units, also known as micelles or supermolecules, play an important part in the chemistry of high polymers, and of the proteins in particular; in this case the value of the molecular weight obtained from osmotic data represents, not the weight of the chemical molecule, but that of the group of associated molecules. For this reason the concepts *molecule* and *molecular weight* are to be used with caution in the field of high polymer chemistry; ambiguity in the meaning attached to the term *molecule* in a particular case is to be avoided. It should be made clear whether the term denotes the osmotically active particle or the chemical molecule, that is, the group of atoms united by primary valences.

Since at infinite dilution the associated units will separate into their component "chemical" molecules, the particle weight at infinite dilution may be calculated by extrapolation and the result regarded as the "chemical" molecular weight. It is clear that only mean values are obtained if the preparation is a mixture; a value obtained for the molecular weight of a preparation of cellulose has the same significance as, for example, a value obtained for the molecular weight of a paraffin or petroleum fraction. For the exact characterization of any particular preparation, it would be necessary to have data on the distribution of the molecular population about the mean molecular weight. Experiments of this kind have so far yielded only qualitative results.

It must, however, be emphasized at this point that a knowledge of the molecular weight of homologous polymeric mixtures is not as important as it is for homogeneous substances, for which it may throw light on constitution, or be used to test the validity of chemical formulas. In general, it is sufficient to have data indicating merely the order of the molecular weight; the technologist may even dispense completely with such figures, and make direct use of certain physical properties which depend on the molecular weight—for example, the viscosity—for the characterization of his products. This corresponds to the method of describing certain commercial products of low molecular weight, such as gasoline, lubricating oil, or paraffin.

4. Morphological and Thermodynamic Approach to the Chemistry of High Polymers

Since most natural high polymers occur as solids, and since synthetic high polymers are used in the solid condition, it is necessary to pay more attention to this state of aggregation than is usual in the chemistry of substances of lower molecular weight: the reactions of an organic compound are generally investigated in the liquid or in the dissolved state; the physical constants of such compounds are also usually determined in the liquid state.

While the physical state of a given liquid is defined by its temperature and that of a solution by its temperature and composition, these variables do not suffice to define the physical properties of a solid. As an example, it will be remembered that the degree of subdivision plays a decisive part in the reflection of light by a solid; furthermore, mechanical properties are to a very large extent dependent on the type of aggregation of a given material, that is, on its texture, so that similar properties are encountered in substances differing in chemical constitution but similar in texture (silk, nylon, and rayon, or rubber, neoprene, and buna are striking examples).

In metals and silicates, the crystallites of which the sample is composed are often microscopically visible, so that their texture is relatively easy to investigate, and as a result of microscopic studies we have at the present time a considerable knowledge of the texture of inorganic industrial materials. In most organic high polymers, however, the largest regularly built structural units (known as crystallites or micelles) lie beyond the limit of resolution of the microscope, and in many high polymers even submicroscopic crystalline regularities are lacking. For this reason other methods have been devised for the study of texture.

In order to obtain as complete a picture of the whole structure as possible, it is desirable to consider: (*1*) the spatial arrangement of the atoms within the molecules, and to construct a complete, three-dimensional molecular model; (*2*) the arrangement of the molecules to form crystallites; and (*3*) the arrangement of the crystallites in the macroscopic solid body, as, for example, in fibrils, fibers, laminae, or films, or in commercial products. For biological objects, this means extending structural analysis into the unknown region between objects on the limit of resolution of the microscope (that is, about 1000 Å. in diameter) and the atoms themselves (with a diameter of only a few Å.).

Accordingly, we are led, not only to consider the size, shape, and structure of submicroscopic biological units (for example, genes or the causative agents of virus diseases), but also the general discussion of submicroscopic histology. The object of this inquiry is to obtain a picture of submicroscopic structure complete down to the atoms, a goal which, up until now, has been reached only in the case of cellulose fibers and chitinous tendons. In the much larger field of organized tissues composed of protein, our knowledge (with few exceptions) is still very incomplete, and the goal still far away.

Throughout this book the morphological point of view will be followed systematically. A knowledge of fine structure will enable us to understand those complex and as yet incompletely known reactions of solid and swollen polymers which occur only in certain definite regions of the system and are therefore known as *topochemical reactions*. Such studies provide the basis for a new branch of biochemistry, as yet little explored: the submicroscopic topochemistry of vital processes.

If we are to be in a position to draw conclusions about fine structure from direct observations on high polymeric or biological specimens, it is necessary, on the one hand, to have as complete knowledge as possible of their chemical composition, and on the other hand, to base our conclusions on data for bond strengths and atomic and molecular structure obtained from physical studies of substances of lower molecular weight. It is necessary to proceed as does the organic chemist who automatically makes use of the characteristic chemical reactions of compounds of lower molecular weight in the study of high polymer chemistry.

Although the fundamental data on atomic forces and molecular structure collected by the physicists have been assimilated by inorganic chemistry, so that chemical, physical, and morphological points of view are now united in a general synthesis, in classical organic chemistry one is still somewhat skeptical of the advantages to be gained by following this lead. The in-

troduction of wave mechanics, however, has undoubtedly led to a fundamental advance in our knowledge of molecular structure and bond strengths. What were formerly only empiric generalizations are now seen to be a strict consequence of more fundamental general principles, and can be applied to the field of high polymers. On the other hand, use will often be made of thermodynamics, in order to determine the causes of the behavior of high polymers. The usual equations of applied thermodynamics, such as the phase rule or van't Hoff's law, are based on assumptions which in most cases are not fulfilled by systems of high polymers. For this reason it is often necessary to return to first principles and derive anew equations suitable for our purposes.

Inasmuch as the equations of thermodynamics are derived mathematically from two or three propositions of general validity (the three laws), their validity is as firmly established as these propositions. Thermodynamic treatment of data obtained by sound experimental procedure will always lead to correct conclusions. But these conclusions have the disadvantage that there is no direct relation between them and our pictures of the atomic structure of matter; their significance cannot be visualized. It is at this point that statistical thermodynamics steps in. By its aid, the thermodynamic behavior of simple systems, for example of an ideal gas, can be explained in terms of concrete models. The attempt has often been made, however, as in the well-known book by Fowler and Guggenheim, for example, to provide a statistical interpretation of the physicochemical behavior of all kinds of systems. The unbiased reader will observe that only in rare instances do equations based on model hypotheses accurately reproduce the behavior observed. This is due chiefly to the fact that the macroscopic models proposed are grossly inadequate, since they cannot represent the essentially wave mechanical features of the binding forces.

In the field of high polymers, the conditions are even more complex than in that of compounds of lower molecular weight. It is not to be expected, therefore, that we should reach more than a qualitative statistical interpretation of thermodynamic behavior, *e.g.*, rubberlike elasticity or the behavior of solutions of chain polymers. Statistical calculations on the basis of every conceivable sort of model are immensely popular at the moment, and the extent of the literature available is considerable. But in these pages a certain reserve will be maintained toward this trend in interpretation and the mistake will not be made of regarding macroscopic models

as enlarged molecules which behave, with respect to all their properties, like the molecules themselves. We are convinced that the time will come when speculation in terms of inadequate models will be abandoned; the way to further knowledge will then be sought along what Lewis has called "the safe and broad highway of thermodynamics."

A. PHYSICAL AND PHYSICO-CHEMICAL INVESTIGATION OF HIGH POLYMERS

One of the major problems of polymer science is the complete analysis of the composition, structure, and texture of high polymers. Chemical methods will be described together with the substances to which they are applied; in contrast to this, it is convenient to treat as a whole the physical and physico-chemical methods of investigation and the relevant theoretical background.

I. INVESTIGATION IN SOLUTION

1. Characterization by Means of Solvents and Swelling Agents

We shall consider first what conclusions may be drawn from experiments with swelling agents and solvents—conclusions as to chemical character, structure, internal arrangement, and molecular weight.

The mere fact that a substance swells in certain liquids, that is, increases in volume without passing into solution to any considerable extent, leads to the conclusion that it is a high polymer, and, furthermore, that groups are present which have a definite affinity for those of the solvent. From such imbibition experiments, therefore, the chemist may draw conclusions about chemical composition, just as he may by studying the solubility of an unknown substance of low molecular weight; he is able to decide, for example, whether an excess of hydrophobic or hydrophilic groups is present, or whether basic or acid groups occur in the substance.

If the substance swells in water, liquid ammonia, formamide, or formic acid, we may conclude that the residues contain for the most part hydrophilic groups such as OH, $CONH_2$, $R \cdot O \cdot R$, COOH, and CO. It is true that chain-polymer substances, consisting of strongly hydrophilic structural units, are often practically insoluble in water, and swell only to a very limited extent. But in these cases we are dealing with crystalline polymers of high molecular weight, the crystallites of which are characterized by a high lattice energy, *e.g.*, polyglycylglycine, cellulose, chitin, and silk, or synthetic products such as nylon. Products such as these, however, often dissolve in strong acids and in concentrated solutions of certain salts, such

as lithium iodide and calcium thiocyanate, and, generally, in ammoniacal solutions of cupric oxide. If the substance dissolves in these media without being soluble in water, it is to be concluded that the primary valence chains are built up from groups with a high dipole moment or with a hydrogen bond, and that they are arranged in a crystal lattice. When solutions in salts, acids, or alkalis are to be studied, it is desirable to work at a low temperature in order to exclude, as far as possible, hydrolytic breakdown.

Contrasting with polymers which consist of hydrophilic structural units are the lipophilic high polymer hydrocarbons and chlorides, which swell in hydrocarbons and in halogen derivatives of hydrocarbons. Intermediate between the two groups are the polymeric esters which swell in esters, ketones, chlorinated hydrocarbons, and similar substances.

If it is found that a high polymer swells more in mixtures of solvents than in pure solvents, it is to be concluded that several characteristic groups are present, each of which is solvated by one of the solvents (for example, alcohol–ether is a very efficient swelling agent for incompletely nitrated cellulose containing OH and ONO_2 groups).

(*a*) *Anisotropic Swelling*

Morphological study of the process of swelling may lead to conclusions about the arrangement of the molecules in a given specimen, for, since the swelling agent penetrates between the chains and separates them laterally, it follows that a greater increase in two dimensions as compared with the third indicates that the chains run for the most part parallel to this latter direction. We may take as an example the swelling of tendon in directions at right angles to the long axis, that is, at right angles to the direction of the primary valence chains, or the analogous experiments on the swelling of muscle in a direction at right angles to the fiber axis. If, as is frequently the case in thin foils, the increase in dimensions in one direction (at right angles to the plane of the foil) is particularly large, then, if the foil consists of chain molecules, it is to be concluded that the chains lie at right angles to this direction. Swelling in one direction may also result from a laminated structure of primary valence nets; examples of this are graphite oxide (graphitic acid) and montmorillonite (see Chapter B, sect. II).

(*b*) *Unlimited and Limited Swelling*

If the solvent penetrates between the chains without forming an insoluble compound with them, the substance either: (*1*) swells *continuously* until a viscous solution is finally formed in which the high polymeric substance is

uniformly distributed; or (*2*) swelling is *limited* and leads to a final state in which the more or less swollen substance is in equilibrium with the solvent. A form of limited swelling comparable to the partial miscibility of liquids of low molecular weight may also occur. As experiment shows, and as can be demonstrated by thermodynamic considerations, at equilibrium the amorphous high polymeric phase may contain a relatively large amount of the liquid of low molecular weight, while only a minute quantity of the high polymer is present in the swelling medium. Frequently the high polymer is completely insoluble in the swelling agent. It is then legitimate to conclude that in the partially swollen material cross linkages knot together all the molecules to form a three-dimensional network, as Meyer and Mark[1] suggested years ago.

The nature of these cross-links and of the network can be determined in most cases. In the first place, the network may consist of crystallites, possessing a regular lattice, which do not dissolve in the swelling agent. The solvent only penetrates into cracks and into the amorphous regions between the crystalline particles. Examples are provided by the swelling of cellulose and of gelatin. In general, it is possible to bring the substance completely into solution by the choice of a suitable swelling agent, or by raising the temperature.

In the second case, linkage may be due to strong van der Waals' forces, and the swollen jelly may be brought into solution by adding a suitable solvating agent; an example is the variety of gel rubber which remains when rubber is dissolved in nonpolar solvents, but which may be brought into solution by the addition of small quantities of suitable polar media such as butyl alcohol, etc. In this case the links in the network consist of associated dipoles in the form of C⁻ O or C—OH groups or of hydrogen bonds produced, perhaps, by the oxidation of a small number of links in the chains.

In the third case, primary valence bridges are present between neighboring chains. When such bridges are present, the cross-linked structure can occasionally be destroyed by chemical means and the substance may be brought into solution. In order to gain insight into the nature of the breakdown reaction, the temperature coefficient of breakdown may be determined (by comparing the length of time necessary to obtain the same amount of dissolved product at different temperatures). From this coefficient the energy of activation may be calculated; if this is between 15,000 and 30,000 calories, it is evident that solution occurs as a consequence of

[1] K. H Meyer and H. Mark, *Ber.*, **61**, 1947 (1928). K. H. Meyer, *Biochem. Z.*, **208**, 23 (1929).

the rupture of primary valence linkages. In this way it is possible to show that the rupture of primary valences is responsible for the formation of gelatin from collagen[2] or of soluble from insoluble pectins (in these particular cases, by hydrolysis).

Swollen particles of jelly often remain after the action of a swelling agent has caused part of the substance to pass into solution. These particles possess but slight mechanical rigidity, and their volume does not change on the addition of more solvent. We may conclude then that the dissolved fraction consists of free chains of finite length, while the jelly consists of a loose three-dimensional net, in which the cross links prevent solution. Examples of such systems are rubber, which may be separated into sol and gel rubber and starch granules which show limited swelling, while water-soluble amylose goes into solution.

(c) *Nature of Solutions of High Polymers*

The properties of dilute solutions can be described in terms of a series of equations derived from certain simplifying assumptions. If the validity of such equations, *e.g.*, those relating to osmotic pressure, is tested on solutions of high polymers, however, satisfactory agreement is only to be found in those polymers whose particles or molecules have a compact "globular" shape. Even within the range of very dilute solutions, chain polymers show considerable departure from the properties of ideal dilute solutions. In order to find a range of dilution over which the equations for "ideal dilute solutions" are still valid, it is necessary to dilute the solution so far that the effects to be measured become too small to be assessed with accuracy by the methods available at present.

The cause of this characteristic behavior is revealed by the fact that even dilute solutions of chain polymers show rubberlike elasticity, like that of a rubbery solid.[3] From this one may conclude that the chain molecules hang together in solution. The formation of this "felt" can be deduced from the relation between concentration and viscosity, which should be linear if the particles do not influence each other; but in this case the viscosity increases much more rapidly than linearly with the concentration, since the mutual interference and felting of the molecules is more and more marked the higher the concentration. The occurrence of felting at high dilutions is due to the extreme fibrillar form of the molecules; in a 0.1% solution of rubber of molecular weight 200,000, for example, calcula-

[2] E. Cherbuliez, J. Jeannerat, and K. H. Meyer, *Compt. rend. soc. phys. his. nat. Genève*, **54**, 122 (1937); *Z. physiol. Chem.*, **255**, 241 (1938).

[3] R. Eisenschitz and B. Rabinowitsch, *Ber.*, **64**, 2522 (1931).

tion shows that each molecule must be in contact, on an average, with ten others. The solutions which we have to consider and which serve all sorts of industrial purposes (such as varnishes, spinning solutions, etc.) are, therefore, in no sense molecularly dispersed solutions. They are to be regarded as felts or solids swollen with liquid, in which free "colloidal particles" or "macromolecules" simply do not occur.

It is to be regretted that, even at the present, measurements made on such systems continue to be interpreted on the assumption that they are solutions containing freely mobile particles, concerning whose properties it is legitimate to draw conclusions from the data collected. Every type of transitional stage between compact (globular) and threadlike (fibrillar) particles is to be found and, correspondingly, all stages between felts and molecularly dispersed solutions are known.

From polymers of low or moderate molecular weight (below 5000) solutions can be prepared on which measurements are possible and in which the relation between concentration and viscosity, for example, is practically linear. Such measurements can be interpreted on the assumption that the molecules are truly "dissolved."

2. Determination of Molecular Weight of Dissolved High Polymers

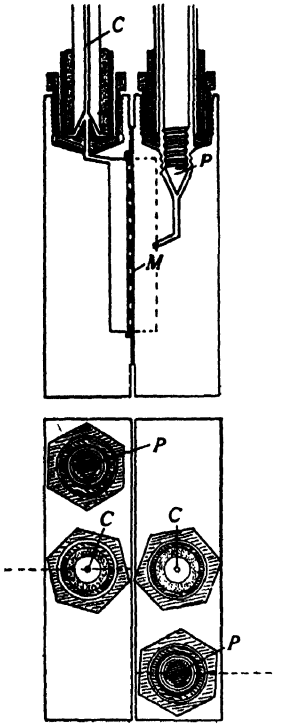
Only when a high polymeric substance is soluble is it possible to undertake a determination of the weight of the osmotically active particle. If, as is almost always the case with high polymers, we are concerned with mixtures, mean values for this molecular weight will be obtained. This will be discussed in detail in the following pages.

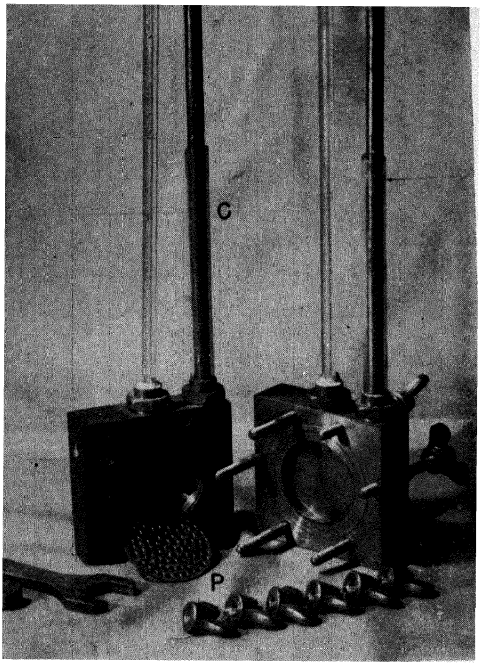
As already explained in Volume II of this series, methods depending on the raising of the boiling point or the lowering of the freezing point are out of the question, since, in order to obtain measurable effects with substances of high molecular weight, one must use solutions of too high a concentration. It is therefore necessary to look for more sensitive methods. Those to be considered include the direct measurement of osmotic pressure, which may be used up to molecular weights of about 400,000, and measurements of the equilibrium or rate of sedimentation in the centrifugal field of an ultracentrifuge, the range of application of which is much greater.

(*a*) *Determination of Molecular Weight by Measurements of Osmotic Pressure*

We shall describe, first of all, the method for the direct determination of osmotic pressure (suitable for use in any laboratory) and the application of this method to the study of high polymers. Reliable measurements of the osmotic pressure of high molecular

substances are by no means easy to make; we shall accordingly describe a procedure which experience has shown to be satisfactory (Figs. 1 and 2).[4] We recommend the brass or silvered brass cell described by Herzog and improved by Meyer, Wolff, and Boissonnas.[5]





Figs. 1 and 2. Osmotic cell (Meyer, Wolff, and Boissonnas[5]).

The membrane, *M*, lies on a metal sieve and is clamped between two metal blocks (75 mm. × 15 mm. × 75 mm.). Each block is bored to receive a capillary tube, *C*, and a tube which is fitted with a needle valve, *P*. Wood's metal is used as cement between metal and glass parts; the membrane is a collodion membrane or a membrane of denitrated collodion. The compensation method described by van Campen[6] is employed in making the determination. The necessary arrangement of capillaries and tubes by

[4] C. G. Boissonnas and K. H. Meyer, *Helv. Chim. Acta*, **20**, 783 (1937).

[5] R. O. Herzog and H. M. Spurlin, *Z. physik. Chem., Bodenstein-Festband*, 239 (1931). K. H. Meyer, E. Wolff, and C. G. Boissonnas, *Helv. Chim. Acta*, **23**, 430 (1940). See also the cells used by R. Carter and B. R. Record, *J. Chem. Soc.*, **1939**, 660, and by R. M. Fuoss and D. J. Mead, *J. Phys. Chem.*, **47**, 59 (1943).

[6] P. van Campen, *Rec. trav. chim.*, **50**, 915 (1931).

which the external pressure is applied is shown in figure 3. The whole apparatus is contained in an air thermostat with a glass window; the differences in height between the columns of liquid are measured from outside by means of a cathetometer. The pressure difference between solution and solvent in millimeters of water at 4°C. is given by the relation:

$$P = (B - O)d_s - (B' - O)d_c + (A - A')d_m$$

in which d_s, d_c, and d_m represent the densities of the solution, the solvent, and the liquid in the manometer. The significance of the other symbols is clear from the figure.

At a given arbitrary adjustment of the various columns of liquid it is found, for example, that solvent passes from the solution through the membrane into the pure solvent. The corresponding difference in pressure between the two halves of the cell is then greater than the osmotic pressure. If the pressure difference is adjusted to a lower value, it may be found that solvent flows in the opposite direction. The osmotic pressure lies, therefore, between these two pressures. By gradually reducing the pressure differences in further measurements it is possible in one day to determine the osmotic pressure with an error of less than 1%. If the measurements are repeated over a period of several days so that the pressure is measured as a function of time, it is possible to extrapolate back to zero time.

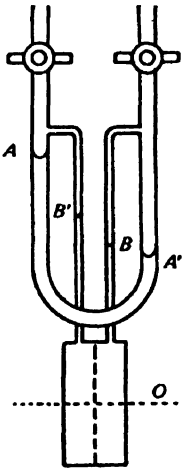


Fig. 3. Arrangement for measuring osmotic pressure by the compensation method.

Care is necessary in using the compensation method. The dissolved substance may tend to clog the membrane; the establishment of equilibrium then exhibits a sort of induction period which may last for days during which time there is practically no transfer of solvent. If this effect occurs it is, of course, impossible to use the compensation method, and the establishment of equilibrium must be awaited. Solutions of methyl amylose behave in this way.

A further difficulty is experienced in handling solutions in solvents with a high surface tension. The use of capillary manometers is inadvisable in such cases since errors due to incomplete wetting or changes of concentration at the surface of the solution (particularly in the case of dilute solutions) become considerable. This happens especially with aqueous solutions.

At infinite dilution, the osmotic pressure is proportional to the number of particles present in a constant volume. In extremely dilute solutions of pure substances, the molecular weight, M, is, therefore, inversely proportional to the osmotic pressure, P, per unit of concentration, c. According to van't Hoff:

$$M = 2.31_7 \times 10^5 (1 + 0.00367t)c/P$$

where t is the temperature in °C., c is the concentration in grams per liter,

and P is the pressure in millimeters of water. If the mean molecular weight of a mixture is calculated from the osmotic pressure, then:

$$\bar{M} = \frac{1}{\sum f_i / M_i}$$

where M_i are the individual molecular weights and f_i are the appropriate fractions of the total mass. It is immediately apparent that, in the case of a mixture, the effect of the large particles with a small value of f_i/M_i is less than that of the small particles.

The proportionality between the number of particles and the osmotic pressure, postulated by van't Hoff, holds only at infinite dilution since the exact equation only becomes identical with that of van't Hoff when $c = 0$. At finite concentrations deviations occur which, in the region of measurable pressures, are small only in the case of compounds of low molecular weight, or of systems with large compact molecules. In the case of chain polymers, deviations appear even at the lowest concentrations which can be used to obtain a measurable pressure.

In place of the van't Hoff equation an empiric relation suggested by Ostwald is often used:

$$P = ac + bc^2 \qquad P/c = a + bc$$

where $a = 2.31_7 \times 10^6 (1 + 0.00367t)/M$, and b is a constant.

It is preferable, however, to make use of the method of graphic extrapolation by plotting P/c (the reduced osmotic pressure) against c. Duclaux and Dobry[7] have made numerous determinations by this means. It is the method almost invariably used at present and is altogether to be preferred to the method of calculated extrapolation by means of equations without theoretical foundation.[8]

By extrapolating to zero, the true particle weight can be measured even for chain polymers. This is not necessarily identical with the weight of the "chemical molecule." The particle (micelle, supermolecule) may be formed by the association of several "chemical molecules." Such associated particles may be of the same size and composition or of different sizes and variable composition, so that the value obtained is only a mean. While it is true that at infinite dilution the associated particles separate into the constituent chemical molecules, it is certain that extrapolation

[7] A. Dobry, *J. chim. phys.*, **32**, 50 (1935).

[8] Such equations have been proposed and used by G. V. Schulz, *Z. physik. Chem.*, **A158**, 237 (1932); **A159**, 374 (1932), **A160**, 409 (1932); **A176**, 317 (1936)

only gives the particle weight for the range of the measurements, *not* the molecular weight at zero concentration. For experimental reasons it is often not possible to extend the range of observations to that region of the curve in which dissociation is considerable. Only when certain considerations exclude the formation of micelles is it legitimate to regard the particle weight as identical with the chemical molecular weight, that is, with the weight of the primary valence chain. It is therefore desirable to make observations in various solvents of the highest possible dissociating action, and to see whether the values for molecular weight agree one with another. Measurements on ethyl cellulose by Steurer[9] may serve as an example.

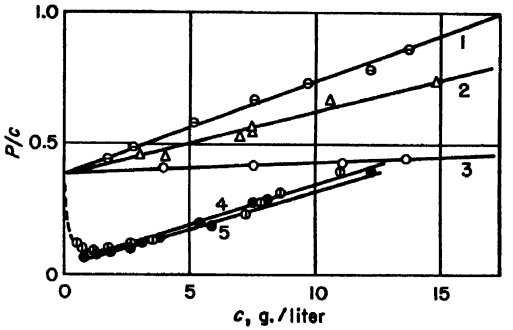


Fig. 4. Reduced osmotic pressure P/c of ethyl cellulose in: (*1*) benzene-ethanol 90:10; (*2*) chloroform, (*3*) dioxane; (*4*) toluene; (*5*) benzene (Steurer[9]).

The extrapolation of the values for P/c in benzene gave a multiple of the molecular weight measured in chloroform and dioxane (see Fig. 4). It is therefore probable that the identical particle weights in the two latter solvents are to be identified with the weight of the primary valence chains.

Furthermore, compounds may be converted into derivatives with the same number of links in the chain ("polymeric analogues," to use Staudinger's term) if care is taken to avoid degradation. The derivatives may be soluble in other solvents and further observations of osmotic pressure can be made; for example, polyalcohols may be converted into polyesters. If extrapolation to $c = 0$ gives particle weights from which the same degree of polymerization for compound and derivative can be calculated, it is extremely probable that the substances are present in solution as primary valence chains, and that the particle weight is equivalent to the chemical

[9] E. Steurer, *Z. physik. Chem.*, **A190**, 1 (1942).

molecular weight. Naturally, this indicates nothing about the condition of these substances in solutions which are *not* greatly diluted and in which association may be considerable.

In the case of proteins, the particles present in aqueous solution are almost always stoichiometrically defined, associated particles; on changing the *p*H or adding urea or formamide, many of these dissociate into single molecules.

If the substance whose molecular weight is to be determined is a multivalent electrolyte and is only soluble in a salt solution (a case which occurs frequently among the proteins), the transference of diffusible ions from the exterior into the solution of the colloid leads to an increase in the osmotic pressure. This phenomenon, first recorded by Donnan[10] and worked out fully by Adair[11] in the case of hemoglobin, necessitates a special correction in the calculation of the molecular weight. The whole question has been reviewed by Bolam.[12]

As a consequence of capillarity effects and fluctuations in temperature, the measurements of pressures less than 2 mm. of water are extremely inaccurate. These errors are not eliminated if the amount of liquid which rises in the capillary is weighed instead of being measured by volume. The former method is used in the osmotic balance, constructed by Jullander and Svedberg.[13] For special purposes the balance may prove to be useful. Since in the case of chain polymers it is necessary to make measurements down to a concentration of 0.5% in order to be able to extrapolate to zero concentration with sufficient certainty, the inaccuracy of measurements at very low pressures fixes the upper limit of molecular weight determinable by this method at 500,000. For determinations of still higher molecular weights it is necessary to use the ultracentrifuge.

(*b*) *Determination of Molecular Weight by the Ultracentrifuge*

It is well known that gaseous molecules in a uniform gravitational field distribute themselves in such a way that their concentration diminishes exponentially with height. The fall in concentration depends on the molecular weight of the gaseous particles, on the temperature, and on the gravitational field; so that if we know the rate at which the concentration diminishes with height, we can determine the molecular weight. Particles

[10] F. G. Donnan, *Z. Elektrochem.*, **17**, 572 (1911); *Chem. Revs.*, **1**, 73 (1925).

[11] G. S. Adair and M. E. Robinson, *Biochem. J.*, **24**, 1864 (1930).

[12] Summary, in T. R. Bolam, *Kolloid-Beihefte*, **39**, 139 (1934). T. R. Bolam, *Donnan Equilibria*, Bell, London, 1932.

[13] I. Jullander and T. Svedberg, *Nature*, **153**, 523 (1944).

suspended in a solvent distribute themselves in a similar way; but it is possible to demonstrate changes in concentration only in the case of particles whose size is much greater than that of molecules, such as the particles of the gamboge emulsion used by Perrin for the determination of Avogadro's number. In the case of smaller particles the concentration cannot be measured with sufficient accuracy to detect these differences. The concentration differences may, however, be increased if the gravitational field is replaced by the centrifugal field of a high-speed centrifuge. The development of this method is due to the Swedish investigator Svedberg[14] who, with a number of collaborators, has made a series of important investigations in the field of high polymers. In these experiments the centrifugal force has been increased to values corresponding to a million times the gravitational field.

The Svedberg ultracentrifuge consists of a rotor about 10 cm. in diameter into which, in an eccentrically placed, radial cavity, a small cell is placed, containing the solution to be studied. The fall in concentration which takes place along the cell in the course of centrifuging is measured optically, either by determining the absorption (in the case of colored substances or those absorbing ultraviolet light) or by measuring the refractive index changes (Lamm[15]). The solution is allowed to remain under the influence of the centrifugal field until equilibrium between sedimentation (as a result of the centrifugal force) and diffusion (the consequence of thermal agitation) is established. For each species of molecule the equation:

$$M = \frac{2RT \cdot \ln c_2/c_1}{(1 - V\rho)\,\omega^2(x_2^2 - x_1^2)}$$

holds (assuming that we are dealing with an ideal dilute solution) where c_2 and c_1 are the concentrations at two points at distances x_2 and x_1 from the axis of rotation, V is the partial specific volume of the dissolved substances, ρ is the density of the solvent, and ω is the angular velocity.

The homogeneity of the substance is tested by measuring the concentration, and calculating the molecular weight at various distances from the axis of rotation. If M is constant, we are dealing with a substance composed of molecules of the same size. If, however, the value changes, several species of molecules are present. If the individual molecular weights

[14] Summaries, in T. Svedberg, *Ber*, **67**, 117 (1934); *Ind. Eng. Chem., Anal. Ed.*, **10**, 113 (1938). See also his book, *The Ultracentrifuge*, Oxford Univ. Press, London, 1940.

[15] O. Lamm, *Z. physik. Chem.*, **A138**, 313 (1928); **A143**, 177 (1929); *Nature*, **132**, 820 (1933).

are not too close to each other, an excess of one component or the other accumulates at the base or at the top of the liquid column when such a solution is exposed to centrifugal fields of varying intensity; by this means it is possible to make an approximate analysis of the molecular weights. As compared with measurements of osmotic pressure, this method has the advantage that it enables one to judge of the homogeneity or inhomogeneity of the substance investigated.

In the case of chain polymers, deviations occur like those observed in measurements of osmotic pressure.

(c) *Determination of Molecular Weight and Measurement of Homogeneity from Sedimentation Velocity in the Ultracentrifuge*

The ultracentrifuge may be used for the determination of molecular weights in another way, and thus provides a check on the values obtained by the method which we have just described. In the present method the experiments involve measurements of diffusion constants and of the velocity of sedimentation. The diffusion constant, D, is obtained by observation of the change in concentration with time along a column of solvent, which at the beginning of the experiment was bounded at one end by a solution of the substance in the same solvent. For working with small quantities, Lamm[16] has developed a suitable diffusion cell and a sensitive measuring device. The velocity of sedimentation at a very high angular velocity is determined in the ultracentrifuge. By calculating the velocity of sedimentation for unit field strength (dividing by $\omega^2 x$), one can obtain s, the characteristic sedimentation constant for the dissolved substance in a given solvent, at a particular temperature. The molecular weight may be calculated from the equation:

$$M = \frac{RTs}{D(1 - V\rho)}$$

Although this equation is of general validity when the solution is infinitely dilute, the method must be used with circumspection in the determination of the molecular weights of chain polymers, since the concentration at which such molecules no longer interfere with one another is so low that measurements of diffusion constant or sedimentation constant can only be made with great difficulty. In order to obtain the sedimentation velocity of the substance in a state of molecular subdivision, it is always necessary to

[16] O. Lamm, *Nova Acta Regiae Soc. Sci. Upsaliensis*, [4] **10**, No. 6 (1937). O. Lamm and A. Polson, *Biochem. J.*, **30**, 528 (1936)

make observations at several concentrations and to extrapolate to zero concentration.

The method is specially suitable for determinations of molecular weights in proteins, the particles of which are mostly compact. Since the particles are almost invariably associated units, the term "molecular weight" as used in protein chemistry always implies the weight of the aggregate and not of the individual molecule. Generally speaking, even at the high dilutions at which it is customary to use the ultracentrifuge, the protein molecules do not dissociate completely; this dissociation can often be effected by adding certain reagents.

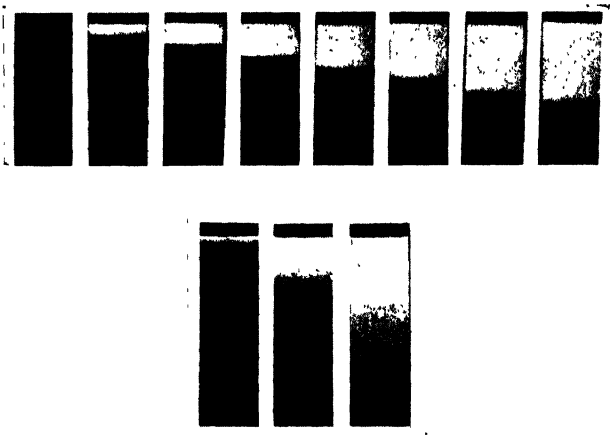


Fig. 5. *Above*: sedimentation of hemocyanin in a centrifugal field of 37,000*g*; time interval of 3 min. between the photographs; monodisperse solution. *Below*: sedimentation of gold sol under similar conditions, polydisperse solution.

Determinations of the velocity of sedimentation require stronger centrifugal fields than those necessary for the determination of the sedimentation equilibrium. The value of the former method lies in the fact that it is possible to determine with certainty whether the substance investigated is homogeneous, that is to say, whether it consists of particles of identical weight. In this case the particles are thrown down equally rapidly and one obtains a sharp boundary between solution and solvent which is displaced during centrifuging (Fig. 5). If the solution is polydisperse, the boundary is indistinct; when only a few species of molecules are present, a boundary layer can be recognized for each species. Using Toepler's

schlieren method, the boundary appears as a dark band. The gradient of the refractive index at any point can be measured as a function of the distance of this point from the center of rotation by an optical device sug-

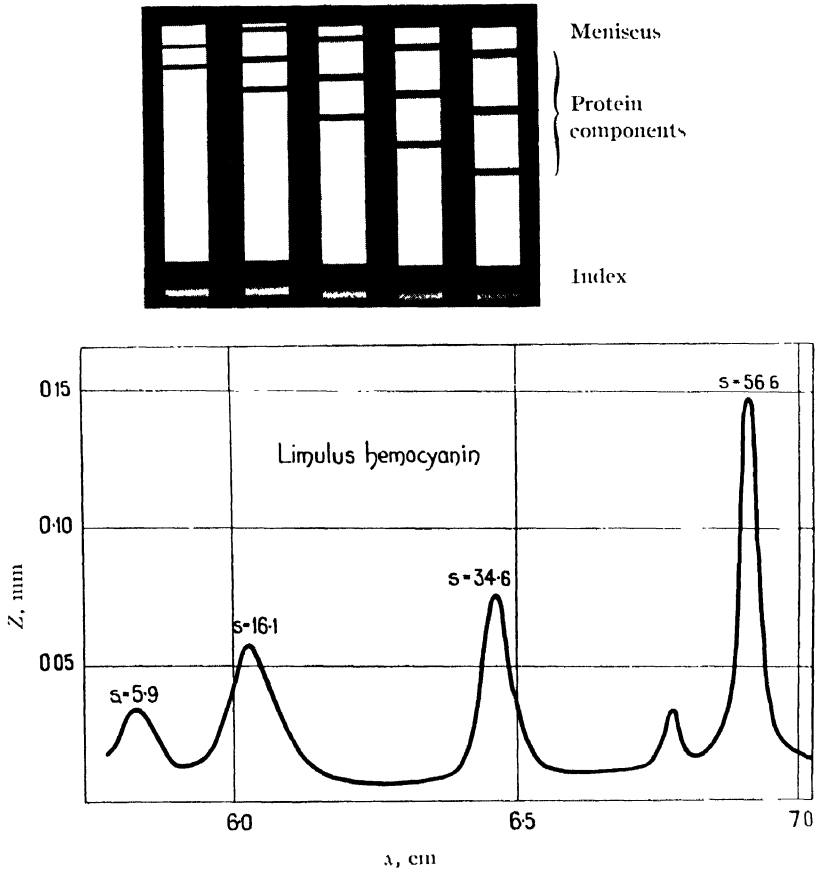


Fig. 6. Sedimentation pictures for *Limulus* hemocyanin (Eriksson-Quensel). *Above*: obtained by Toepler schlieren method at *p*H 6.8, showing the fastest three sedimenting components of $s = 56\,5 \times 10^{-13}$, 34.6×10^{-13}, and $16\,1 \times 10^{-13}$. Centrifugal force 120,000g. Time between exposures, 5 min *Below*: obtained by the Philpot method at *p*H 6.8, showing four main components and also a small amount of a fifth Centrifugal force, 120,000g. Time after reaching full speed, 35 min

gested by Philpot. A sudden change of the refractive index corresponds with a high value of the gradient and appears as a peak on the diagram (Fig. 6). More slowly progressing variations of the index are indicated as

broad maxima; these are characteristic for heterodisperse systems (Fig. 7).

Recently the practice has developed of characterizing particles by the sedimentation constant without attempting to measure the diffusion constant or to calculate a molecular weight.

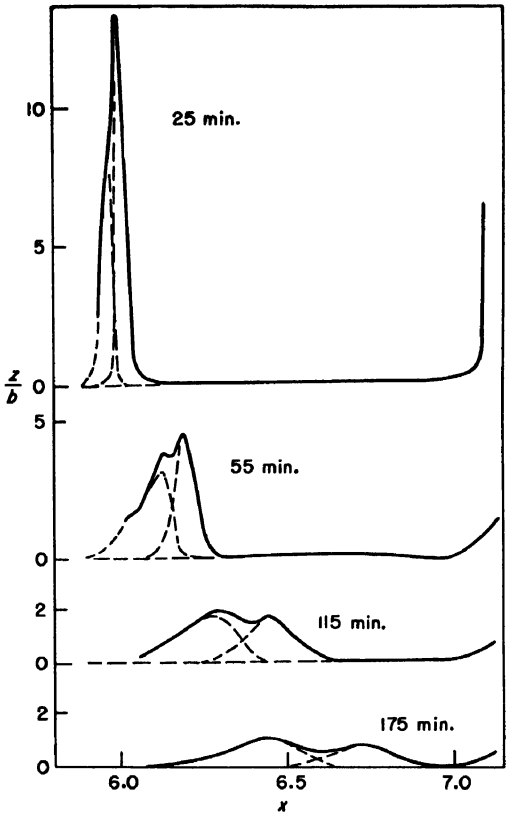


Fig. 7. Sedimentation diagrams of native wood cellulose (Svedberg). Two maxima. cellulose, hemicellulose and polyoses. $M_{cellulose}$ 800,000. z/b = gradient, x = distance from center of rotation

(*d*) *Estimation of Particle Size by Ultrafiltration*[17]

The filtration of a solution through suitably prepared membranes of cellulose or nitrocellulose makes possible the separation of larger particles,

[17] A general review of ultrafiltration, J. D. Ferry, *Chem. Revs.*, **18**, 373 (1936)

if these are more or less spherical. The membranes are calibrated with substances of known particle size, such as hemoglobin (M = 70,000), and it is then necessary to determine which membrane retains a given particle. By such means it is possible to estimate the size of the particle in question. This method, developed by Zsigmondy,[18] Bechhold,[19] Erbe,[20] and Elford,[21] has proved useful for the characterization of larger particles, in particular, virus particles, but it is unsuitable for chain polymers.

(*e*) *Approximate Determination of Weight and Form of Molecules from Diffusion*

If a layer of a solution of given concentration is covered by a layer of pure solvent or a solution of lower concentration, the solute diffuses from the first into the second as a result of thermal agitation of the molecules. This phenomenon is described by:

$$dm = -DA\frac{dc}{dx}dt$$

where dm is the amount of solute that passes in the time dt through a boundary of area A, at which the concentration gradient is dc/dx. D is a constant characteristic of the system and can be deduced from determination of the rate dm/dt of diffusion. In dilute solutions this constant has the value:

$$D = kT/f = RT/F$$

$F = N_A f$ being a frictional constant. The latter may be computed for spherical molecules of radius r by the use of Stokes' law:

$$f_0 = 6\pi\eta r$$

where η is the viscosity of the solvent. From this, if M is the molecular weight and V the partial specific volume of the solute:

$$f_0 = 6\pi\eta\left(\frac{3MV}{4\pi N_A}\right)^{1/3}$$

and thus the molecular weight M may be found (N_A is Avogadro's constant).

[18] R. Zsigmondy, *Angew. Chem.*, **39**, 398 (1926).
[19] H. Bechhold, *Kolloid-Z.*, **66**, 329 (1934); **67**, 66 (1934).
[20] F. Erbe, *ibid.*, **59**, 32, 195 (1932); **63**, 277 (1933).
[21] W. J. Elford, *Trans. Faraday Soc.*, **33**, 1094 (1937).

Instead of the more precise diffusion cell, Anson and Northrop[22] utilize an apparatus in which the solution is separated from the solvent by a porous membrane. The rate of diffusion through the membrane is determined analytically and, if the membrane has been calibrated with the aid of proteins of known molecular weight, a rough estimation of the molecular weight of an unknown protein may be obtained.

The situation is less simple if the particles are nonspherical. Perrin[23] obtained, from hydrodynamical consideration, the increase of f for ellipsoidal particles in comparison with f_0 for spherical ones of the same weight and volume. In order to apply Perrin's equation, M must first be determined by means of osmotic pressure measurements and from this the value of f_0 is derived. If V is known, the real value of f may be computed from diffusion experiments. The quotient f/f_0 may be used to find the axial ratio a/b for particles having the form of prolate ellipsoids; Perrin gives the following numerical relations (Table I):

TABLE I

f/f_0	a/b	f/f_0	a/b
1.000	1.0	1.1	3.0
1.003	1.2	1.3	6.0
1.01	1.4	1.5	9.0
1.03	1.8		

Oncley[24] has tried to take account of the increase in weight and volume due to hydration. Assuming a thin layer of water, he recalculates the axial ratio for different proteins and especially serum proteins by Perrin's method. His figures should be regarded as being of a qualitative nature.

(*f*) *Determination of Molecular Weight and Particle Shape by Means of Light Scattering*

Light entering a colorless suspension of particles is scattered if the refractive index of the particles differs from that of the medium in which they are suspended. If the particles are optically isotropic, spherical, and small in diameter as compared with the wave length of the light, the component scattered at 90° to the incident beam is completely polarized. If the par-

[22] J. H. Northrop and M. L. Anson, *J. Gen Phys.*, **12**, 543 (1929). See W A. James, E. A. Hollingshead, and A. R. Gordon, *J. Chem. Phys.*, **7**, 89 (1939).

[23] F. Perrin, *J. phys. radium*, **7**, 1 (1936).

[24] J. L. Oncley, in *Proteins, Amino Acids and Peptides.* By E. J. Cohn and J. T Edsall, Reinhold, New York, 1943

ticles are anisometric and anisotropic, the light scattered in this way is in part depolarized. The ratio of the intensity of the scattered light polarized in a plane parallel to the direction of propagation of the incident beam to that of the component polarized in a plane at right angles to this is known as the degree of depolarization, Δ.

In the case of submicroscopic, spherical, isotropic particles, the degree of depolarization is zero. According to Lotmar,[25] in considering departures from the zero value, we have to distinguish between suspensions of metallic,

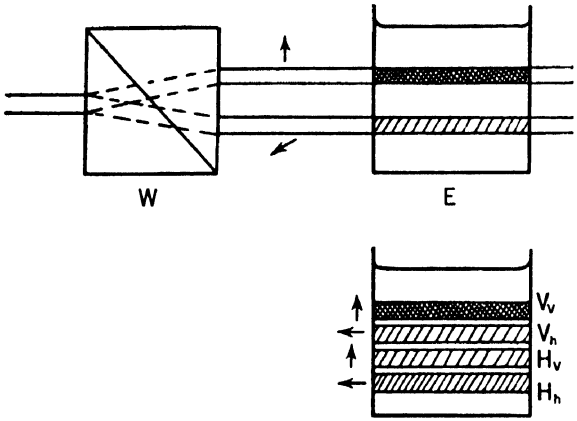


Fig. 8. Arrangement used by Krishnan for the determination of the degree of depolarization (Lotmar). W = Wollaston prism; E = experimental vessel

light-absorbing particles and of dielectric particles. In the former category, particle shape is the important factor, while in the case of dielectric particles internal anisotropy is of decisive importance.

The following method, recommended by Raman and Krishnan for the quantitative observation of the polarization of scattered light, is particularly informative. The incident beam is split into two beams, polarized at right angles to each other, by a Wollaston prism. These are the component H (polarized in the plane defined by the direction of observation and the direction of incidence) and the component V. The scattered light is observed at right angles to the direction of incidence and is split into four components by means of a second Wollaston prism (see Fig. 8): a horizontal and vertical component of beam H, namely H_h and H_v, and a horizontal and a vertical component of beam V, V_h and V_v. The component

[25] W. Lotmar, *Helv Chim. Acta*, **21**, 792, 953, 1780 (1938).

V_v is always the strongest and in the case of very small, spherical, isotropic particles this alone is present. If the particles are anisotropic, the three other components are visible, and are of equal intensity. This is the case generally for particles of molecular dimensions. With larger particles, H_h is brighter than H_v and V_h, which are of equal intensity. With spherical, isotropic particles, H_h and V_h disappear; with anisotropic particles the intensity of these components is appreciable.

This method has been used by Krishnan[26] to demonstrate polymolecular association in liquid mixtures, *e.g.*, carbon disulfide and methyl alcohol,

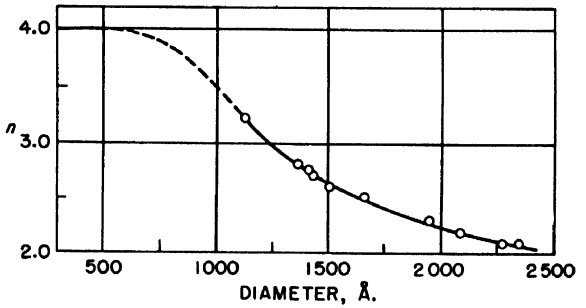


Fig. 9. Variation of wave length exponent n with the diameter of spherical dielectric particles (Heller).

aniline and cyclohexane; it has also been used by Gehman and Field[27] in studying solutions of rubber, and by Herzog, Kratky, and Petertil[28] for solutions of cellulose derivatives.

According to Heller[29] use can be made of the relation between the intensity of the scattered light and the exponent of the wave length in determining the diameter of coarser, spherical particles, *e.g.*, in emulsions, such as are commonly used in the preparation of vinyl polymers. According to the equation mentioned below due to Rayleigh, the intensity of the scattered light, i, is proportional to $1/\lambda^4$ if the diameter of the particles is smaller than $\lambda/10$; with larger particles the exponent decreases from 4 to 2. Turbidity is measured for different wave lengths, and the exponent, x, is calculated by means of the equation:

$$\tau = K\lambda^{-x}$$

[26] R. S. Krishnan, *Kolloid-Z.*, **84**, 8 (1938).
[27] S. D. Gehman and J. E. Field, *Ind. Eng. Chem.*, **29**, 793 (1937).
[28] R. O. Herzog, O. Kratky, and E. Petertil, *Trans. Faraday Soc.*, **29**, 60 (1933)
[29] W. Heller and E. Vassy, *J. Chem. Phys.*, **14**, 565, 566 (1946).

The diameter of the particles can then be read off from the curve given in figure 9.

In the case of small particles which do not interfere with each other in solution, the following relation, obtained by Putzeys and Brosteaux[30] by a modification of an earlier expression due to Rayleigh, is valid:

$$\frac{i_0}{I} = \frac{9\pi^2}{2\lambda^4}\left[\frac{n_2^2 - n_1^2}{n_2^2 + 2n_1^2}\right]^2 \frac{6(1+\Delta)}{6-7\Delta} V \times \frac{M}{N_A \rho}$$

In this equation, I is the intensity of the incident light, and i_0 is the intensity of the scattered light referred to unit volume and measured at unit distance and at 90° to the direction of the incident light; n_1 is the refractive index of the medium and n_2 is that of the suspended substance. V is the total volume of particles present in unit volume; M is the molecular weight; ρ is the specific gravity of the particles; N_A = Avogadro's number. Measurements made on solutions of approximately spherical protein molecules, such as ovalbumin and amandin, excelsin and hemocyanin, gave values for M in agreement with those obtained from ultracentrifuge measurements.

In practice the apparatus can be calibrated each time it is used with a solution of a homodisperse substance of known molecular weight, chemically related to the substance under investigation. For example, Bücher[31] used an apparatus calibrated with edestin (molecular weight = 300,000) for determining the molecular weight of the crystalline enzyme enolase. In the case of heterodisperse suspensions, the "molecular weight" is given by the sum of the scattering powers of the individual particles. A mean molecular weight is obtained but this mean value is a *weight average*, different from the mean value obtained by osmotic methods.

The Einstein[31a] equation for the turbidity of liquid mixtures has been applied by Debye[32] to solutions of macromolecules. If the particles are small as compared with the wave length λ of the incident light, the turbidity τ—which is the proportion of the incident light removed from the primary beam by scattering as it passes through a layer of the suspension 1 cm. thick—is given by the following equation:

$$\frac{c}{\tau} = \frac{3\lambda^4 N_A}{32\pi^3 n^2 (\partial n/\partial c)^2}\left[\frac{1}{M} + \frac{2B}{RT}c \ldots\right]$$

[30] P. Putzeys and J. Brosteaux, *Trans. Faraday Soc.*, **31**, 1314 (1935).

[31] T. Bücher, *Die Chemie*, **56**, 328 (1943); *Biochim. Biophys. Acta*, **1**, 467 (1947).

[31a] A. Einstein, in J. Alexander, ed., *Colloid Chemistry*, Vol. I, Reinhold, New York, 1926, page 323.

[32] P. Debye, *J. Applied Phys.*, **15**, 338 (1944).

where c is the concentration, n is the refractive index of the medium, N_A is the Avogadro number, and B is a constant. By measuring c/τ at several concentrations and extrapolating to $c = 0$, B can be excluded and M can be calculated. Debye's theory has been applied by Doty, Zimm, and Mark[33] to the study of various high polymers.

If mixtures of two liquids of different refractive indices are used, conclusions about the adsorption of the two media on the polymer can be drawn from the scattering data.[34]

P. and P. P. Debye have recently shown that conclusions about the shape of the scattering molecules, particularly of chain molecules, can be drawn from the relation between the intensity of the scattered light and the angle through which it is scattered. Stein and Doty[35] have commented as follows on Debye's theory and its experimental application:

"If a linear dimension of the molecule is greater than about one twentieth of the wave length, the scattering from different parts of the same molecule must be considered separately. Thus the light scattered from different parts of the molecule differs in phase, due to the difference in path length, and a greater path difference is produced in the backward than in the forward direction. As a result of this interference effect, there will be an all-round decrease in scattered intensity, with a greater reduction in the backward direction and a consequent dissymmetry in the angular distribution of intensity of the scattered light (radiation envelope).

'For first-order interference from a polymer of homogeneous molecular size and shape, the radiation envelope may be adequately characterized by measuring the ratio of scattered intensity at two angles symmetrical about 90° (for example, 51° and 129°).

"For a straight rod-shaped molecule, the angular variation of intensity, I, is given by:

$$I = \frac{1}{x}\int_0^{2x} \frac{\sin x}{x}\, dx - \left(\frac{\sin x}{x}\right)^2$$

where $x = \pi \mathfrak{L}\sigma'/\lambda$, λ = wave length of light in solution, $\mathfrak{L}$ = length of the molecule, $\sigma' = 2 \sin \theta/2$.

"For a randomly coiled molecule:

$$I = \frac{2}{v^2}\left[e^{-v} - (1 - v)\right]$$

where

$$v = \frac{2}{3}\frac{(\pi R\sigma')^2}{\lambda^2}$$

R^2 is the root mean square separation of the ends of the chain molecule."

[33] P. M. Doty, B. H. Zimm, and H. Mark, *J. Chem. Phys.*, **13**, 159 (1945). *Cf.* J. Waser, R. M. Badger, and V. Schomaker, *ibid.*, **14**, 43 (1946).

[34] R. H. Ewart, C. P. Roe, P. Debye, and J. R. McCartney, *J. Chem. Phys.*, **14**, 687 (1946).

[35] R. S. Stein and P. Doty, *J. Am. Chem. Soc.*, **68**, 159 (1946).

Experiments with fractionated cellulose acetates of known chain length showed that the second formula represented the experimental results more accurately in the case of high molecular fractions; from this it may be concluded that the molecules are curled. On the other hand, at molecular weights below 80,000, the molecules are practically straight.

It is to be noted that measurements of scattered light are made difficult by the fact that dust particles or small quantities of coarser particles may very easily falsify the results. The suspensions examined must therefore be carefully freed from coarser particles by centrifugation.[35a]

(g) *Determination of Molecular Weights from Measurements of Viscosity*

In the methods considered up to this point, the molecular weight is obtained from the experimental data by means of equations derived from theoretical considerations. The calculation of the molecular weight from viscosity data, however, depends on the use of an empirically determined relation between molecular weight and viscosity. The method is therefore to be judged on a different basis from that used for osmotic methods. It has been shown to be useful for obtaining rapidly a very rough estimate of the order of magnitude of the molecular weight of chain polymers.

Measurements of the viscosity of cellulose solutions have been used for a long time for determining the degree of degradation of cellulose, since it has been found empirically that the viscosity diminishes as breakdown proceeds. This relation of viscosity to molecular weight was first pointed out by Ostwald[36] and Biltz,[37] but as early as 1909 the polymerization of styrene had been followed viscometrically by Stobbe.[38] From 1927 on systematic studies of the viscosity of cellulose derivatives, rubber, and other high polymers were made in the laboratories of the I. G. Farbenindustrie A.-G.[39] and led to the characterization of celluloses and cellulose products on the basis of their viscosity in solutions of ammoniacal copper oxide. In 1930 Staudinger,[40] who as early as 1926 had drawn attention to

[35a] R. H. Blaker, R. M. Badger, and T. S. Gilmann, *J. Phys. & Colloid Chem.*, **53**, 794 (1949).

[36] W. Ostwald, *Grundriss der Kolloidchemie.* 2nd ed., Steinkopff, Dresden, 1911.

[37] W. Biltz, *Ber.*, **46**, 1532 (1913); *Z. physik. Chem.*, **83**, 703 (1923).

[38] H. Stobbe and G. Posnjak, *Ann.*, **371**, 259 (1909).

[39] See leaflet issued by I. G. Farbenindustrie A.-G. Ludwigshafen, *Die Viskosität der lyophilen Sole*, 1929. H. Fikentscher, *Cellulosechemie*, **13**, 58 (1932). H. Fikentscher and H. Mark, *Kolloid-Z.*, **49**, 135 (1929).

[40] H. Staudinger, *Ber.*, **59**, 3031 (1926). H. Staudinger and W. Heuer, *ibid.*, **63**, 222 (1930). Also many later papers, for example, H Staudinger and G. V. Schulz, *ibid.*, **68**, 2320 (1935).

the relationship between molecular weight and viscosity, put forward the view that the specific viscosity of homologous polymers is proportional to the molecular weight. According to his empirical equation:

$$\eta_{spec}/c = K_m M$$

in solutions of low concentration; from this, knowing K , the molecular weight, may be calculated:

$$M = \eta_{spec}/K_m c$$

For this purpose the relative viscosity, η_{rel}, that is, the ratio of the viscosity of the solution to that of the pure solvent, must be determined. By subtracting unity, the specific viscosity, η_{spec} is obtained.

$$\eta_{spec} = \eta_{rel} - 1$$

The viscosity is measured in dilute solution, in which case, according to Staudinger, η_{spec} is proportional to the concentration, so that η_{spec}/c, the reduced viscosity, is constant. With high polymers, however, this quantity

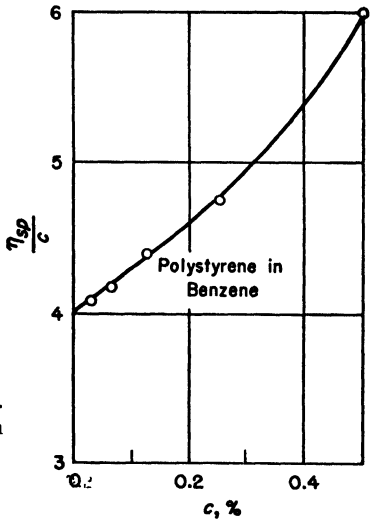


Fig. 10. η_{sp}/c as a function of c. Extrapolation to zero concentration gives the intrinsic viscosity $[\eta]$.

is not constant, even at very great dilutions, as is clear from figure 10 which is based on measurements by Daneš.[41] Following a recommendation made by Elöd,[42] the reduced viscosity extrapolated to zero may be

[41] V. Z. Daneš, *Kolloid-Z.*, **68**, 110 (1934).

[42] E. Elöd and H. Schmid-Bielenberg, *Z. phys. Chem.*, **B25**, 27 (1934).

used. This *limiting viscosity* is the same as the *intrinsic viscosity* of Kraemer, which is the limiting value of $\ln \eta_{rel}$.

$$[\eta] \equiv \lim_{c=0} \left[\frac{\eta_{spec}}{c}\right] \equiv \lim_{c=0} \left[\frac{\ln \eta_{rel}}{c}\right]$$

The concentration is expressed as grams per 100 cc. The term *intrinsic viscosity* will be used here. It seems advisable to abandon other terms such as "viscosity number" (c in grams per 1000 cc.)[43] or "characteristic viscosity constant" (Hess).

The simplicity of the Staudinger relation, together with the ease with which measurements can be made, has led to its uncritical acceptance, so that both in scientific and technical literature values for molecular weights are found which are indeed only fractions of the true molecular weights. Even the molecular weights for high polymeric vinyl derivatives, or for cellulose given by Staudinger himself, have been shown to be too low, as is made clear by a comparison between molecular weights obtained from osmotic measurements and those calculated from the Staudinger relation.[44] Numerous studies of this kind made in recent years have led to the following general conclusions.

In the case of homogeneous high polymers composed of unbranched chains, the intrinsic viscosity is a continuous function of the chain length, differing from system to system. If, therefore, the viscosities of several members of any homologous polymeric series are known, it is possible—knowing the viscosity of any intermediate member—to obtain its molecular weight by interpolation. This procedure is analogous to the characterization of mixtures of hydrocarbons by their boiling point or density, from which the mean molecular weight can also be estimated by comparison with the corresponding properties of pure, standard hydrocarbons.

Observations on several homologous polymeric series have shown that the ratio of the intrinsic viscosity to the molecular weight (Staudinger's K_m value) first increases in the region of the lower members, reaches a maximum, and finally decreases continuously, with transition to the higher members.

The following considerations hold for mixtures of high polymers. The mean value of the molecular weight, as calculated from the viscosity, has an entirely different meaning from the mean value for the molecular weight obtained from osmotic pressure measurements, so that the two methods

[43] G. V. Schulz and F. Blaschke, *J. prakt. Chem.*, **158**, 130 (1941).

[44] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **19**, 218 (1936). K. H. Meyer and E. Wolff, *Kolloid-Z.*, **89**, 194 (1939).

yield different values for the mean molecular weight. The method of interpolation can only be used in the case of products which are not highly heterodisperse. With preparations containing substances of low molecular weight, a value for the mean degree of polymerization or mean molecular weight has scarcely any meaning.

In the case of fractions (of high molecular weight but not too heterodisperse) of a polymeric homologous series, the following purely empirical equation expresses the relations satisfactorily (Mark,[45] Houwink[46]):

$$\log M = A + B \log [\eta] \text{ or } [\eta] = KM^a$$

where the exponent a has values from 0.6 to 1.0 (see Table II).

TABLE II
Viscosity–Molecular Weight Relationship[a,b]

Polymer	Solvent	$K \times 10^4$	a
Cellulose	Cuprammonium oxide	0.85	0 81
Cellulose acetate	Acetone	1.5	0.82
Cellulose nitrate	Acetone[c]	5 0	0.8
Cellulose acetobutyrate	Acetone	1.37	0.83
Pectin nitrate	Acetone	5 9	1 0
Polyisobutylene	Diisobutylene	3.6	0.65
Rubber	Toluene	5 02	0.67
GR-S copolymer	Toluene	5 4	0.66
Polystyrene emulsion polymer	Toluene	3.7	0.62
	Butanol	7.0	0 53
Polystyrene, bulk polymer	Toluene	0 01	1.12
Methyl methacrylate	Benzene	0 94	0.76
Polyvinyl acetate	Acetone	2.8	0.67
Dimethylsiloxan	Toluene	2.	0.66

[a] These values are valid only for a limited range of molecular weight and cannot be considered very precise; in using them only a rough estimation of the molecular weight is possible.

[b] Most of the values are taken from a review by I. Goldberg, W. P. Hohenstein, and H. Mark, *J. Polymer Sci.*, **2**, 503 (1947).

[c] H. Mosimann, *Helv. Chim. Acta*, **26**, 369 (1943).

Viscometry is very useful as a means of following changes in molecular weight during degradation or synthesis. The method was used for this purpose for the first time by Stobbe and Posnjak,[47] who followed the polymerization of styrene. Both polymerization and degradation of high polymers by chemical agents can be conveniently followed by observing viscosity changes. If, for example, a high polymeric compound such as cellulose is converted into a derivative, let us say an ester, and subsequently re-

[45] H. Mark, *Der feste Körper*. Hirzel, Leipzig, 1938, p. 103.

[46] R. Houwink, *J. prakt. Chem.*, **157**, 15 (1940).

[47] H. Stobbe and G. Posnjak, *Ann.*, **371**, 259 (1909).

converted into the initial substance, and if in this final state it displays the same viscosity as it did initially, we may conclude that no degradation has occurred, either in esterification or in saponification. The derivative has the same degree of polymerization as the initial material and may be regarded as a polymeric analogue.

From the fact that a high polymeric compound can be converted into an analogous derivative and recovered unchanged, Staudinger concludes that the solution of the compound does not contain micelles, but "macromolecules", that is, isolated primary valence chains. This remarkable deduction, which is often referred to in Staudinger's papers and is the basis of his views on the "macromolecular" structure of solutions of cellulose and starch, is entirely misguided. Clearly, a substance yielding a micellar solution which under specific conditions exhibits a certain viscosity will also yield exactly the same micellar solution with the same viscosity after conversion to a derivative and reconversion to the original compound, if no degradation has occurred (for example: sodium oleate → oleic ester → sodium oleate; polyacrylic acid → ester → polyacrylic acid).

Viscometry may also be used for the characterization of fractions obtained by fractional precipitation from homologous polymeric mixtures. In this way an indication of the degree of homogeneity of a particular substance may be obtained; the substance is separated into a series of fractions, the viscosities of which can then be compared. By repeated fractional precipitations, reuniting fractions of similar viscosity, and subjecting these to the same treatment, it is possible to obtain insight into the composition; suitable formulas have been devised by Dostal and Mark.[48] The method is often of great value in characterizing commercial products.

Systematic studies of the relation between the viscosity of chain polymers and the concentration of the solution have been made by Fikentscher,[49] in part in collaboration with Mark. He made the following important observation: If two preparations of different chain polymers at the same concentration have the same relative viscosity, their viscosities are approximately the same at other concentrations. The relation between the viscosity and the concentration may be expressed by the following empirical equation:

$$\frac{\log \eta_{rel}}{c} = \frac{75K^2}{1 + 1.5Kc} + K$$

[48] H. Dostal and H. Mark, *Naturwissenschaften*, **24**, 796 (1936).
[49] H. Fikentscher, *Cellulosechemie*, **13**, 58 (1932).

where c is concentration in grams per 100 cc. of solution and K is the parameter of the curve (and therefore independent of the concentration) and will be called the viscosity coefficient (Fikentscher's *Eigenviscosität*).

The Fikentscher equation was derived from observations on the systems rubber–benzene, rubber–chlorobenzene, nitrocellulose–benzyl alcohol, polyvinyl acetate–acetone, and several others, and has proved useful in the case of other systems, such as polystyrene in benzene,[50] gum arabic in dilute aqueous barium chloride solution,[51] and polyisobutylene.[52] In order to determine the viscosity coefficient (the K value) of any normal chain polymer, it is sufficient to measure the relative viscosity at any one concentration. If it is found that considerable deviations from Fikentscher's curve occur in any concentrated polymer solution, it may be concluded that association of the molecules leads to deviation, *e. g.*, with gelatin,[53] or that the molecules present are more compact in shape.[54] In converting viscosity coefficients to intrinsic viscosities, the Fikentscher equation reduces to:

$$[\eta] = 2.3(75K^2 + K)$$

Both intrinsic viscosity and viscosity coefficient, as well as the absolute viscosity measured at definite concentrations, are used for the characterization of commercially important high polymers, such as vinyl polymers and cellulose derivatives. In many cases the interpolated molecular weight or the degree of polymerization (D.P.) is added, but this is unnecessary for purposes of characterization.

(*h*) *Molecular Weight of Mixtures*

In determinations of the molecular weight of substances of low molecular weight, one starts with substances which are as pure as possible and whose solutions are usually homodisperse, that is, contain but one kind of molecule or particle. As we have already seen, however, when dealing with synthetic high polymers, and usually when dealing with natural high polymers, one must work with mixtures of molecules of different weights. Their solutions are markedly heterodisperse and determinations of molecu-

[50] V. Z. Danes, *Kolloid-Z.*, **68**, 110 (1934).

[51] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloid-Beihefte*, **36**, 429 (1932). See also H. L. Bredée and J. De Booys, *Kolloid-Z.*, **79**, 31 (1937).

[52] G. V. Schulz and G. Sing, *J. prakt. Chem.*, **161**, 161 (1943).

[53] H. Bincer, *Kolloid-Z.*, **59**, 82 (1932).

[54] R. Houwink and K. H. Klaassens, *ibid.*, **79**, 138 (1937).

lar weight give a mean value. The mean value of any property in a population can be determined in various ways, each of which yields a different result, and no value can be said to be the true mean value. Various methods of determining molecular weights also give appreciably different results when applied to the same mixture, and this is due to the different methods determining different mean values.

If we determine molecular weights by the osmotic method, we have on the one hand the total weight of substance dissolved in a given volume, and on the other hand the number of particles. In making the calculation, the weight (that is, the sum of the weight of all the particles) is divided by their number, and we obtain the arithmetic mean. In certain cases the same value may be obtained by chemical analysis. If a characteristic end group that can be estimated quantitatively is present in each molecule (for example, the free aldehyde group of a polysaccharide), analysis will enable the number of molecules present to be estimated.

Let n_i be the number of particles of molecular weight M_i; each particle consists of i monomeric residues. Then:

$$\bar{M}_{os} = \frac{\sum M_i n_i}{\sum n_i}$$

This value—the arithmetic mean—is known as the *number average.*

If the molecular weight is determined from viscosity measurements, the mean value arises in a different way. If we assume that intrinsic viscosity is proportional to molecular weight,[55] then the mean value for the molecular weight, $\bar{M}_w$, is given by the arithmetic mean of the square of the weights divided by the arithmetic mean of the weights:

$$\bar{M}_w = \frac{\sum M_i^2 n_i}{\sum M_i n_i} = \frac{\sum M_i^2 n_i}{\sum n_i} \Big/ \frac{\sum M_i n_i}{\sum n_i}$$

This value is known as the *weight average* and is significantly different from the arithmetic mean.

The intrinsic viscosity, however, is not proportional to the molecular weight, but is related to it by the following approximate equation:

$$[\eta] = KM^a$$

accordingly:

$$\bar{M}_\eta = [\sum n_i M_i^{a+1} / \sum n_i M_i]^{1/a}$$

[55] E. O. Kraemer and W. D. Lansing, *J. Phys. Chem.*, **39**, 153 (1935).

Let us take as an example a mixture consisting of 10 particles of molecular weight 100, and 5 particles of molecular weight 1000. Then we have:

$$\bar{M}_{os} = \frac{10 \times 100 + 5 \times 1000}{15} = 400$$

$$\bar{M}_{w} = \frac{10 \times 10^4 + 5 \times 10^6}{10 \times 10^2 + 5 \times 10^3} = 850$$

If $a = 0.6$:

$$\bar{M}_{\eta} = \left[\frac{10 \times 100^{1.6} + 5 \times 1000^{1.6}}{1000 + 5000}\right]^{1/0.6} = 811$$

In general, $\bar{M}_w$ and $\bar{M}_\eta$ are not very different; both are larger than $\bar{M}_{os}$. There is no constant relation between $\bar{M}_w$ and $\bar{M}_{os}$, however; the relation depends largely on the composition of the mixture, that is, on a distribution function, such as $n_i = f(M_i)$.

In the majority of cases this distribution function is unknown. Attempts to determine the distribution by experimental means—by observing velocity of sedimentation or by fractional precipitation—are fraught with difficulties. In some cases the attempt has been made to derive a distribution function on the basis of certain plausible assumptions. Kuhn[56] has derived the following equation for the distribution function of a product of the degradation of infinitely large molecules by a homogeneous reaction:

$$\varphi_i = i\alpha^2(1 - \alpha)^{i-1}$$

where φ_i is the fraction by weight of molecules present with i linkages which can be split and α is the degree of degradation, that is, the number of ruptured linkages divided by the total number of linkages present which can be split.

The same distribution is to be expected, according to Flory,[57] in polymerization or condensation reactions which proceed stepwise (Chap. D, sect. I 1). In these, α is given by the quotient of the number of as yet unclosed linkages and the total number of linkages which can be closed. B, that is $(1 - \alpha)$, is also known as the *extent of reaction.*

If Kuhn's distribution prevails (and if $[\eta]$ is proportional to M) the mean molecular weights determined by viscometry and by osmotic pressure

[56] W. Kuhn, *Ber.*, **63**, 1503 (1930).
[57] P. J. Flory, *J. Am. Chem. Soc.*, **58**, 1877 (1936).

measurements (as well as by chemical analysis) are related by the following equation:[57]

$$\bar{M}_w/\bar{M}_{os} = 2 - \alpha$$

so that at a low degree of degradation the mean viscometric molecular weight is almost twice as large as that, for example, obtained from an end-group determination.

This would hold if, in fact, a preparation with Kuhn's distribution could be obtained and measured. But in the process of preparation the smallest particles are often lost in washing or the largest in filtration. Above all, under industrial conditions degradation never gives Kuhn's distribution, since homogeneous breakdown only occurs in solution and under certain well-defined conditions. Since for industrial purposes it is usual to work with heterogeneous systems (for example, ripening of viscose, mastication of rubber, heat degradation of nitrocellulose, gelatin manufacture), the mixtures obtained are much more highly heterodisperse than Kuhn's distribution allows. Moreover, polymerization yields highly heterodisperse mixtures, if, as is nearly always the case, it proceeds as a chain reaction. In these instances the osmotic and viscometric molecular weights diverge even more widely.

This has been proved for certain substances by Kraemer and Lansing,[58] who attempted to apply an exponential distribution function, defined by a single parameter, β, obtained from the sedimentation velocity. This is the *coefficient of heterogeneity.* It has been shown that such an empirical distribution function can be derived in many cases. In these the relation between $\bar{M}_\eta$ and $\bar{M}_{os}$ is determined by β only. Table III gives a number of experimental results. In a homodisperse (homogeneous) product, $\beta = 0$ and $\bar{M}_\eta = \bar{M}_{os}$.

The results obtained with fractionated products are worthy of note; it is clear what little success attends the attempt to prepare a homodisperse product from a heterodisperse mixture. Kraemer concludes that at present the molecular weight of high polymers which give a heterodisperse solution is best represented by $\bar{M}_\eta$ and β. It is quite impossible, however, to make general use of this method. One must be content with accurately determined values for $\bar{M}_{os}$ and $\bar{M}_\eta$; from the difference between these certain conclusions regarding the state of dispersion may be drawn. In practice, however, only the value of $\bar{M}_{os}$ or that of $\bar{M}_\eta$ is quoted.

[58] E. O. Kraemer, in T. Svedberg and K. O. Pedersen, *The Ultracentrifuge.* Oxford Univ. Press, London, 1940.

TABLE III
Selected Values for Coefficient of Heterogeneity

Solution	β	$\bar{M}_\eta/\bar{M}_{os}$	Reference
Gelatin	1.7	4.11	Lansing and Kraemer (1935) reported by Krishnamurti and Svedberg (1930)
Polychloroprene	1.0	1.63	Kraemer and Lansing (1935)
Sol rubber	0.7	1.27	Kraemer and Lansing (1935)
Cellulose acetate (commercial)	1.0–1.3	1.63–2.28	Kraemer and Lansing (1935) Nichols (unpublished)
Polystyrene (fraction)	1.1	1.81	Kraemer and Lansing (1935)
Methyl cellulose (commercial)	1.2	2.02	Signer and von Tavel (1938)
Methyl cellulose (fractions)			Signer and von Tavel (1938)
M.W. 38,000	1.0	1.63	
24,000	1.0	1.63	
14,000	0.5	1.13	

From the foregoing it follows that a value for the mean molecular weight only has meaning if it is clear how the figure in question was obtained. According to the distribution prevailing, two entirely different products may give identical or different values for $\bar{M}_\eta$ and $\bar{M}_{os}$. Since it is very difficult to obtain data for the heterogeneity of a preparation, it should always be clearly stated how the product was prepared. Merely to give a value for the "*mean degree of polymerization*," as is frequently done at present, is therefore positively misleading.

(*i*) *Molecular Weight Determination by Precipitation Titration*

As we shall see later, the solubility of chain polymers in suitable solvents or solvent mixtures falls off with increasing molecular weight. If a precipitant is run from a burette into a solution of a chain polymer, opalescence occurs with a smaller volume of precipitant the higher the molecular weight of the polymer. Jirgensons'[59] method of determining molecular weights depends on this phenomenon. It is admittedly only of use for relatively homogeneous fractions of a homologous polymeric series. Jirgensons showed that the concentration of precipitant at the critical point is a linear function of the reciprocal of the molecular weight.

A calibration curve is prepared from fractions of known molecular weight, plotting critical concentration against molecular weight. It is then possible to read off the molecular weights corresponding to other critical concentrations. Jirgensons gives as examples the behavior of frac-

[59] B. Jirgensons, *J. prakt. Chem.*, **159**, 303 (1941); **160**, 21, 65 (1942); **161**, 30 (1943).

tions of polymethacrylic acid in benzene (precipitant, cyclohexane), and of nitrocellulose in acetone (precipitant, water).

We conclude that this method is only applicable to polymers of moderate molecular weight and only to such as are strictly homologous polymers, such as polyvinyl chlorides, cellulose nitrates, etc. If, however, there is the possibility of even slight chemical differences between molecules—if, for example, we have a mixture of cellulose chains nitrated to varying degrees—then precipitation will depend not only on the molecular weight but also on the degree of nitration. Jirgensons has also applied his method to products of protein breakdown; these results, however, can only be of qualitative significance.

3. Determination of Sign of Charge on Particles and Test of Homogeneity by Means of Electrophoresis

If a solution of an electrolyte is electrolyzed, cations are removed from solution and deposited on the cathode, while the same thing happens to the

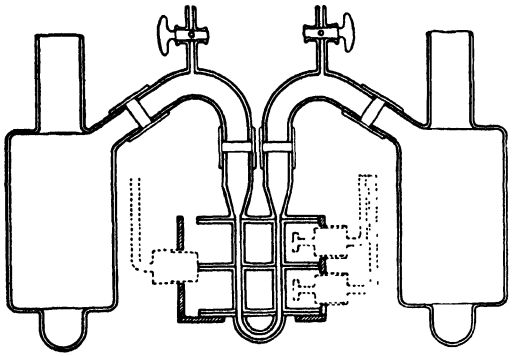


Fig. 11. Diagram of the Tiselius electrophoresis apparatus.

anions at the anode. Within the solution the ions migrate under the influence of the electric field; this migration is known as *electrophoresis*. The relative rate of migration of the charged particles is directly proportional to their charge, and varies inversely with the size of the particles. Electrophoresis may be used to separate smaller ions from larger ions by enclosing the solution in a cell bounded by two semipermeable membranes. Electrodes are placed outside the membranes, and the liquid in the neighborhood of the electrodes is constantly renewed. Small ions migrate through the semipermeable membranes, while the large ions remain within the cell and are usually precipitated. The method is known as *electro-*

dialysis. It was introduced by Dhéré[60] and has been used extensively by Pauli.[61] Electrophoretic phenomena may also be used for the investigation of substances in solution; the migration of the dissolved substances under the influence of the electric field is observed by means of optical or analytical methods of various kinds. In the latter case samples are taken from time to time after the commencement of electrophoresis. In this

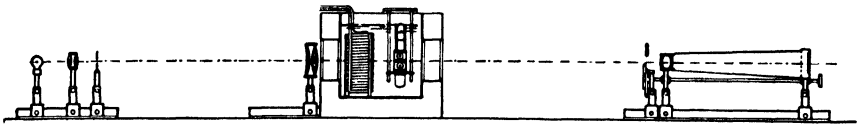


Fig. 12. Optical setup for the observation of electrophoresis by the schlieren method. From left to right: lamp, condenser, slit, schlieren objective, cells, and electrodes in cooled thermostat, diaphragm, camera.

way it is possible to determine the isoelectric point of electrolytes of high molecular weight, that is, the *p*H of the solution at which the substance in question does not migrate under the influence of the electric field.

It is also possible to test the homogeneity of homodisperse electrolytes of high molecular weight by this method. The solution of the substance in question is subjected to electrophoresis (if necessary, at various hydrogen ion concentrations). The method has been improved by Tiselius[62] and is used extensively. In order to prevent disturbances due to convection,



Fig. 13. Electrophoretic diagram of horse serum (after 80 min.).

Tiselius advises working in a thermostat at 4°C., where the thermal expansion coefficient disappears, and recommends the use of electrode vessels of much greater capacity than the U-shaped observation tube (Fig. 11). The U tube is divided into several parts that can be filled separately and can be caused to slide over each other by means of a mechanical device in such a way that when the solutions are brought in contact no mixing occurs and the boundary line remains sharp.

[60] C. Dhéré, *J. physiol. path. gén.*, **12**, 645 (1910); *Kolloid-Z.*, **41**, 243, 315 (1927).

[61] See W. Pauli and E. Valkó, *Elektrochemie der Kolloide.* Springer, Wien, 1929.

[62] A. Tiselius, *Kolloid-Z.*, **85**, 129 (1938). H. Svensson, *Arkiv. Kemi Mineral. Geol.*, **A22**, 1 (1946).

At the beginning of electrophoresis the solution is in the lower cell and is covered with a buffer solution. The change in refractive index which occurs

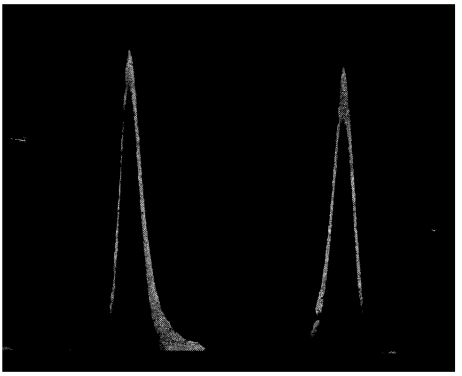


Fig. 14. Electrophoretic diagram of crystalline α-amylase. Optical system: Philpot-Svensson; buffer: Michaelis *p*H 6.5; ionic strength $\mu = 0.1$; temp.: 4.0°C.; time: 7320 seconds; electrical field: 2.74 volt/cm.; descending boundary at the left; one component.

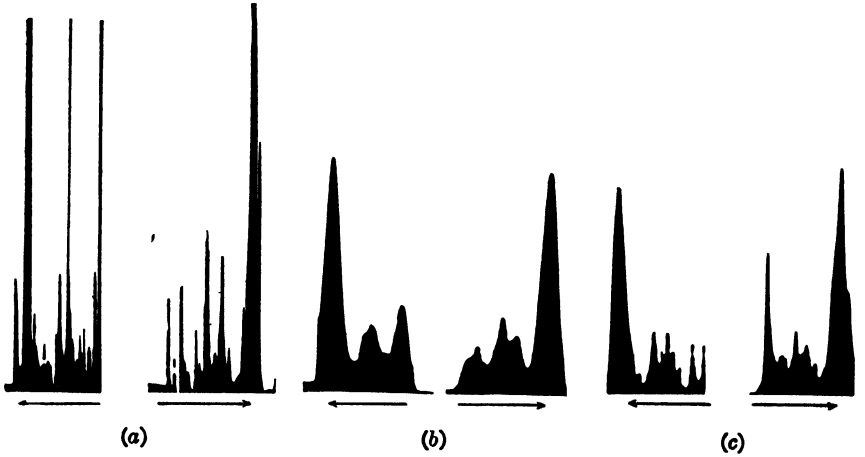


Fig. 15. Electrophoretic diagrams of human serum in the presence of 2.8 *M* urea: (*a*) after 4.8 volts/cm. had been applied for four hours; (*b*) after standing twelve additional hours without voltage; (*c*) after voltage was applied an additional thirty minutes, then switched off for one hour. Optical system Longsworth; ascending boundary at the left.[62a]

[62a] D. H. Moore, in A. Weissberger, ed., *Physical Methods of Organic Chemistry*, Part II, 2nd ed., Interscience, New York, 1949, page 1646.

at the transition from the colloidal solution to the buffer can be made visible as a dark band across the cell by the *schlieren* method of Töpler (Fig. 12). As the colloid migrates, the gradient of refractive index, and hence the black band, also migrates. If substances of different velocities of migration are present, the gradient separates into several subsidiary gradients, and the band into several bands, each of which is associated with one component (Fig. 13). By various means (methods of Philpot, Longsworth, and Svensson), it is possible to record simultaneously the position and magnitude of the gradient. The latter is a measure of the concentration of the solute, and the position which the gradient reaches in a given electric field after a certain time is a measure of its mobility. Figures 14 and 15 are examples of two diagrams of this type.

4. Determination of Shape of Dissolved Particles

(*a*) *Shape of Particles and Anomalous Properties of Solutions*

It has been mentioned previously that chain polymers in solution display several characteristic phenomena, such as the peculiar relationship between osmotic pressure and concentration, and the strikingly high viscosity of even dilute solutions. Wherever these properties are found, we may conclude with certainty that very long chain molecules are present. The characteristic property of swelling slowly and passing into solution gradually and with difficulty is found only in the case of chain polymers. If solutions of substances of high molecular weight do not display these properties, it is certain that the particles present in solution are not uncoiled chain molecules; they are more compact units and may be spherical, platelike, or elongated. Experiments on viscosity and flow birefringence may give further information.

(*b*) *Particle Shape and Viscosity*

Suspensions of spherical rigid particles obey the Einstein relation:

$$\frac{\eta_{spec}}{c_v} = 0.025$$

where c_v is the concentration in volume per cent. If the density of the suspended substance is ρ, this corresponds to an intrinsic viscosity of $0.025/\rho$. If the solution of a high polymer obeys this relation, it may be concluded that spherical particles are present; if it does not, this may be due to departure from the rigid spherical shape, or to enlargement of the

particles by the absorption of solvent. Solutions of many globulins as well as starch pastes reveal themselves as suspensions of approximately spherical particles.

Solutions of chain polymers usually display much higher viscosities, suggesting that the chains are unfolded. If two chain polymers of the same analytical composition, same molecular weight, and same homogeneity display different viscosities under identical conditions, it may be concluded that they differ in constitution. If they are polymers of the same monomers, the difference in constitution must lie in a different arrangement of the residues.

Among paraffins of moderate molecular weight, it is the cyclic forms which show the highest viscosities; these are followed by normal paraffins and lastly by branched paraffins. As Staudinger has pointed out, in high polymers, those isomers whose solutions are least viscous may be assumed to be branched. Use has been made of this fact in the study of synthetic rubbers. The general topic of viscosity will be dealt with in detail at a later stage (Chap. I, sect. V 1 and 2).

(c) *Study of Particle Shape by Means of Flow Birefringence*[63]

The birefringence of a suspension or solution which is set in motion has been used by Freundlich and Zocher[64] for the study of suspensions of rod-shaped ultramicroscopic particles, such as vanadium pentoxide, benzopurpurin, and other sols. The method was subsequently refined by Signer[65] and applied to the study of solutions of high polymeric substances.

Many solutions, when set in motion (as, for example, when placed in a rotating cylinder), show perceptible birefringence; in this case one is dealing with suspensions of particles of pronouncedly anisotropic structure and relatively large size, perhaps as much as 0.01 μ in one dimension. This characteristic birefringence of flow is shown by soap solutions, solutions of benzopurpurin,[66] and other particles (such as the virus particles from plants infected with tobacco mosaic virus).[67] For the measurement of smaller effects, observations are made on a thin layer of liquid, several centimeters deep, enclosed between two cylinders, one stationary and

[63] See Chapter I, sect. VII 3.

[64] H. Freundlich, F. Stapelfeldt, and H. Zocher, *Z. physik. Chem.*, **A114**, 161 (1925).

[65] R. Signer, *Helv. Chim. Acta*, **19**, 897 (1936). G. Boehm and R. Signer, *ibid.*, **14**, 1370 (1931).

[66] H. Zocher, *Z. physik. Chem.*, **A98**, 293 (1921).

[67] F. C. Bawden, N. W. Pirie, J. D. Bernal, and I. Fankuchen, *Nature*, **138**, 1051 (1936).

hollow, the other rotating and solid. The birefringence of the layer of liquid is determined as a function of the shearing velocity, and the angle of extinction is measured.

The mean position of the particles results from the opposed effects of the shearing force and thermal agitation (Brownian movement). If the effect of thermal agitation is considerable (as is the case with small particles), the position which the particles take up most frequently is such that the long axis makes an angle of 45° with the direction of the lines of flow. If the birefringence of the particles is positive with respect to the long axis, the angle of extinction ψ is 45°; if the birefringence is negative, the angle amounts to 135° (see Fig. 16). In the case of larger particles, the influence of ther-

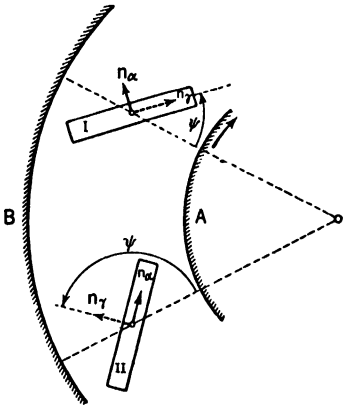


Fig. 16. Flow birefringence (Signer). A = rotating cylinder, B = hollow cylinder, I = particle with positive and II = with negative intrinsic birefringence.

mal agitation is less important, and the particles tend to orient themselves in the direction of shear; the angles of extinction become greater and, when orientation is complete, amount to 90° to 180°. The birefringence $(\eta_\gamma - \eta_\alpha)$ depends on the value of the intrinsic birefringence and the refractive index of the medium, which must be determined separately. If the shearing force remains constant, the birefringence varies with the ratio of the length to the breadth of the particles. With homologous high polymeric, rod-shaped chain molecules this ratio is proportional to the molecular weight. In the case of solid particles the birefringence increases proportionately with the velocity gradient. With particles which, as a result of flow, are deformed from a more or less spherical to an elongated shape (as may happen in the case of long-chain molecules) the birefringence increases more rapidly than the gradient. Observations made by this

method may lead to conclusions about the size, the ratio of the axes, and the deformability of the particles. The polydispersity of the solution produces a characteristic shift in the angle of extinction. Signer,[68] who studied the phenomenon in the case of polystyrene, later used this method for the investigation of natural high polymeric compounds, such as nucleic acid and various proteins, and has also attempted to determine the form in solution of particles of methyl cellulose. We shall discuss flow birefringence in Chapter I, sect. VII. With reference to the "migration birefringence" of ionized chain polymers in an electric field, see the report by Kuhn.[69]

II. INVESTIGATION OF THE SOLID STATE WITH X-RAYS*

By far the most important and most informative method of studying atomic arrangement, particularly in the solid state, is that of x-ray analysis. By this means the arrangement of the molecules in many solid polymers has been investigated, and in numerous instances the constitution of the molecule has also been elucidated. Since we shall have frequent cause to discuss the results of x-ray analysis (and to consider certain misinterpretations which are widely current), the principles of this method will be treated in some detail in the following pages. The "usual" methods have been developed with relation to indisputably crystalline materials and therefore the question arises how far it is legitimate to extend the arguments used in the case of true crystals to more or less crystalline high polymers.

1. Solid State of High Polymers in General

In the past the term *crystalline solid* has been taken to mean a substance consisting of visible crystals; by crystal is meant a solid with plane surfaces meeting at definite, characteristic angles, the basis of systematic crystallography. A characteristic property of most crystals is their birefringence, and structures have often been referred to as crystalline (particularly biological structures) only because they were birefringent and in spite of the fact that they displayed no other characteristics of crystalline materials.

* Contributed by A. J. A. van der Wyk.

[68] R. Signer, *Trans. Faraday Soc.*, **32**, 296 (1936). R. Signer, T. Caspersson, and E. Hammarsten, *Nature*, **141**, 122 (1938). G. Boehm and R. Signer, *Helv. Chim. Acta*, **14**, 1370 (1931); *Klin. Wochschr.*, **11**, 509 (1932).

[69] W. Kuhn and H. Kuhn, *Helv. Chim. Acta*, **27**, 493 (1944).

As a result of the study of crystal structure by x-ray analysis and the demonstration that all crystals are built up of a regular periodic three-dimensional arrangement of atoms, ions, or molecules (long-range order), it has become customary to accept the presence of such an arrangement, revealed by sharp x-ray interference spots or lines, as evidence of the crystalline state. Considered from this point of view, solid substances of low molecular weight fall into two sharply defined categories: the x-ray diagrams of salts, metals, and crystalline organic compounds differ profoundly from those of supercooled glasslike alcohols, for instance. The first group produces numerous interferences, the occurrence of which is almost always associated with the presence of visible crystal surfaces in the solid. The second group, like liquids in general, gives a small number of diffuse interference rings. This indicates that, while the arrangement of the scattering centers is not statistically random, the arrangement is one of short-range order only. The positions of the neighbors of a particular molecule are determined, within rather narrow limits, by that molecule, while the positions of more remote molecules are not so determined.

In high polymers the boundary between crystalline and amorphous materials is not so sharply defined. The interference spots in the x-ray diagrams of almost all crystalline organic high polymers are fewer and often broader than in typical crystalline substances. This indicates that the lattice is imperfect and that the crystalline regions are very small. Frequently, only a few diffuse interference spots are present, indicating a molecular arrangement neither crystalline nor amorphous but intermediate between the two conditions. If crystal interferences are absent, and birefringence alone reveals a certain degree of orientation—usually parallel arrangement of chain molecules—the object is no longer referred to as crystalline. It is very common to find, even in pure substances, that the x-ray diagram shows an "amorphous" ring in addition to "crystal" interferences, so that a mixture of crystalline and amorphous material may be assumed to be present. Lastly, visible plane crystal surfaces are exceptional in high polymeric solids.

A further characteristic feature of many high polymers (for example, cellulose and starch) is the occurrence of numerous modifications in each of which a slightly different molecular arrangement prevails. The facts reported are all understandable if one considers how structures such as a fiber of native cellulose or one spun in a precipitating bath arise: bundles of chain molecules are formed by the parallel arrangement of molecules during growth or in spinning. Here and there the molecules snap together to form a lattice; in other parts their arrangement is less perfect. Ac-

cording to the conditions under which this bundling together takes place, various arrangements may arise, and it seems unnecessary to regard all the forms observed as distinct modifications.

We shall have occasion to refer repeatedly to the peculiarities of the solid state in high polymers (for example, in rubber, cellulose, and viruses); fusion and softening will be dealt with in detail in Chapter C, section VI.

2. Scattering of X-Rays

If a parallel bundle of monochromatic x-rays of sufficient *hardness*—that is to say, electromagnetic waves of a single, very small wave length—encounters any substance, the electrons of the atoms of this substance will be compelled to execute vibrations of the same frequency as the radiation. These electronic vibrations generate new electromagnetic waves (secondary waves) of the same frequency as the incident radiation. The secondary waves emitted by each irradiated electron are therefore x-rays. The intensity of the radiation scattered by a particle of charge e and mass m is proportional to e^4/m^2, and since this factor is several thousand times greater for an electron than it is for any nucleus, the radiation scattered by the nucleus can be neglected. The *scattering of x-rays by matter is therefore almost entirely due to the electrons it contains.* The application of x-rays to the study of molecular structure depends on these facts, whether we are concerned with single atoms, or small or large molecules, no matter what their state of aggregation.

According to classical electrodynamics, if electromagnetic waves encounter a free electron, this emits a wave whose amplitude is independent of the direction and the frequency.

If the incident radiation is natural (that is, nonpolarized x-radiation), the intensity (that is, the square of the amplitude) is proportional to:

$$\frac{1 + \cos^2 2\vartheta}{2}$$

where 2ϑ is the angle between the direction of the incident radiation and that in which the scattered light is observed. This *polarization factor* arises from the fact that electromagnetic waves are only propagated in a direction at right angles to that of the electrical vibration. This results in a minimal intensity of the secondary wave in directions normal to the primary beam, and maximal intensities in directions parallel and opposed to the primary beam. In what follows, it will always be assumed that the polarization factor is taken into account.

(a) *Scattering by Free Atoms and Molecules*

Let us consider first a hypothetical helium atom with its two electrons a small distance, r, apart, and fixed in position, placed in a beam of x-rays.

The intensity of the scattered radiation will now depend upon the direction because of interference between the two secondary waves of identical frequency emitted by the two electrons. The amplitudes of the two waves reinforce each other maximally (sum) when the path difference, γ, of the two diffracted rays is an exact whole number, n, of wave lengths, *i.e.*, if $\gamma = n\lambda$, and the intensity is zero if $\gamma = (n + 0.5)\lambda$. From figure 17 it can easily be seen that the path difference is given by:

$$OP + PO' = \gamma = r[\sin \varphi + \sin (2\vartheta - \varphi)]$$

The angle, $2\vartheta_{max}$, defining the direction of the maximum, therefore depends on the distance r and on the angle of orientation φ. In reality, however,

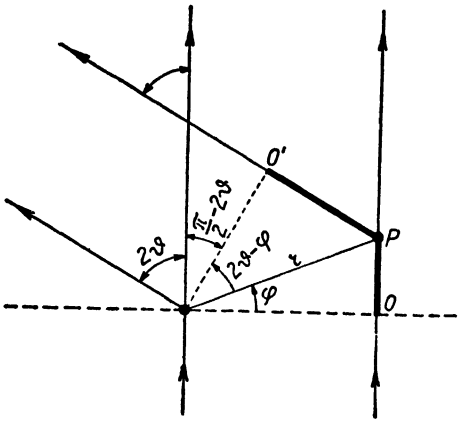


Fig. 17. Path difference = $OP + PO'$.

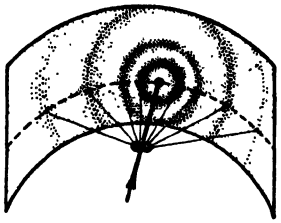


Fig. 18. Appearance of maxima and minima of scattered radiation on cylindrical film strip.

the position of electrons in an atom cannot be determined and therefore a representation of the scattering from a hydrogen or a helium atom by one or two scattering points does not correspond to reality. Instead, we have to consider the distribution of negative electricity about the nucleus, the so-called electron density. A complication now arises from the fact that, with a continuous distribution of charge, which may be computed from wave mechanics, there is an interference effect. Even a single hydrogen atom does not scatter x-radiation equally in all directions, though it contains only one (practically free) electron. However, as the distribution of charge is known, the amplitude of the scattered radiation in any direction can be calculated essentially along the same lines, for every kind of atom.

The ratio F of this amplitude to that for a single free point electron is called the *atomic factor*. The maximum value of F amounts to Z, the atomic number and corresponds to $2\vartheta = 0$. At larger angles, F falls off through intraatomic interference, at first steeply, then more slowly. Thus, for carbon atoms, $F = 6$ for $2\vartheta = 0$, but drops to 4.6 at 17.8° and to 3 at 36° with copper radiation ($\lambda = 1.54$ Å.). This falling off is more marked the more voluminous the kind of atom considered.

It must be emphasized that it is the square of F which enters into the expression for the intensity of the scattered radiation. Furthermore, the ratio of the intensities of scattered radiation from two different kinds of atoms in the same direction, 2ϑ, depends largely on the angle 2ϑ.

The introduction of atomic factors makes it possible to regard the atoms as punctiform scattering centers with scattering powers dependent on the angle. This possibility is often made use of to describe the scattering by a molecule. If we can postulate to a first approximation a dumbbell model, *i.e.*, if we neglect changes in the electron distributions of the two atoms due to the bond between them (and with heavy atoms or weak bonds the error introduced in this way is relatively small), the following will hold.

Let us consider the simplest case of a molecule consisting of two identical atoms, irradiated by a bundle of parallel, monochromatic x-rays. Essentially we shall have the relationships indicated in figure 17. The maxima of scattered radiation, according to the condition:

$$\gamma = n\lambda$$

$$\gamma = r\,[\sin\varphi + \sin(2\vartheta - \varphi)]$$

will reveal their presence if a strip of film is curved into a circle about the molecule as center (Fig. 18), but the intensities of these maxima will diminish much more rapidly with increasing 2ϑ than was the case with two electrons, since not only the polarization factor but also the atomic factor, F, diminishes rapidly between 0° and 90°.

For molecules with light atoms, such as O_2, CO, or N_2, the approximation cannot hold: the deformation of the electron clouds of the two atoms on combination is much too great. For hydrogen molecules the dumbbell model is essentially inadequate. If we consider the scattering of *electron rays*, the position is quite different since electrons are scattered chiefly by the nuclei. For this reason a dumbbell model is more readily applicable to electron interference studies.

In a gas at atmospheric pressure, the spacing of the molecules is so large that the intensities of the waves scattered by the individual particles are

simply summed. The scattering obtained by passing a fine beam of monochromatic x-radiation through a small volume of a gas is therefore essentially the same picture as would arise from the irradiation of a single rotating molecule. The intensity is given by a periodic function: maxima and minima occur in circles symmetrically about the primary beam. Such scattering pictures have been observed in gases the molecules of which contain several heavy atoms, such as CCl_4, but experiments with N_2 or O_2 give intensity curves falling off without maxima or minima. In this case the approximation by a dumbbell model is not valid. The scattering curve observed for H_2 is, in fact, scarcely distinguishable from that for He.

The conclusions reached above are of special interest for the converse problem: the determination of atomic arrangement from scattering curves obtained experimentally. It is clear that an interpretation which depends on a model hypothesis may go completely astray, particularly with light atoms.

(*b*) *Scattering by Liquids and Amorphous Substances*

The atoms or molecules of a liquid are so closely packed that the waves emitted by the individual scattering centers interfere with each other. The

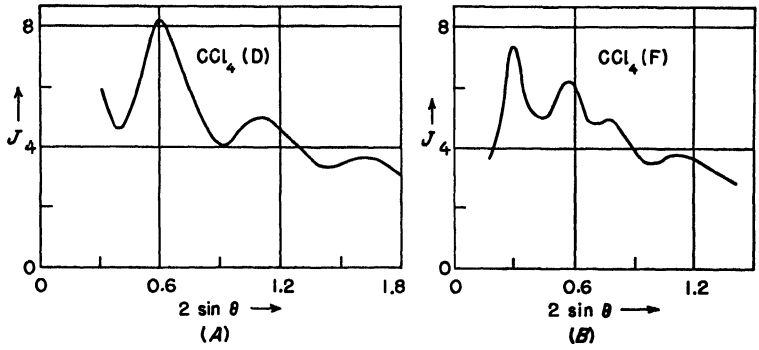


Fig. 19. Scattering of x-rays (λ = 1.54 Å.) (*A*) by carbon tetrachloride vapor (Debye), (*B*) by liquid carbon tetrachloride (Menke).

scattering curves of the same substance in gaseous and liquid states are therefore quite different (Fig. 19*A* and *B*). Theoretical treatment of the scattering effect is simplest for monoatomic liquids, since the scattering power of spherically symmetrical atoms is independent of their orientation, and since in such liquids there is no risk of the electron density of a single atom being markedly influenced by its neighbors.

At an early date it proved possible to calculate the scattering curve of liquid mercury, in satisfactory agreement with the experimental data. Conversely, it is possible (albeit with considerable mathematical difficulty) to calculate from the scattering curves obtained by experiment, a distribution curve for the atoms in the liquid. Such a curve shows, for example, the number of atoms surrounding a given atom in a spherical shell of radius r. A maximum in this curve (*not* in the scattering intensity curve) implies real mean spacing of neighboring atoms.

The application of Fourier analysis to scattering curves (to be discussed later) has considerably extended the field of application of the x-ray interference method, and has made it possible for Warren, for example, to interpret the scattering of x-rays by quartz and other glasses. It must be emphasized, however, that in this case (in contrast to that of crystals) we are not as yet dealing with an objective method: it is not as yet possible to interpret scattering data (in the case of amorphous and liquid substances) without taking into account a number of approximaations and special assumptions. The results hitherto obtained with amorphous or liquid organic substances, for example, the interpretation of the scattering curves for alcohol, paraffin, amorphous rubber, or amorphous vinyl polymers, will not stand objective criticism.

(*c*) *Scattering by Crystals*

In contrast to the amorphous substances so far considered a crystal has a strictly periodic structure. In all directions the same atomic arrangement is repeated at regular intervals or *periods*. If two neighboring and exactly similar scattering centers have been located in a crystal, identical scattering centers will be found at equal intervals along the straight line through these two centers. The diffraction picture—that is, the angular distribution of interference beams—arising from such a series of points is closely related to that of two punctiform scattering centers (sect. 2*a*); the maxima and minima appear in the same directions (Eq. 1). Calculation shows, however, that the diminution in intensity of the scattered radiation on either side of each maximum will be steeper, the greater the number of identical scattering centers along a given straight line. If there are several hundred scattering points, the total scattered radiation will be practically confined to very small angular zones about the direction of the maxima, while in regions between, practically no scattered radiation is to be found. The diffraction picture of a crystal appears therefore as a discontinuous figure. The diffracted radiation from a long row of points can

thus be represented schematically by a set of coaxial cones, the axis of which coincides with the direction of the row. A family of curves of the second order (conic sections) will be formed on a photographic plate placed at right angles to the primary beam at a considerable distance from scattering points (Fig. 20).

A crystal is a periodic structure in *three* dimensions, however, so that every single scattering center belongs not to a single series of identical scattering centers but, simultaneously, to many (at least three) other series of different periodicities and extending in different directions. The radiation from each individual row of identical points can again be represented

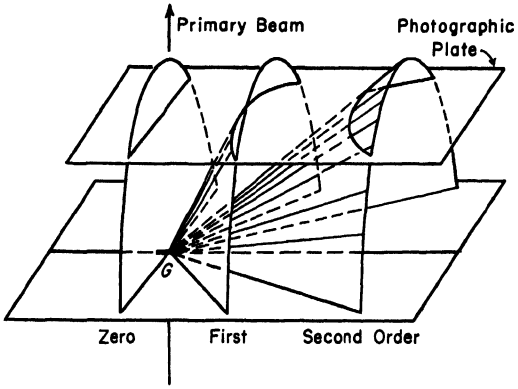


Fig. 20. Scattered radiation from a horizontal point row G (Ewald[69a]).

by a set of cones, whose axis coincides with the row. Each of these sets of cones would describe a family of curves on the photographic plate (Fig. 21). Only if, at one point, at least three curves belonging to different sets intersect, is the condition:

$$n\lambda = r[\sin \varphi + \sin (2\vartheta - \varphi)] \tag{1}$$

simultaneously satisfied for three different series of scattering centers (and hence for all other series). Only in this direction can an interference beam appear and record its presence on the plate as a spot.

In general, therefore, if a crystal is set up in a beam of parallel, *monochromatic* x-rays, no interference beams will be detected. Only if the crystal is rotated will it pass successively through different positions in which the angle of orientation, φ, has the correct value. During this rotation of

[69a] P. P. Ewald, *Kristalle und Roentgenstrahlen.* Springer, Berlin, 1923.

the crystal the interference beams appear one after the other. If, on the other hand, *polychromatic* ("white") radiation containing a continuous series of wave lengths is used, many interferences will appear simultane-

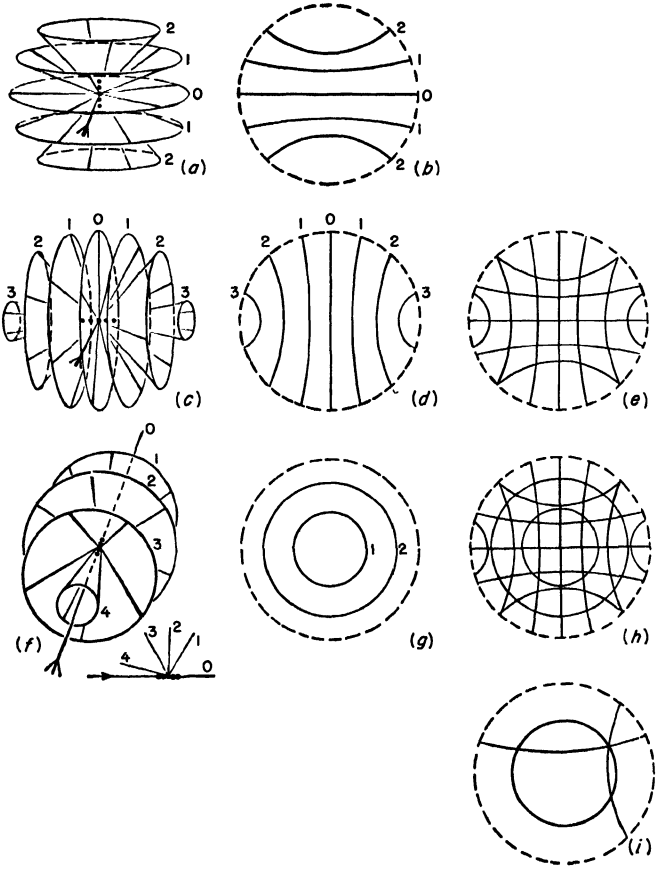


Fig. 21. Three point rows in three different directions give rise to three families of cones *a*, *c*, and *f*, which would be recorded on a photographic plate as three families of conical sections *b*, *d*, *g*. But only if a triple intersection occurs, as in *i*, an interference beam will result (Bijvoet, Kolkmeyer, and MacGillavry[69b]).

ously even when the crystal is at rest. This condition corresponds to the Laue method.

[69b] J. M. Bijvoet, N. H. Kolkmeyer, and C. H. MacGillavry, *Roentgenanalyse von Kristallen*. Springer, Berlin, 1945.

Let us return to equation (1). It can easily be transformed into:

$$n\lambda = 2r \sin \vartheta \cos (\varphi - \vartheta)$$

If the line bisecting the angle between primary beam and interference beam is inserted in the figure, it is at once clear from figure 22 that the spacing, D, between two such bisecting lines is given by the relation:

$$D = r \cos (\varphi - \vartheta)$$

The condition for interference can therefore be written in the following way:

$$n\lambda = 2D \sin \vartheta \qquad (2)$$

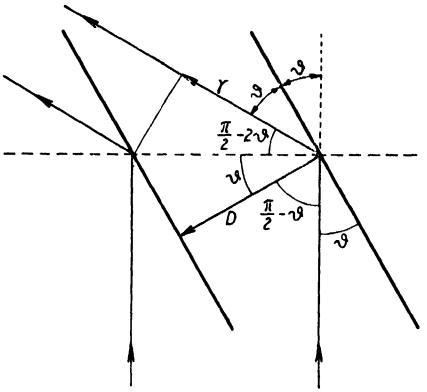


Fig. 22. Bragg's equation. Path difference $\gamma = \frac{D}{\cos \vartheta} \sin 2\vartheta$.

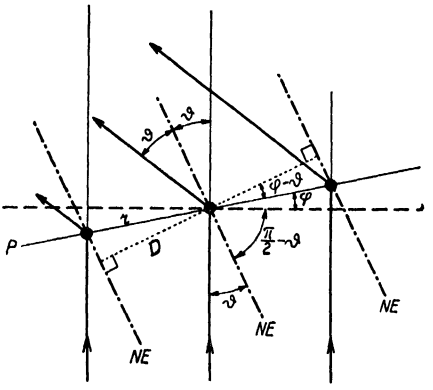


Fig. 23. Diffraction by the point row P can be thought to be reflection from the net planes NE.

This relation, known as the Bragg equation, can be derived, though not entirely correctly, as follows. Let us consider the bisectors, NE, in figure 23 as the projections of planes vertical to the plane of the drawing; the significance of these so-called net planes in the crystal lattice will be considered in the next section. If we now accept the fiction that the net planes are able to reflect x-rays, the equation follows directly from the figure.

In spite of the elegance of this procedure, it must not be forgotten that it rests on a fictitious assumption, namely, the reflecting power of the net planes. That this is indeed a fiction without physical reality is immediately apparent if, for example, the net planes are "roughened," *e.g.*, by thermal agitation of the scattering centers. The interference beam in question is not broadened (as a reflected beam) but its intensity is diminished. The process taking place in a crystal is not reflection but interference. For this reason, we have avoided the term "reflection" in what follows.

(*d*) *Lattice Theory*

The totality of point rows constitutes a *translation lattice.* Hitherto we have considered crystal structure symbolically as such a point lattice, in accordance with Laue's conception of the interference phenomenon. The crystal lattice might be just as well described, however, in terms of the sets of planes just defined. For example, in figure 24 a series of points, P, is shown with a corresponding set of planes. This description corresponds to the idea of Bragg of the reflection of x-rays.

The distance, D, between the planes is known as the net plane spacing. We can represent it by a vector, $\boldsymbol{D}$, normal to the set of net planes. It is precisely this vector which appears in the Bragg equation.

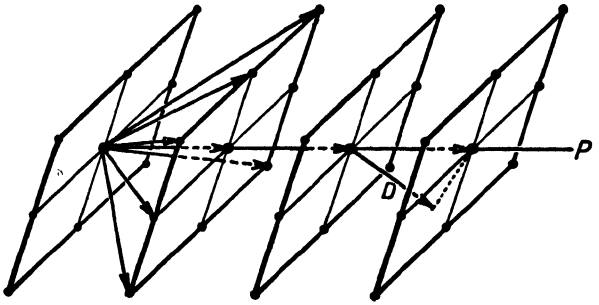


Fig. 24. A set of net planes may be associated with a point row P.
D = net plane spacing.

The net plane spacings, *i.e.*, the vectors $\boldsymbol{D}$, could also be used to describe the whole translation lattice, but it is more useful, as Ewald has shown, while keeping the direction of these vectors, to make use of the reciprocal value of the net plane spacings, that is, $1/D$, as the length (magnitude) of the vectors. In this way the *reciprocal lattice* arises. Each point of the reciprocal lattice, that is, each end point of the vectors of magnitude $1/D$, represents a *set* of net planes. The diffraction picture on the photographic plate is nothing but a more or less distorted photograph of the reciprocal lattice of the crystal under investigation. For a given wave length, it is therefore possible to construct, for each observed interference spot, the corresponding point of the reciprocal lattice, and from this to derive the sets of net planes; as is seen from figure 24, however, an unlimited number of point rows, with larger or smaller spacing of the scattering centers, may be associated with any single set of net planes. There is no general relationship between net plane spacing and scattering center spacing.

This is demonstrated in figure 25*A*. Neither the directions, nor the magnitudes of the two vectors are the same; only in the case of orthogonal lattices do they occasionally coincide (Fig. 25*B*).

In contrast to the scattering center spacing, the net planes and their spacings have only a formal, purely geometric significance which disappears as soon as the object ceases to be strictly periodic in structure. It is thus a contradiction in terms to speak of net planes and net plane spacings in amorphous or liquid substances. Unfortunately, the practice has arisen of calculating "spacings" from the observed angle of diffraction by means of the Bragg equation, and, without any justification to regard these as atomic or even molecular spacings; the error is not diminished by introducing terms such as "mean," "most frequent," or "effective" atomic or

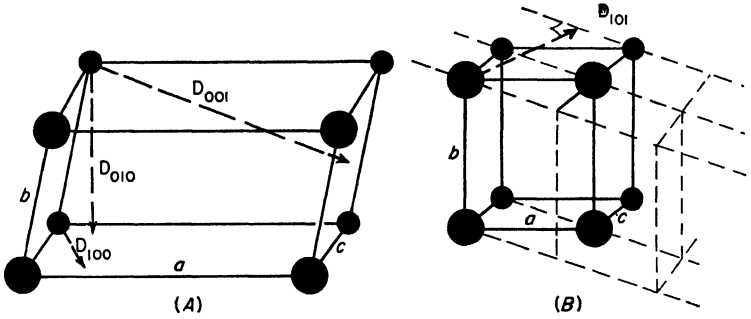


Fig. 25. (*A*) Simple triclinic lattice; no net plane spacing corresponds to any scattering center spacing. (*B*) Simple tetragonal lattice; only net plane spacings of (100), (010), and (001) coincide with scattering centers spacing, but, *e.g.*, D_{101} is not a spacing between such centers.

molecular spacing. The absurdity of this conception is demonstrated by the diffraction patterns of liquid paraffins, no maximum of which corresponds to the C—C spacing.

In general, the position of the maxima and the question of whether they are broad (as in amorphous substances) or contracted to lines or points (as in crystals) does not permit any conclusion to be drawn about atomic or molecular spacings. These can only be determined if the *intensities* of the different interference beams are taken into consideration. Before proceeding to a discussion of these, the following points must be emphasized; they can readily be demonstrated with the help of the reciprocal lattice.

(*1*) The interpolation of new scattering centers in an existing translation lattice can only lead to a change in the intensity and even to the disappearance of some of the inter-

ferences, but never to the appearance of new interferences, that is, of such as are not related to the original translation lattice.

(*2*) In monochromatic light the primary beam and the interference beams are physically equivalent. The primary spot is nothing more than the interference 000; like other interferences it may be influenced by the structure of the crystal—for example, it may be broadened or weakened or even suppressed. This fact will be taken into consideration when we come to discuss small-angle scattering.

(*e*) *Intensity of Interference Beams*

The intensity of an interference beam lying in a particular direction relative to the primary beam can be represented as the product of several fac-

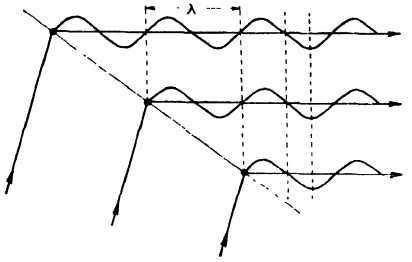


Fig. 26. All secondary waves of the interference beam from a simple lattice have the same phase.

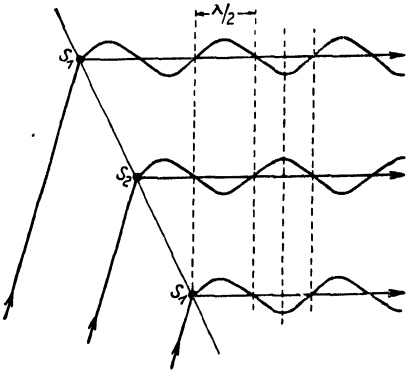


Fig. 27. Interpolated scattering centers S_2 create waves with phase shift 0.5.

tors, two of which, the polarization factor and the atomic factor, have already been mentioned. Of the other factors only those will be considered here which are directly related to the crystal structure.

Let us first consider a row of identical scattering centers lying along a single lattice line. Let us orient this series of points in a monochromatic parallel beam of x-rays so that an interference beam is formed in a given direction; the secondary waves 1, 2, 3... are then displaced exactly 1, 2, 3 ...whole wave lengths with respect to the wave at the origin (Fig. 26). For interferences of the *h*th order the phase differences are exactly *h*, *2h*, *3h*...whole wave lengths. When an interference beam emerges, therefore, all "identical" scattering centers, that is, such as are displaced relative to each other by a whole multiple of the translation period, emit secondary waves whose phases differ by whole numbers and which therefore strengthen

each other maximally. This consideration is valid for all possible interference beams. It follows from this that all interference beams arising from a simple lattice would have the same intensity if the factors already mentioned did not bring about a diminution in intensity with increasing angles of diffraction.

Let us now suppose that a number of new scattering centers, S_2, are inserted midway between the original centers, S_1, as in figure 27, and let us orient this double series of points in the primary beam in such a way that an interference of the hth order arises. Since the secondary waves from the points S_1 are shifted h whole wave lengths relative to each other, the waves from the points S_2 will show path differences of half h relative to the original wave train. According to whether h is an even or an odd number, the waves from S_2 will maximally strengthen or weaken the waves sent out by S_1. If the scattering powers of S_2 and S_1 are equal, the interference in the first case will be 2^2, that is 4 times as strong, while in the second case, its intensity will be reduced to zero, that is, the interference is extinguished.

In three-dimensional space, the order of the interference beam is determined, not by a single number h, but by a threefold group of numbers hkl. We have seen that in a simple lattice the intensity of the interferences is independent of the order, that is, independent of the triple index, hkl, but in the compound lattice, obtained by the interpolation of new scattering centers in the simple lattice, this is not the case. The intensities of the interferences of the simple lattice must be multiplied by a factor S^2, dependent on hkl, in order to obtain the values of the intensities for the compound lattice. The vector S_{hkl} is known as the *structure factor*.

Any lattice, no matter how complex, can be supposed to arise by interpolating in their proper positions, the various scattering centers, starting from a simple translation lattice. Each type of scattering center will contribute to each interference beam emitted. Its scattering power determines the amplitude, and its position in the lattice determines the phase, of this contribution. The resulting amplitude is obtained by the summation of all the products of scattering power and phase factor. Thus, if ϕ_j is the phase of the secondary waves of the jth species of scattering center, and S_j is its scattering power, the intensity of the interference hkl is proportional to S^2_{hkl}:

$$S^2_{hkl} = [\sum S_j \cos 2\pi\phi_j]^2 + [\sum S_j \sin 2\pi\phi_j]^2 \qquad (3)$$

where $\phi_j = hu_j + kv_j + lw_j$, and u_j, v_j, and w_j are the coordinates of the scattering center of species j (Fig. 28).

From these considerations it follows that *all* scattering centers in the

crystal participate in the formation of *each* interference beam by either increasing or decreasing its intensity. Attempts to relate a single species of scattering center to a given interference are therefore misguided unless

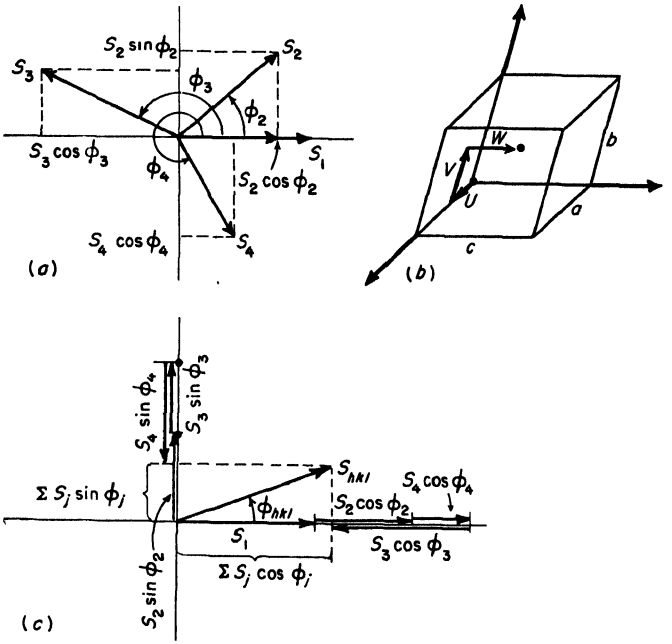


Fig. 28. Graphical determination of the structure factor: (*a*) 4 species of scattering centers, with scattering powers S_j and phase angles $\phi_j(\phi_1 = 0)$, and their components on two rectangular axes; (*b*) coordinates of a scattering center u, v, and w are expressed as fractions of a, b, and c, respectively; (*c*) demonstrating equation (3).

the contributions of all other species happen to cancel out for the selected beam.

(*f*) *Crystal Analysis*

Summarizing the various points already raised, we see that the direction of the interference beams and thus the position of the spots in the x-ray diagram of a crystal is determined by the translation lattice, while the intensity of each interference depends in a definite way on the scattering power and on the arrangement of the scattering centers. It is relatively easy to calculate the diffraction pattern of a crystal of known structure;

x-ray analysis, however, is concerned with the converse problem, and the calculation of the structure from the diffraction pattern is a much more difficult task.

Of all the factors which determine the intensity of an interference, only S^2 includes the parameters of the scattering centers. It is therefore necessary at the outset to determine the values of S^2 from the observed intensities. This in itself, both experimentally and theoretically, is by no means a simple problem.

Let us suppose, however, that this task has been accomplished. We must then determine the three parameters, u_j, v_j, and w_j, for each of the s scattering centers of the elementary cell from all the available values for S^2. Accordingly (see Eq. 3), it is necessary to know the scattering power, F_j, of all the scattering centers. For this purpose it is reasonable to regard the atoms as the scattering centers, since the spherically symmetrical atomic factors are known with sufficient accuracy as functions of the scattering angle, 2ϑ. In doing so, however, we implicitly make two by no means harmless assumptions; these will be considered at the end of this section. For the moment we need only bear in mind the uncertainty thereby introduced.

Even when all F_j values are known, and all values of hkl and of S^2 have been determined from the diagram, the coordinates of the atoms cannot be calculated directly. The mathematical form of S^2 necessitates a method of calculation depending on trial and error. This means that we must guess the arrangement of the atoms and see if the S values calculated from it are correct. In making this guess, data derived from the chemical constitution and physical properties of the crystal, and particularly from its symmetry, are taken as guides (see section 4).

If the crystal has a high degree of symmetry, or if the elementary cell contains only a few atoms, the symmetry so limits the number of possible arrangements that the atomic parameters are to a large extent (or even completely) determined. In organic substances, and particularly in high polymeric substances, these conditions are practically never fulfilled and the symmetry of the lattice yields fewer indications of structure than it did in the investigation of simple inorganic compounds in the early days of x-ray analysis.

Once a plausible arrangement has been worked out, the structure factors for the interferences of lowest indices: (100), (010), etc., are calculated and compared with the observed values. If there is no qualitative agreement, the arrangement must be rejected and another model tried. If there is moderately good agreement, an attempt can be made to improve this by small displacements of the atoms. If the attempt is successful, the S^2

values of higher indices are also calculated and compared. These are much more sensitive to small changes in the parameters.

In this way an attempt is made to obtain the fullest possible agreement between the calculated values of S^2 and those derived from the x-ray diagram. If the number of observed intensities is sufficiently large, that is, about three times the number of atoms in the elementary cell, and if there are no discrepancies when the calculated values for S^2 are compared with those obtained from the diagram, it may be assumed that the solution is correct. In practice, it is rarely possible to show that an arrangement found in this way is the only one which will satisfy these requirements.

The application of the method of trial and error is time-consuming and the result cannot be regarded as final. In order to use the method at all, we have had to pass from the translation lattice to the atomic lattice. As mentioned previously, two hypotheses underlie this transition:

(*1*) The atoms can be represented as scattering points with the scattering power, F, of the free atom. In section 2*a*, we have already seen to what errors this "simplification" may give rise, particularly in the case of light atoms.

(*2*) The atoms are immobile in the lattice. For hard crystals of high melting point and for heavy atoms this may be a useful approximation, but for light atoms it would seem to be of very doubtful value. It is true that one may compensate for this error, to a certain extent, by introducing an empirical temperature factor; but in the majority of cases data for the calculation of such a correction are hard to obtain.

Lastly, it must not be forgotten that the hydrogen atoms are usually neglected entirely—a practice which, as Ewald remarked, is in accordance with the nature of the crystallographer rather than with that of the crystal.

Atomic coordinates obtained by the method of trial and error are thus in the nature of approximations, and there is no point in pushing the accuracy of measurement and of numerical calculation too far, since there is no hope of obtaining detailed information about the position of the atoms in this way. Fortunately, methods are available which can be used to check and to make more precise the results obtained by the method of trial and error. These methods, which do not suffer from the same uncertainty will be discussed in the next section.

(*g*) *Fourier Synthesis*

As in a gas or a liquid, the electrons in a crystal are the cause of the scattering of the x-rays. The translation lattice requires that the electron density, $\rho(u,v,w)$, at the point with coordinates u, v, and w, be repeated as are the periods of the translation lattice. Along the a axis, for example,

$\rho(u)$ can be represented by a strictly periodic curve of period a, as in figure 29.

Fourier showed that any periodic function of the period a can be reproduced by superimposing a cosine and a sine curve of the same periodicity, a, together with their "overtones" (periods $a/2$, $a/3 \ldots a/i \ldots$), if the amplitudes, A_i and B_i, of the individual waves are given suitable values.

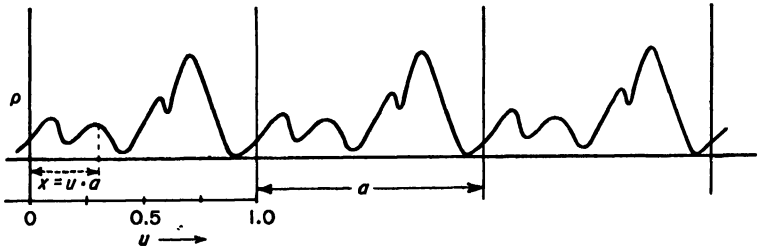


Fig. 29. The electron density along a lattice line is periodic, with the same periodicity a as the lattice line itself.

The electron density can therefore be represented by a Fourier series:

$$\rho(u) = A_0 + A_1 \cos 2\pi \cdot u + A_2 \cos 2\pi \cdot 2u + A_3 \cos 2\pi \cdot 3u + \ldots$$
$$+ B_1 \sin 2\pi \cdot u + B_2 \sin 2\pi \cdot 2u + B_3 \sin 2\pi \cdot 3u + \ldots$$
$$= \sum_{n=0}^{\infty} (A_n \cos 2\pi \cdot nu + B_n \sin 2\pi \cdot nu)$$

By this means the electron density is resolved into standing harmonic waves. Let us now consider one of these density waves of period n. At the point $u = x/a$ this makes a contribution to the electron density of:

$$\rho_u^n = A_n \cos 2\pi \cdot nu + B_n \sin 2\pi \cdot nu$$

Now the following important facts can be demonstrated: this nth harmonic density wave scatters in two directions only: in the direction of the primary beam, $2\vartheta = 0$ (which does not interest us at the moment), and in the direction of the interference beam of the nth order—in this one direction other density waves make no contribution to the scattered radiation. The structure factor of the interference beam of the nth order is therefore given by:

$$S_n^2 = (A_n^2 + B_n^2)/4$$

and the intensity of the beam is proportional to this expression. In general, this equation is insoluble, since for each structure factor we must determine two coefficients, A_n and B_n, that is, the amplitudes of the nth harmonic of the electron density, $S_{n=h}$. If, however, a center of symmetry exists in the observed direction (here the a axis), or if it is possible on any other grounds to say something about the phase of the density waves, solution is possible.

These considerations can be applied to the problem in three dimensions, and it is then found that the electron density at any point can be deter-

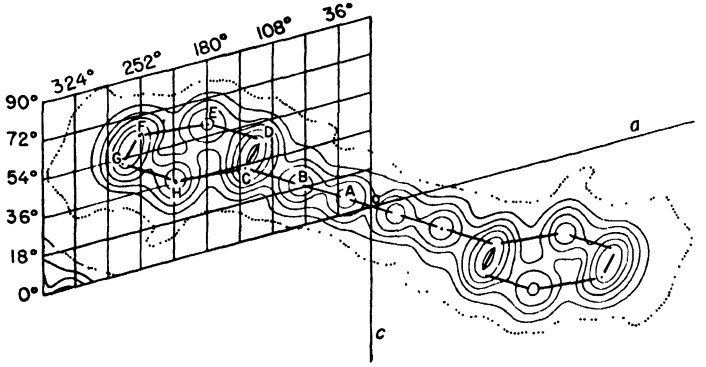


Fig. 30. The electron density in diphenyldiacetylene. The flat molecule makes a small angle with the plane of drawing a,c plane (Wiebenga[69c]). A, B, C, etc., mark points of maximum electron density.

mined from the totality of observed intensities. It is customary to represent the electron density by the projection of ρ along any one suitable crystallographic direction or on a plane at right angles to this. The distribution is represented graphically like the altitude on a map by means of contour lines, each of which indicates a constant value of ρ. The electron density is highest in the neighborhood of the atomic nuclei. The peaks of the map therefore have the coordinates of the atomic nuclei; the height of the peak is proportional to the atomic number and indicates the nature of the atom (Fig. 30).

In the application of Fourier synthesis of electron densities the following points must be borne in mind.

(*1*) In practice the method can be used only if the unit cell has a center of symmetry. In no case can this be decided by x-ray methods.

[69c] E. H. Wiebenga, *Z. Krist.*, **A102**, 193 (1940).

(*2*) Even if a center of symmetry is present we can only calculate S^2_{hkl}; the *sign* of each S_{hkl} must be determined by some other method. In many cases this is difficult.

(*3*) No temperature factor is introduced in calculating the value of S^2_{hkl} from the observed intensities, so that Fourier synthesis reveals the density distribution corresponding to the temperature of observation. This is a great advantage as compared with the method of trial and error.

(*4*) Details in electron density determined by the Fourier method are only significant if a great many intensities have been measured. The number of intensities is necessarily limited, if only because of the finite value of the wave length of x-radiation used. The Fourier series is therefore always interrupted, and hence false details may arise in the maps. This is equivalent to the limitation of the resolution of a microscope by the wave length of the light used and the formation of "ghosts" by the finite aperture of the objective. On the other hand, these interrupted Fourier series have the important mathematical property that, if account is taken of extra terms (that is, of S values of interferences of still higher order), those already found are not modified.

In favorable instances Fourier synthesis of a crystal diagram can yield more information than the method of trial and error. It permits the determination not only of the position of the atomic nuclei, but also of the kind of nuclei and the distribution of electrons between them. In this way the mode of linkage of the atoms is brought within the scope of x-ray studies. Moreover, the method affords the possibility of completing or improving results obtained by the method of trial and error, since the two most important sources of uncertainty, neglect of the linkage clouds and the influence of temperature (both of which are of special importance in dealing with light atoms), do not affect the results.

(*h*) *Atomic Spacings: Patterson Synthesis*

In the preceding paragraphs we have seen that the maxima of the function $\rho(u,v,w)$, that is, the peaks of the map, mark the positions of the atomic nuclei. The distance between two peaks at $(u_1v_1w_1)$ and $(u_2v_2w_2)$ is therefore identical in direction and magnitude with the distance between the atoms 1 and 2.

Now it is obvious that the product: $\rho(u_1v_1w_1) \times \rho(u_2v_2w_2)$, of the electron densities at any two points in the unit cell can only have large values if the two points $u_1v_1w_1$ and $u_2v_2w_2$ coincide with peaks. If, for example (Fig. 31), we determine the product of the density at a point (u) times the density at the point $(u + \Delta u)$ on the a axis, then $\rho(u) \times \rho(u + \Delta u)$ can only be large if the two points (u) and $(u + \Delta u)$ coincide with maxima of electron density on the a axis. If this operation is repeated at constant point distance, Δu, for all points along the a axis, and if the individual

products are added together, the sum of the products will itself only be large if Δu corresponds to the spacing of two maxima. The function:

$$P_a = \int_0^1 \rho(u) \times \rho(u + \Delta u)du$$

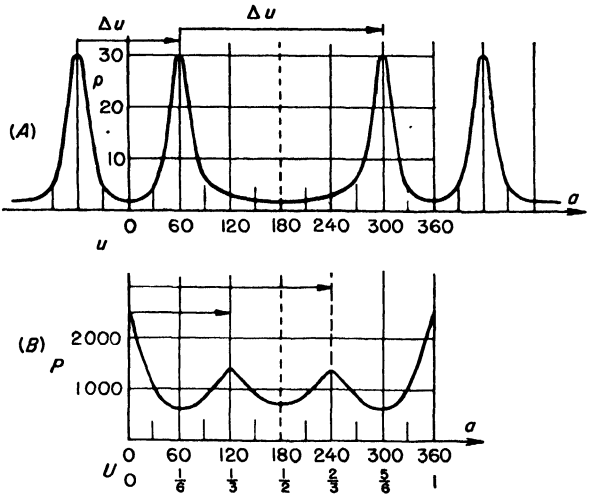


Fig. 31. (A) Assumed electron density along a axis; the identity period has been subdivided into 360 divisions. Centers of symmetry at 0 and 180. Two atomic spacings Δu can be distinguished. (B) Every sum of products $\rho(u) \times \rho(u + \Delta u)$ for Δu = constant has been plotted against $\Delta u \equiv U$. The *abscissae of the maxima of P* indicate the *distances between the maxima of* ρ.

shows a peak if Δu is an atomic spacing. By calculating the products, integrating, and transferring to three dimensions, one obtains a Patterson series:

$$P(UVW) = 4 \sum_h \sum_k \sum_l S_{hkl}^2 \cos 2\pi(hU + kV + lW)$$

where $U = \Delta u$, $V = \Delta v$, and $W = \Delta w$. In order to determine atomic spacings in the crystal lattice we must therefore calculate $P(UVW)$ for each point in the unit cell. Since only S^2 occurs in this expression, and since the values for this quantity can be determined without ambiguity from intensities, P can be calculated without any doubt as to sign. As with Fourier series the results are represented graphically by projection. The highest peak in figure 31 corresponds to $U = 0$, since all the atoms are present at zero spacing from themselves. The distance from the origin

to the other peaks gives the interatomic spacings within the crystal in magnitude and direction. In the case of atoms of the same kind the height of the peak varies with the frequency of occurrence of the corresponding spacing; it increases with the atomic number.

An essential feature of the Patterson method is its objectivity. It not only provides an excellent means of testing results obtained by other methods, but it can also be used with advantage to secure a basis for the method of trial and error or for Fourier synthesis. The combination of all three methods leads to a more certain and more complete knowledge of the structure of the crystal and of the chemical units it contains than can be obtained by any single method of investigation.

3. X-Ray Diagrams of High Polymers

(*a*) *Powder Diagrams*

In the preceding sections we have seen how the x-ray diffraction picture of a crystal arises, and how the arrangement of the atoms can be deduced from this picture. It has not as yet been possible, however, to prepare single crystals of the majority of high polymers; only certain proteins have been studied as individual crystals. In all other cases the material to be investigated is polycrystalline.

The properties of polycrystalline substances depend not only on the structure of the crystals, but also on the manner in which they are associated. Their arrangement is spoken of as *texture*, and the small crystals or crystalline regions (mostly very incompletely crystalline) are known as *crystallites*. When no direction is favored in the texture, that is, when the crystallites are distributed in an entirely irregular manner, the object is macroscopically isotropic. The x-ray diagram of such an object resembles that obtained from a powdered crystal and is therefore known as a *powder diagram*. Powder diagrams are obtained, for example, from spontaneously frozen rubber or gutta-percha. One can imagine their derivation from a crystal diagram by supposing that the crystal is rotated about all possible directions during exposure in the primary beam. In this way all possible interference beams arise, and are recorded on the photographic plate as concentric circles, the center of which is the point at which the primary beam strikes the plate. The lattices of high polymers for the most part have a very low degree of symmetry, so that numerous interference beams are emitted. The spacings of the net planes are generally large, so that the scattering angles are small, and many interference circles therefore lie close together. Moreover, on account of their small size, the lattice

of the crystallites is usually nonideal so that the interferences are broadened. For these three reasons the interference circles overlap to such an extent that, quite apart from theoretical difficulties, it is impossible to interpret such powder diagrams. Interpretation of powder diagrams of starch, amylose, or other high polymers such as have often been published, represent only one of an infinite number of possibilities, and no significance can be attached to them. Nevertheless, even in the case of high polymers of unknown structure, powder diagrams may lead to important conclusions: (*1*) they may be used in the same way as other physical constants, such as boiling point or melting point of compounds of low molecular weight, for the identification of high polymer substances. As compared with these, they have the advantage that not one but several physical characteristics are recorded—as many as the number of observable interference rings. (*2*) Relatively sharp interferences indicate the presence of crystalline regions which could not be detected by other methods but which are of importance in determining the mechanical, optical, and electrical properties of high polymers. (*3*) Chemical reactions may be demonstrated and followed by means of powder diagrams.

(*b*) *Fiber Diagrams*

In many polycrystalline solids the crystallites are not arranged in a completely irregular manner, but one particular crystallographic direction favors a particular orientation. The type of texture of this kind which is of most importance to us is fiber texture, in which the crystallites are so arranged that one and the same lattice line (which can be chosen as the axis of the lattice) is parallel (or approximately parallel) to a given direction called the *fiber axis*. Such an arrangement is revealed macroscopically by the optical and mechanical properties of the object; it occurs frequently in high polymers. Cellulose fibers and the chitin of arthropod tendons display well-developed fiber texture, presumably determined by the manner of growth. In many other high polymers, as in mercerized cellulose and in certain starch derivatives, this texture may be produced artificially by stretching, whereby the crystallites already present are rotated until one axis is parallel to the direction of stretching. Moreover, a similar arrangement of the crystallites arises in the racking of many amorphous, rubberlike high polymers: the direction of stretching becomes the fiber axis of the specimen as it crystallizes.

In the fiber the crystallites thus lie parallel to the fiber axis but in all positions arising from rotation about this axis. If the fiber is irradiated

with a parallel beam of x-rays traversing simultaneously a large number of small crystallites, a diffraction pattern is formed equivalent to the diagram obtained by rotating a single crystallite about the fiber axis; the *fiber diagram* is therefore essentially similar to a rotating crystal photograph (see Chap. A, sect. II 2*c*). As in the latter, the interference beams will be recorded as points on the photographic plate, though they are usually much less sharp.

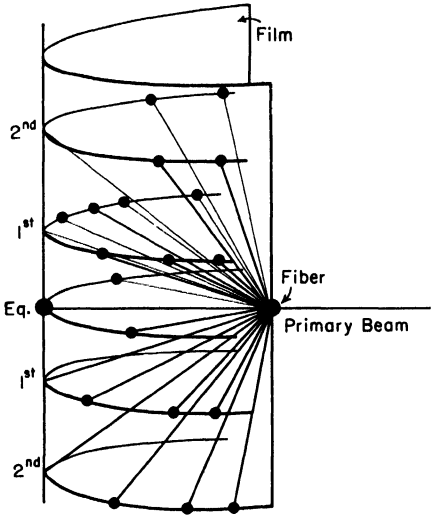


Fig. 32. Layer lines on a cylindrical film: Eq. = Equator. Compare also figure 21.

If the fiber axis is oriented at right angles to the primary beam, the spots are arranged on hyperbolae in photographs on flat film (compare Fig. 20); while on cylindrical films, and if the fiber axis is coaxial to the film, they will be arranged on straight lines at right angles to the fiber axis. These are the *layer lines*. One layer line, the *equator*, passes through the point at which the primary beam strikes the film; the others lie symmetrically about the equator and are numbered in sequence from the equator outward (Fig. 32).

The significance of the distance of a layer line from the equator is immediately obvious when we recall that the fiber axis is a lattice line and therefore passes through a series of identical scattering points. We can again make use of equation (1). Since the primary beam is now at right

angles to the series of points, $\varphi = 0$ and equation (1) for the nth layer line reduces to:

$$n\lambda = r\,[\sin\varphi + \sin(2\theta - \varphi)] = r \sin 2\theta_s$$

where the layer-line angle, $2\theta_s$ can be calculated, from the linear distance from the equator, A_n, and the distance, A_F, between fiber and film (see Fig. 33):

$$\tan 2\theta_s = \frac{A_n}{A_F}$$

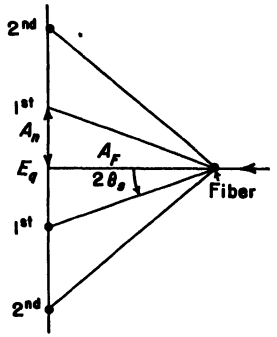


Fig. 33. Radial section of figure 32: $\tan 2\phi_s = A_n/A_F$.

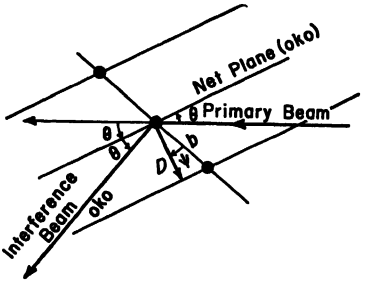


Fig. 34. Net plane distance D and fiber period b; $D = b \cos \Psi$.

From the layer-line spacing, A_n, the translation period, r, along the fiber axis is obtained. If this is chosen as the b axis, the general interference condition (1) is transformed into the Polanyi equation:

$$b = \frac{n\lambda}{\sin 2\theta_s}$$

The choice of the fiber axis as chief crystallographic axis is entirely arbitrary; all that we know is that b is a lattice line and therefore *may* be chosen as the axis. Whether or not this choice leads to a simple and useful description of the lattice only emerges in the course of analysis. The latter is only possible if the fiber axis is an ortho axis (sect. 2*d*), since otherwise the equations are so complicated and the number of unknowns is so large that the result is uncertain. Whether the fiber axis is an ortho axis or not can be decided by measuring the scattering angle of the *diatropes*,

i.e., interferences lying on the meridian above and below the point at which the primary beam strikes the plate. In order to measure these it is necessary to oscillate or rotate the fiber about the direction at right angles to both fiber axis and primary beam. From the diatropes appearing in such photographs the net plane spacing can be calculated according to the Bragg relation:

$$D_{0k0} = \frac{k\lambda}{2 \sin \vartheta_B}$$

where the angle ϑ_B is obtained from the distance, A_D, from the primary spot to the diatrope and the distance, A_F, from the film to the fiber by:

$$\tan 2\vartheta_B = \frac{A_D}{A_F}$$

b is only an ortho axis if the net plane spacing, D, is equal to the period of translation, b; for from figure 34 one can obtain immediately: $D = b \cos \psi$, where ψ is the angle between the vectors $\boldsymbol{b}$ and $\boldsymbol{D}$.

It may be mentioned here that the diatropes do not in general lie on the layer lines. This is only so if by chance $D = b \cos \theta_s$, that is, if $\psi = \theta_s$. If the fiber axis is an ortho axis, that is, $\psi = 0$, this is certainly not the case.

After b has been determined, and if we are dealing with an ortho axis, three unknowns remain: the translation periods a and c and the angle β. The task of determining these is considerably more difficult than that of determining b, and in practice can only be carried out (by graphic methods or by calculation) if sufficiently sharp diagrams containing many interferences are available. The literature must be consulted for special methods.

In principle these methods consist of a means to arrange systematically the projections of the vectors of the reciprocal lattice—of which one can only calculate the magnitudes (from the scattering angle 2ϑ) and the vertical components (from the period b)—in the ac plane in such a way that a reciprocal lattice net is formed. From this the translation lattice can then be derived unequivocally.

(*c*) *Diagrams of Objects with Orientation of Higher Order*

Experience has shown that the fiber diagram does not suffice for the determination of the lattice in the case of high polymers. Even if the fiber axis, b, is an ortho axis, the determination of a, c, and β depends, as we have seen, on a trial-and-error method which only rarely leads to an

unambiguous result and usually leaves open a number of different possibilities. From these one, and frequently only one, can be selected if it is possible to say something about the angles between a few net plane spacings. Such data may be obtained from diagrams of objects with orientation of higher order.

In certain natural products, for example, the cell wall of the giant unicellular alga, *Valonia ventricosa*, as well as in that of *Chaetomorpha linum*, regions can be found in which the cellulose crystallites are not merely approximately parallel to each other, but are so arranged that a second crystallographic direction favors a certain orientation. This type of texture can also be produced artificially: if threads of rubber or swollen cellulose fibers are rolled in the direction of the fiber axis, the crystallites, which are already oriented parallel to each other, arrange themselves so that one of the $(h0l)$ planes lies approximately parallel to the plane of rolling. The second orientation is usually only developed to a limited extent, but it suffices for an approximate determination of the angles between some of the net planes. Oscillation photographs may be of use here; the object is oscillated through a definite angle about the fiber axis during the exposure. Goniometer photographs, in which the film also moves, provide more certain information and the angle between two net planes can be measured directly if the orientation is sufficiently good. If, for example, the fiber diagram suggests two possibilities: either that the two net plane spacings $(h_1k_1l_1)$ and $(h_2k_2l_2)$ enclose an angle of 25° or of 85°, and if the goniometer diagram permits a value of only $(90 \pm 20)°$ for this angle, the first possibility is excluded, and the translation lattice is determined unambiguously.

(*d*) *Unit Cell: Dimensions and Space Group*

The parallelipiped defined by the three translations a, b, and c is known as the *unit cell.* All the symmetry properties of the translation lattice, that is, its space group, are also to be found in this unit.

The translation lattice can be regarded as built up by packing such cells in all directions; the crystallite is built up by arranging the material content of the cells in the same way. If the translations and the angles are known, the volume, V, of the unit cell can be obtained and, multiplying by the density, ρ, of the crystalline substance, the mass of its contents, $V\rho$, can be calculated. This mass is necessarily a whole multiple of the absolute formula weight, M/N_A, where N_A is Avogadro's number and M is the weight of one mole (in substances of low molecular weight) or the weight

of the repeating unit (in the case of high polymers). The number of units, Z, in the unit cell is therefore:

$$Z = V\rho(N_A/M)$$

This relation also provides a check on the previous calculations, since Z must be a whole number within the limits of error.

When the unit cell and from this the translation lattice have been determined, and when, therefore, all interferences have been ascribed to definite net planes, it is possible to consider the symmetry. The more regular the arrangement prevailing within the unit cell, and therefore the more symmetry elements there are present, the larger is the number of extinctions (see sect. II 2*e*). Except for a center of symmetry, each element of symmetry provokes the extinction of a group of interferences; the relationship between every possible element of symmetry and the corresponding group of extinctions is tabulated, *e.g.*, in the *International Tables for the Determination of Crystal Structures*. The center of symmetry provokes no extinctions and therefore cannot be revealed by x-rays. Its absence may sometimes be demonstrated by the optical activity of the object.

The ensemble of all the symmetry elements present is called the *space group*. All possible elements of symmetry can combine in 230 different ways, thus the number of space groups is also 230. Of these, many may be excluded at once if the translation lattice has no higher than, for example, monoclinic symmetry. From the remaining, those can be excluded for which any observed interference belongs to a group of extinctions that is characteristic of any of their elements of symmetry.

Thus, the final assignment of the space group is in reality a question of the intensity of certain groups of interferences: whether greater than or equal to zero. As it is impossible to ascertain if the intensity of any interference spot is exactly zero, or if it is only too weak to be observed, there always remains a certain ambiguity. The lack of precision is much greater with organic fibers than with ordinary crystals, because all high-order interferences from the former are generally too weak to be observed. Conversely, it should be stated that if a group of interferences characteristic of a given element of symmetry is extremely weak, the configuration must be such that the element of symmetry in question is approximated.

Finally, it should not be forgotten that we must aim at the determination of the coordinates of the atoms in the unit cell. This task is simplified, but by no means accomplished, by the determination of the symmetry; knowledge of the latter only renders the use of Patterson and Fourier

synthesis, as well as of the method of trial and error, easier and less time-consuming.

(*e*) *Deviations from Ideal Diagram*

Although the diagrams obtained for high polymers are fundamentally similar to those of macroscopic crystals, there are many deviations from



Fig. 35. Fiber diagram of chitin tendon of rock lobster; orientation rather imperfect, diatropes (*i.e*, meridional interferences) strongly marked.[69d]

the ideal diagram. Under certain circumstances these deviations may affect the determination of the translation lattice; they may also influence considerably the calculation of structure factors from the observed blackening on the film. The deviations can be divided into two groups: (*1*) those due to imperfections in the orientation of the crystallites; and (*2*) those due to imperfections in the lattice.

In many fiber diagrams the interferences are drawn out into arcs, because the *b* axes of the crystallites are only approximately parallel to the fiber axis. If this irregularity is slight the disturbance at the equator is insignificant, but in the region of the meridian the course of the layer line is changed and the intensity is falsified. It is readily seen that if the texture

[69d] Meyer and Pankow, *Helv. Chim. Acta*, **18**, 589 (1935).

is ideal the meridional interferences ought not to be visible in a fiber diagram, but in most actual fiber diagrams of high polymers, meridional interferences occur (Figs. 35 and 36). The latter is also true for interferences adjacent to the meridian. The intensities of these spots cannot be used for calculation. The intensities in oblique diagrams, such as Fig. 5 in Chap. C, sect. IV 3 in which the fiber axis is inclined at the Bragg angle to the primary beam, are equally unsuitable. In such photographs the intensity of the "focused" meridional interference appears increased several

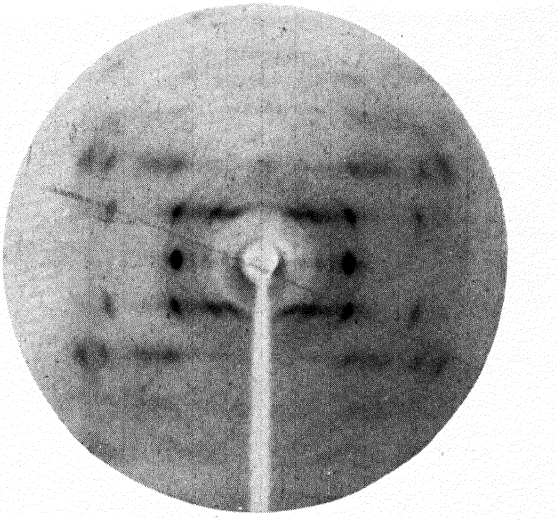


Fig. 36. Fiber diagram of racked film of amylose acetate; orientation very good, sharp spots on the equator, diatropes hardly visible.[69c]

hundred times over that of the "unfocused" net planes, so that direct comparison is impossible. Several examples of mistakes of this kind occur in the literature.

Not only imperfect orientation of the crystallites, but also double orientation or "twinning" occurs sometimes. This can only be detected by means of goniometer photographs of specimens with orientation of higher order, and then only if the orientation is sufficiently good, since otherwise the superposition of two interferences of approximately the same scattering angle will give the appearance of a maximum corresponding to no actual

[69c] P. Zuppinger, Thesis. Geneva, 1946.

interference beam. This occurred in the early study of rubber and led to an erroneous determination of the translation lattice.

In a last analysis all these complications are due to the lack of macroscopic crystals on which optical measurements can be made to complete the x-ray data. In a fiber, it is not even possible, for this reason, to determine whether one or several modifications are present. The occurrence of several modifications is by no means exceptional in high polymers such as cellulose.

The second type of deviation from the ideal diagram is due to disturbance in the lattice of the crystallites. A lattice disturbance common to all crystals is a consequence of their necessarily finite dimensions; the lattice is of course disturbed at the boundaries of the crystals. Since two scattering centers give a continuous diffraction picture with broad maxima, while with an infinite series of such centers the intensity is confined to definite directions, there must be a region between these two extremes where the maxima have a measurable breadth, and where the equidistant scattering centers number up to a few hundred.

Many attempts have been made to correlate the dimensions of the crystallites with the half-breadth, *i.e.*, the angular range where the intensity is equal to or higher than one half of the maximum. For very thin crystal flakes, Bragg has deduced an approximate relationship:

$$B = \frac{51\lambda}{t \cos \vartheta}$$

where B is the half-breadth, t the thickness of the plate, and λ and ϑ have their usual meanings. For needlelike crystals Laue has obtained much more complicated equations.

Whatever formulas may be applied, the numerical results should be used with extreme caution. There are, in fact, several reasons which make the calculations very hazardous. Thus, it is assumed that the lattice inside the crystallites is undisturbed. Further, the crystallites are assumed to be imbedded in a medium of zero scattering power and it is supposed that their boundaries are crystal faces. Though these assumptions may be considered as useful approximations in ordinary crystal powders, none of them prevail in fibers of high polymers.

According to the first assumption the lattice disturbances are supposed to occur exclusively at the boundaries of the crystallites, but they may also occur in the interior. As soon as for any reason the distance between two centers of a row of scattering centers is greater or smaller than the period of the row, the secondary waves from the region posterior to the disturb-

ance become out of step with the secondary waves emitted by the centers anterior to the disturbance. It is a matter of indifference whether the disturbance is due to mechanical distortion, to the interpolation of foreign atoms, or to the presence of unoccupied positions in the lattice. The result is a broadening of the maxima which cannot be separated from the broadening due to the disturbance at the boundary.

This brings us to the second point. The crystallites in a fiber of, *e.g.*, cellulose, are not bounded by well-determined crystal planes but the boundary must be pictured as a rather ill-defined zone of transition between a crystalline region and the amorphous portion of the fiber. Thus, even if all other complications are taken into account, the dimensions of the crystallites in a fiber are much less sharply defined than, *e.g.*, in a metallic wire. Besides it does not seem reasonable to suppose that all crystallites have the same dimensions as is assumed in the calculation. It is therefore extremely difficult to ascribe a definite physical meaning to the dimensions calculated from the half-breadth. For this reason, the half-breadth of a few conveniently chosen interference spots might well be used for the purpose of comparing the amount of lattice disturbance, produced by such treatments as mercerizing or stretching of a given fiber. There is no point in complicating the technical use of experimental data by applying any formulas to them.

In the preceding paragraph the broadening of the interferences was attributed to disturbances in the point rows. Since a crystal contains innumerable series of such rows disposed in various directions, the irregularity may be greater or less in a given direction than in others. Correspondingly, the interference maxima may be more or less spread out in certain directions than in others. One finds, for example, that the diatropes are relatively sharp in highly stretched mercerized ramie, while in directions at right angles to the direction of stretching there is a considerable broadening of the interferences, sometimes so extensive that the layer lines appear almost continuous. From this one may conclude that irregularities in the lattice, produced by stretching in the direction of the fiber axis, are much slighter in that direction than in directions at right angles. In this special case, therefore, each interference is relatively sharp in the direction of the meridian, while in the direction of the equator it is spread out.

The spreading of the interferences is also noticeable near the primary spot, since, as seen in Chap. A, sect. II 2*d*, this is nothing more than the interference with the index (000). In principle, therefore, no new information can be obtained from the study of what is known as low-angle scattering, that is, from the intensity distribution of the x-radiation scat-

tered through an angle of less than about 5°. From the point of view of calculation, analysis may appear simpler; in fact, however, experimental and theoretical difficulties are so considerable that the results hitherto obtained must be regarded as very uncertain.

In this same low-angle region, the x-ray diagrams of many fibrous high polymers show definite interferences on the meridians indicating the presence of large net plane spacings of 100 Å. and more. These were first observed by Pankow[69f] in gutta-percha and were found to be unrelated to the lattice of the crystallites. Hess and Kiessig[69g] observed similar interferences in polyesters and polyamides and found that they differed from preparation to preparation. In other cases (in collagen, for example), we seem to be concerned with the fundamental period (640 Å.) of a very long unit cell. Hess and Kiessig related the appearance of this large period to a possible periodic alternation of well-oriented and less well-oriented regions along the fiber axis. Bunn[69h] has attempted to interpret such large spacing as indicative of the presence in the object of standing thermal (elastic) waves.

(*f*) *Amorphous and Mixed Fibers*

We have only discussed the x-ray diagram of the crystalline portion of the fibers, but, of course, the amorphous portion also diffracts x-rays, and in some fibers the proportion of amorphous material is quite high. Some high polymers even crystallize with great difficulty or not at all, *e.g.*, nitrocellulose or polyvinyl acetate. Nevertheless, quite typical fibers may be obtained from these substances.

The general appearance of the x-ray diagram of such fibers is not unlike that of the diagrams obtained with ordinary liquids, but some of the maxima are stronger in the neighborhood of the equator, while others are concentrated near the meridian; most vary little with the latitude. This behavior is readily understood if we consider that the chain molecules must all lie fairly parallel to the fiber axis, as is shown by the mechanical behavior. Furthermore, the small difference in specific weight of, for example, amorphous and crystalline rubber suggests that the packing is very similar in both modifications. This is corroborated by the observation that the maxima of the amorphous portion have nearly the same glancing angle as the strongest spots from the crystallites or may be thought of as due to a superposition of several spots. The molecular arrangement in an

[69f] G. W. Pankow, *Helv. Chim. Acta*, **19**, 221 (1936). G. L. Clark, E. Wolthuis, and W. H. Smith, *J. Res. Natl. Bur. Standards*, **19**, 479 (1937).

[69g] K. Hess and H. Kiessig, *Z. physik. Chem.* **A193**, 196 (1944).

[69h] C. W. Bunn, *Proc. Roy. Soc.* (*London*), **A180**, 40 (1942).

amorphous fiber is thus much further from randomness than that of an ordinary liquid.

Up to date no satisfactory numerical interpretation of diagrams from amorphous fibers can be given; the problem is still more difficult than the interpretation of the patterns from ordinary liquids. Attempts have been made to estimate the ratio of crystalline to amorphous material in partially crystallized fibers by comparison of the intensities of suitable spots and diffuse maxima. To this end the assumption is made that, if it were possible to eliminate the amorphous portion, no radiation would be scattered toward regions outside the spots. This assumption is certainly not justified, if only because of the inevitable incoherent radiation. It is furthermore assumed that the intensities of the diffracted waves from crystalline and amorphous material can be simply added: this hypothesis cannot be verified and seems rather doubtful. For these and other reasons, the results obtained are useful for the purpose of verifying the increase or decrease of crystallinity in a given fiber as a result of processing, but should not be considered as indicating the ratio of crystalline to amorphous material; it is even doubtful if such a ratio has a precise significance.

4. Atomic Configuration of High Polymers

(*a*) *Lattice Types*

It has been mentioned (sections 2*g* and 2*h*) that, in order to determine objectively the coordinates of the atoms in the unit cell, a Fourier or Patterson analysis should be made. With high polymers, however, the number of interferences observed is usually much too small to yield sufficient details and, therefore, the method of trial and error must be applied. For this purpose it is useful to consider first the lattice type of the crystallite.

From the mechanical and chemical behavior of the product it is generally possible to decide to which one of the following four lattice types, occurring in high polymers, it belongs.

(*1*) *Molecular Lattice.* Crystals with a molecular lattice can usually be recognized microscopically by the presence of true crystal surfaces. High polymers occurring as crystals of this type (these include a number of proteins) yield solutions of low viscosity. From this fact, as well as from the fact that the molecule is confined in a compact unit cell much shorter than the fully extended protein chains, it follows that the latter must be folded or coiled.

(*2*) *Primary Valence Chain Lattice.* In this type, primary valence chains traverse the whole crystallite, and along the fiber axis the unit cell contains only a small number of residues, usually two or three. In the two remaining dimensions the crystal is held together by secondary valences. This type is characterized by marked anisotropy in

mechanical properties, being strong in one direction, but weak and cleaving readily in the directions perpendicular to the first.

(*3*) *Primary Valence Net Lattice.* Primary valences hold the crystal together in two dimensions, secondary valences in the third. The crystal has a laminated structure and the molecules are primary valence nets; it mostly cleaves readily in planes parallel to these nets.

(*4*) *Primary Valence Three-Dimensional Lattice.* The crystal is held together in all directions by primary valences; the molecule is a three-dimensional network or sponge.

Chains, nets, and three-dimensional networks may also consist of polyvalent ions, the charges of which are neutralized by interpolated metallic ions. This variant is found among the silicates.

(*b*) *Macroscopic Models*

The discussion of possible arrangements may be greatly facilitated by the use of macroscopic models. So, having obtained a rough idea of the shape of the molecule from the lattice type of the crystallites and with the aid of data obtained from chemical studies of the constitution, a model of the molecule or part of it may be constructed or drawn to scale. For this purpose, the following empirical bond lengths are usually adopted.

Bond Lengths (Å.)		
C—C 1.52 ± 0.02	C—H 1.08 ± 0.01	C—N 1.40 ± 0.05
C=C 1.34 ± 0.03	C—O 1.4 ± 0.05	C=N 1.21 ± 0.03
C≡C 1.20 ± 0.04	C=O 1.3 ± 0.05	C≡N 1.08 ± 0.03

The following bond lengths are occasionally required in constructing models:

C—F 1.4	C—S 1.78
C—Cl 1.75	S—S 2.04
C—Br 1.85	C—C 1.39 (aromatic)

The valency angle between single bonds with C, N, or O is 110° to a first approximation (109° 36′ = tetrahedral angle), but deviations up to 10° are by no means rare. Neither can the bond lengths always be assumed to be strictly constant, since the length of a particular bond depends on the other groups united with the two atoms in question. Thus, for example, the length of the CH_2—CH_2 bond is 1.58 Å. in dibenzyl and even 2.01 Å. in acenaphthene. The bond length C—N amounts to 1.33 Å. in diketopiperazine and 1.47 Å. in dimethylamine.

If we are dealing with primary valence chains, the model will not be rigid. Since, however, the chains lie along the fiber axis (*b* axis), and since the

identity period in this direction is known, one can tell at once whether the chains are fully stretched or strongly folded. This knowledge limits the possible shape of the chains. As another example, a comparison of the *cis* and *trans* models of polyprene hydrocarbons rubber and gutta-percha shows that rubber has the shorter, *cis*, configuration, while gutta-percha has the longer, *trans*, configuration (see Chap. C, sect. IV).

The molecules must now be arranged in the translation lattice in such a way that the observed symmetry requirements (space group) are satisfied; at the same time, the rules for "secondary bond lengths" are respected.

Secondary Bond Lengths (Å.)

$CH_2 \ldots CH_2$	3.55	H...H	2.6
$CH_2 \ldots NH_2$	3.55	O...O	2.7
$CH_2 \ldots OH$	3.4	S.. S	3 5
CH_2 ..S	3 5	NH...NH	3 6
CH...CH	3.5	OH...OH	2.75

The figures given are to be taken as approximate minimal values for the distances between atoms (groups) in adjacent molecules or chains. Within a single molecule considerably smaller distances may occur. Thus, in trioxymethylene the C...O distance is 2.18 Å., in paraffins the $CH_2 \ldots CH_2$ distance is 2.52 Å. The lengths of secondary bonds vary even more from case to case than do those of the primary valences. The distance OH...OH seems to be an exception, however; its value seems almost always to lie between 2.7 and 2.8 Å. This reflects the nature of the hydrogen bond.

It is convenient to use rigid atomic models. The out-of-date wire models are not very suitable for our purposes; instead we can use models built up from spheres whose diameters are proportional to the distances between the centers of the atoms bound by primary valences. The diameters of the spheres for C, N, and O are in the ratio of 1.5 · 1.3 : 1.3; hydrogen atoms are usually omitted. One may also, following Stuart, make use of spherical calottes which reproduce both primary and secondary valence spacings.

In both types of models, constancy of bond lengths and valency angles, and in Stuart's models also constancy of secondary valence distances, are assumed. Particularly the latter assumption is in actual fact only a very rough approximation. While discussion may be facilitated by means of such models, their use cannot lead to definite conclusions about the possibility of different atomic arrangements; fine details of configuration are quite beyond their scope. Because their potentialities are often exagger-

ated, we would emphasize that the ease of construction of, and the plausibility of the conclusions drawn from, such models do not prove their correctness. The proof can only be provided by the study of the intensities of the interferences.

In conclusion, it should be noted that the diffraction of x-rays has been interpreted in the preceding pages according to elementary kinematic wave theory. It is known, however, that this theory is only an approximation, since the interaction between the radiation field of the interference beam and the incident primary radiation is neglected. If the complete, dynamic theory is applied, the phenomena become considerably more complex. The kinematic theory may lead to erroneous results in dealing with strong interferences. Moreover, its application is limited to substances (atoms) which do not absorb the incident radiation, that is, do not convert this into radiation of another wave length. Such a conversion can never be entirely avoided, but it is on the whole inconsiderable in dealing with light elements. Summing up, we may say that, while x-ray analysis indeed provides the most certain method as yet available for the investigation of molecular structure, its application demands much time and effort.

III. INVESTIGATION IN SOLID STATE BY OTHER OPTICAL METHODS

1. Electron Microscope

Objects with dimensions smaller than 0.5λ, that is, about 2000 Å., cannot be resolved by the ordinary microscope. Using the ultraviolet microscope, the limit of resolution is finally reached at about 1000 Å. The use of radiant energy of still shorter wave length, such as x-rays ($\lambda = 0.6$ to 3 Å.), is impracticable, since it appears impossible to construct useful lenses for such waves. However, much shorter waves can be produced by replacing photons by electrons.

According to de Broglie, an electron moving in a given direction with velocity u is associated with a transverse wave train, the wave length of which is given by:

$$\lambda = h/mu = \sqrt{(150/V)}\ \text{Å}.$$

where h is Planck's constant, m the mass of the electron, and V the accelerating potential in volts. According to this, an electron beam is equivalent to a light beam of extremely short wave length, *e.g.*, $\lambda = 0.05$ Å. at an accelerating potential of 60 kv. On the other hand, it has long been known that electron beams can be deviated by means of electrostatic or magnetic

fields, so that, for beams of this kind, lenses can be replaced by electric or magnetic fields.

The construction of the electron microscope depends on these two facts. The object to be studied, the thickness of which must not exceed a fraction of a micron, is illuminated *in vacuo* by a beam of fast electrons, focused by an electric or magnetic field acting as a condenser. The emerging

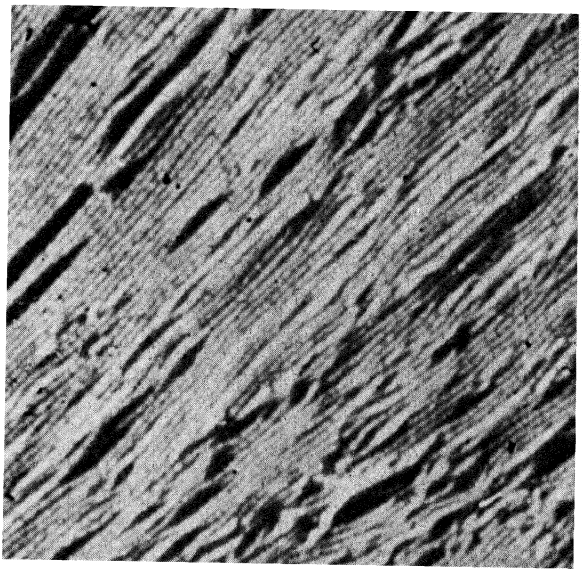


Fig. 37. Electron micrograph of a gold-shadowed replica of tobacco mosaic virus: rods in parallel alignment (×50,000) (Wyckoff).

electron beam passes through a second field which acts as an objective and forms a greatly enlarged image which is then reproduced by a third field acting as a projecting lens. The image thus obtained may either be photographed or observed on a fluorescent screen.

Contrasts arise as a result of diffraction and absorption by the object. With electrons both depend essentially upon the irradiated mass; masses appear therefore as shadows. The resolving power of modern instruments is about 40 Å. Under favorable conditions resolution down to 10 Å. has been obtained. In order to resolve the images of protein molecules, organic fibers, and the like, the object may be shadowed by spraying with cathodic sput-

tered gold in an oblique direction.[70] Pictures are obtained such as that shown in figure 37; hemocyanine molecules appear as little spheres; fibrils of cellulose, tendon, or muscle disintegrated by ultrasonics or by another method have been resolved by this technique.

The electron microscope will certainly be of great value for the investigation of complicated biological structures, although living cells cannot be observed since humidity and air must be excluded because they absorb the electrons; it remains to be seen to what extent it can be used for the determination of interatomic configuration, *i.e.*, chemical constitution.

2. Structural Analysis by Means of Birefringence Studies

(*a*) *Birefringence of High Polymeric Substances*[71]

The second optical method for the investigation of solid high polymers consists in the determination of their birefringence. In the present account we shall not consider the methods involved, but only point out what conclusions may be drawn from the results of such studies of birefringence.

Any object whose refractive index is the same in all directions is described as optically isotropic. In this category belong amorphous substances as well as crystals of the cubic system. All other arrangements give crystals which are optically anisotropic; that is to say, the refractive index varies with the plane of vibration of the incident light. If one direction is optically isotropic, the crystal is optically uniaxial. The birefringence is described as positive or negative according to whether the index of refraction for light whose plane of vibration lies in the singular direction is larger or smaller than that for light polarized in the direction at right angles to this. If two directions are optically isotropic, that is, if the refractive index is different in three directions, the crystal is described as biaxially birefringent. In the case of polymers, truly holohedric lattices never occur; their crystallites are therefore always optically anisotropic. We wish now to show to what extent conclusions about the structure of high polymeric substances may be drawn from their optical behavior.

If the crystallites or oriented regions lie beyond the limit of resolution of the microscope, an assemblage of anisotropic particles may appear optically isotropic. Whether such an optically isotropic preparation is crystalline or not can only be decided from the x-ray diffraction pattern. Thus, for example, the sharp rings of the powder diagram of amylose or

[70] R. C. Williams and R. W. G. Wyckoff, *J. Applied Phys.*, **17**, 23 (1946).

[71] General account of birefringence: Ambronn and Frey, *Das Polarisationsmikroskop.* Akadem. Verlagsgesellschaft, Leipzig, 1926.

of many synthetic high polymers show that ordered regions (crystallites) are present in which the molecules are arranged in a regular crystal lattice.

On the other hand, in the case of systems which can be resolved by the microscope (such as sections of tissues, fibers, etc.) it is possible to determine not only whether the material is anisotropic but also the position of the optical axes with respect to the morphological axes. If now both optical behavior and structure have been investigated in the case of a given high polymeric substance, so that the position of the optical axis with respect to the direction of the primary valence chains is known, it is only necessary to know the orientation of the optical axis in any object composed of this material in order to be able to state the orientation of the chain molecules. The following examples illustrate this fact. The myelin sheath of nerve, consisting of ether-soluble fatty substances, is negatively birefringent with respect to the long axis of the nerve. It is known that the birefringence of a filament consisting of fatty acid or soap is negative with respect to the axis of the filament, and that the molecular axes are at right angles to this direction. The birefringence of the nerve sheath indicates, therefore, that rod-shaped fatty acid molecules are oriented in the sheath at right angles to the nerve axis (Meyer[72]), a result which has received confirmation from x-ray analysis (Boehm[73]). A further example is provided by the birefringence of the cellulose walls of plant cells. From a study of the birefringence it is possible to determine the position of the cellulose crystallites in the cell wall.

(*b*) *Birefringence Due to Tension and Birefringence Due to Orientation*

If isotropic solid bodies are subjected to anisotropic tension, they become birefringent. It is necessary to distinguish this type of tension birefringence from that which appears on stretching films composed of chain-polymer substances, whether stretching results in plastic deformation only (as in the case of a nitrocellulose film) or in elastic deformation (such as occurs when vulcanized rubber is stretched). The cause of this birefringence is the orientation of anisotropic molecules parallel to the direction of stretching. This may take place in two ways: (*1*) the chains may be associated (in the unstretched condition) to form elongated crystalline mi-

[72] K. H Meyer, *Umschau*, **1932**, Heft 20.

[73] G. Boehm, *Kolloid-Z.*, **62**, 1 (1933). More recent works: F. O. Schmitt, G. L. Clark, and J. N. Mrgudich, *Science*, **80**, 567 (1934); F. O. Schmitt, R. S. Bear, and G. L. Clark, *ibid.*, **92**, 44 (1935); *J. Radiol. Iowa City*, **25**, 131 (1935).

celles which, during stretching, become oriented parallel to the direction of stretching; or (*2*) irregularly twisted chains may be straightened out by stretching. The parallel orientation of the chains in films treated in this way has been confirmed in many cases by x-ray analysis.

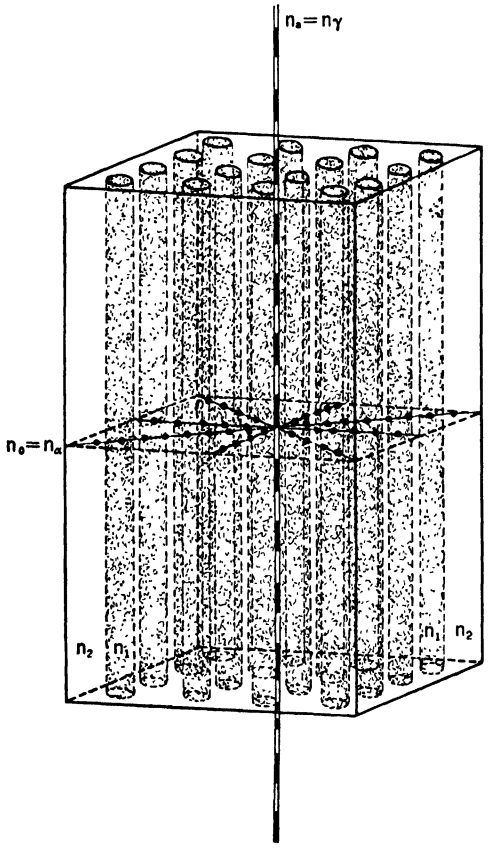


Fig. 38. Diagrammatic representation of the structure of a composite rodlet body (Ambronn and Frey). See page 90.

Birefringence of chain polymers provides, therefore, a measure of the degree of orientation of long molecules parallel to a particular direction. Nägeli attributed the birefringence of biological objects to the presence of "crystalline" micelles, that is, of submicroscopic crystallites, and many biologists have assumed the presence of such crystalline micelles in all cases of marked birefringence. This is unjustifiable. The parallel orientation of

identical molecules may lead to the formation of regular crystal lattices, detectable by x-ray analysis, to which the term crystallite may be applied, but such orientation does not necessarily lead to the formation of crystallites. From the birefringence of a tissue built up from chain molecules, it is possible, therefore, to conclude only that the arrangement of the molecules is anisotropic.

Measurements of birefringence may afford a means of following the behavior of chain molecules during stretching and relaxation, as well as in

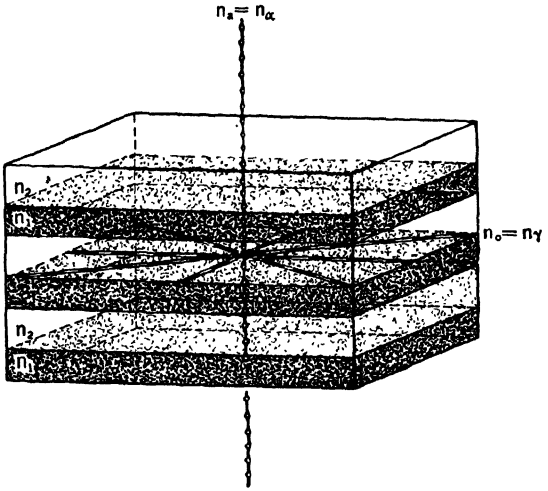


Fig. 39. Diagrammatic representation of the structure of a composite platelet body (Ambronn and Frey). See page 90.

biological processes. The fact that the birefringence of muscle fibrils diminishes during contraction leads immediately to the conclusion that the parallel orientation of the protein chains which make up the fibrils diminishes during contraction.[74]

The occurrence of birefringence as a result of the parallel orientation of chain molecules is more easily observed than the variation of the intensities in x-ray analysis. Thus, for example, stretched polyvinyl acetate may, during relaxation, reach a state of disorientation such that the x-ray diagram shows no orientation of the chains, while the slight birefringence is still measurable (Misch and Picken[75]). In this case, therefore, the study of birefringence has advantages over the method of x-ray analysis. There

[74] K. H. Meyer, *Biochem. Z.*, **214**, 253 (1929).

[75] L. Misch and L. E. R. Picken, *Z. physik. Chem.*, **B36**, 398 (1937).

can be no doubt that a great deal of the material already collected in studies on the birefringence of natural objects would permit conclusions to be drawn about the arrangement of the molecules in the objects studied.

(*c*) *Structural Birefringence*; *Rodlet and Platelet Birefringence*

Birefringence also occurs if isotropic, parallel-oriented rodlets or platelets are embedded in an isotropic substance (*cf.* Figs. 38 and 39). The diameter and spacing of the rodlets and platelets must be small as compared with the wave length of light, large in comparison with atomic dimensions. A "composite body" (*Mischkörper*) consisting of isotropic rodlets is positively and uniaxially birefringent, the optical axis lying parallel to the axis of the rodlets. A "composite platelet body" is negatively and uniaxially birefringent, the optical axis being at right angles to the plane of the platelets. If, in addition, the rodlets or platelets possess intrinsic birefringence (*Eigendoppelbrechung*), the two types of birefringence are superimposed.

In order to determine whether objects which fulfil the conditions for rodlet or platelet birefringence also possess intrinsic birefringence, it is necessary to study the birefringence in media of different refractive indices. The medium in which the rodlets or platelets are immersed (in general, air or water) is replaced by liquids of various refractive indices. If rodlet birefringence is present, the birefringence of the object will be minimal at one particular value of the refractive index of the surrounding liquid. If the minimum is zero, that is, if the birefringence disappears completely, the rodlets (or platelets) are isotropic and possess (at the minimum) the same refractive index as the surrounding medium. If, on the other hand, the minimum differs from zero, the particles are individually birefringent, that is, they display intrinsic birefringence. In the case of particles composed of threadlike molecules the intrinsic birefringence is due to the oriented arrangement of the chains.

It is, of course, necessary to use as imbibition fluids such substances as do not penetrate into the micelles in a "permutoid" (intramicellar) fashion. As a result of the permutoid adsorption of a foreign substance, the optical properties of the material of which the micelles are composed will change. A characteristic example is provided by the change in intrinsic birefringence of incompletely nitrated nitrocellulose. In oriented preparations, nitrocellulose shows positive uniaxial birefringence; as a result of the addition of camphor the birefringence becomes negative.[76] This is because the cam-

[76] J. R. Katz, J. C. Derksen, C. A. Kramers, K. Hess, and C. Trogus, *Z. physik. Chem.*, **A151**, 145 (1930).

phor penetrates between the chains and forms, presumably, a loose molecular compound with nitrocellulose. This complex possesses negative intrinsic birefringence. Even in those cases in which it is not possible to speak of the formation of a molecular compound but only of permutoid penetration, the residual fields of the atoms of the primary valence chains may nevertheless be so distorted by the presence of the foreign molecules that the intrinsic birefringence is modified and may even change its sign.

Liquids whose molecules undergo oriented adsorption on the surfaces of the crystallites (for example, alcohol on nitrocellulose) may also contribute to the birefringence. This is the phenomenon of adsorption birefringence, described by Vermaas.[77] Such liquids are unsuitable for imbibition experiments.

The greater refractive index always lies in the direction in which the molecules or their components are most polarizable. The direction of greatest polarizability is known for various monomers and for the structural units of high polymers; in cyclic compounds this direction lies in the plane of the ring. The sign of birefringence in an oriented chain polymer may therefore indicate the position of cyclic units. If, as in cellulose, the intrinsic birefringence is positive and uniaxial, the rings (in this case, glucose rings) lie in the chain axis. If the birefringence is negative and uniaxial, as in the addition compound of nitrocellulose and camphor, or in polystyrene, the plane of the camphor or benzene rings is at right angles to the chain axis. As we shall see in the case of starch, quantitative information regarding the relation between the plane of the rings and the chain axis may be obtained from the value for the intrinsic birefringence.

Natural rod-shaped textures almost always possess intrinsic birefringence. The formation of the rodlets indicates in itself anisotropic growth, and thus the favoring of one direction as compared with the other two. If, therefore, positive unaxial birefringence has been demonstrated in a natural texture (for example, a fiber), one may be certain that at least a part of the optical behavior is due to intrinsic birefringence and indicates a definite arrangement of the molecules and (if these are primary valence chains) an orientation of these parallel to the fiber axis.

In stretched films of high polymeric organic substances which are free from air, solvents, or swelling agents, rodlet birefringence is of relatively slight importance. It is true that, on stretching, a composite body consisting of crystallites and noncrystalline material may be formed (as in the case of rubber); but the differences in refractive index between crystal-

[77] D. Vermaas, *Z. physik. Chem.*, **B52**, 131 (1942).

lized and noncrystallized components do not amount to more than a few per cent in the case of organic compounds, so that the resulting rodlet birefringence of such composite bodies is in any case very small.

3. Investigation by Means of Polarized Infrared Radiation

Absorption of infrared radiation is due to quantized energy uptake by pairs of atoms which are linked together by attractive forces such as homopolar or ionic bonds. Most of the absorption bonds of organic compounds can be attributed to either stretching oscillations or to bending or rotational vibrations of homopolar bonds. Stretching frequencies are only absorbed by the corresponding linkage if the $\boldsymbol{E}$ vector of the incident radiation is parallel to the direction of the bond. Solid substances in which certain bonds are oriented exhibit, therefore, the phenomena of infrared dichroism and of selective absorption of polarized infrared radiation. In studying the absorption of fibers it has been possible to determine the orientation of certain linkages such as N—H bonds; atomic configurations which had been proposed but were in disagreement with these results could thus be eliminated.[78]

[78] A. Elliot, E. J. Ambrose, and R. B. Temple, *Nature*, **163**, 567 (1949).

B. INORGANIC HIGH POLYMERS

I. INORGANIC CHAIN POLYMERS

The view that certain inorganic substances should be included among the high polymers is fairly recent, and first arose as a result of intensive study of the nature of linkage. It will be seen that certain striking properties encountered in organic high polymers (such as the formation of fibers, and rubberlike elasticity) also occur in inorganic materials, and are to be assigned to the same causes in these as in organic polymers.

1. Polymerized Sulfur (Elastic Sulfur)

—S—S—S—S—S—S—S—S—

(*a*) *Unit Cell and Three-Dimensional Model*

If rhombic sulfur is melted and heated above the melting point, the previously fluid melt becomes brown and extremely viscous at 180°C. At 187°C. the viscosity of pure sulfur reaches its maximum value of 932 c.g.s. units[1]; on slow cooling the viscosity once more diminishes, while if the melt is suddenly cooled, a rubberlike, elastic material, insoluble in carbon disulfide, is obtained. Insolubility and high viscosity indicate that the molecular weight has increased; it is therefore considerably greater than that of rhombic sulfur, the molecules of which are ring-shaped and composed of eight sulfur atoms. If threads of elastic sulfur are drawn out and cooled, they harden and give rise to a typical fiber diagram[2,3] (see figure 1). From all these facts Meyer and Go[3] concluded that elastic sulfur and the various forms of insoluble sulfur consist of very long chains of the type: —S—S—S—S—. While the fiber period along this axis can be determined with certainty from the diagram, the results for the two other identity periods and the angle between them are somewhat uncertain, since, in the case of fiber diagrams without higher orientation,

[1] R. F. Bacon and R. Fanelli, *J. Am. Chem. Soc.*, **65**, 639 (1943).

[2] J. J. Trillat and H. Forestier, *Bull. soc. chim.*, **51**, 248 (1932). See also F. Halla and E. Mehl, *Sitzber. Akad. Wiss. Wien, Math. naturw. Klasse*, Meeting on May 15, 1930.

[3] K. H. Meyer and Y. Go, *Helv. Chim. Acta*, **17**, 1081 (1934).

the indexing represents only one possibility. With this reservation, the monoclinic unit cell suggested by Meyer and Go[3] may be put forward:

$$a = 26.4 \pm 0.1 \text{ Å.}$$
$$b = 9.26 \pm 0.05 \text{ Å. (fiber period)}$$
$$c = 12.32 \pm 0.05 \text{ Å.}$$
$$\beta = 79° 15'$$

The volume of the unit cell calculated from these dimensions amounts to 2959 ± 44 Å. From the experimentally determined value for the density of fibrous sulfur, 2.01, the number of sulfur atoms in the unit cell is 112 (that is, 14 × 8). If the number of atoms in the unit cell is taken as 128

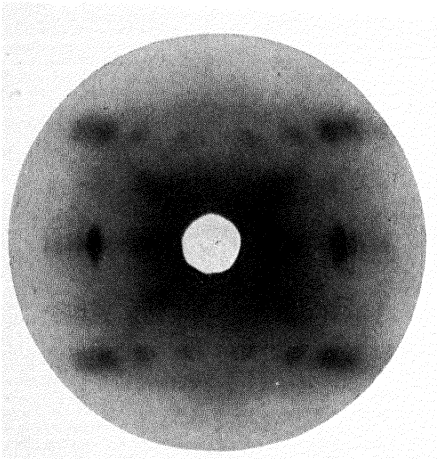


Fig. 1. Fiber diagram of fibrous sulfur (Meyer and Go), Cu, Kα, distance 40 mm.

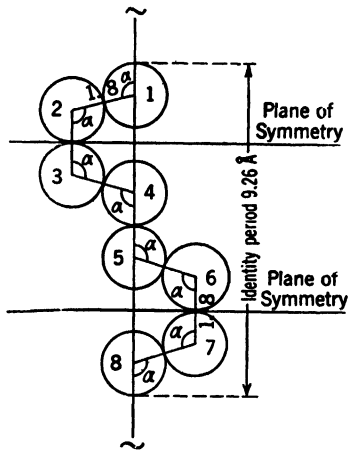


Fig. 2. Probable arrangement of atoms in the molecule of fibrous sulfur.

(that is, 16 × 8) the density of the crystallites should amount to 2.34. Apart from this uncertainty the most probable space group appears to be $P2_1/m$, which contains a set of digonal screw axes, parallel to b, and two reflection planes, $0\frac{1}{4}0$ and $0\frac{3}{4}0$. Consideration of the number of atoms to each chain in the cell and of the space available leads to the chain configuration shown in figure 2 as the most probable configuration. According to Pauling[3a] it is, however, not excluded that seven sulfur atoms disposed in a two-turn spiral form the repeating unit of each chain; this would explain the unusual occurrence of a multiple of seven atoms in the unit cell.

[3a] L. Pauling, *Proc. Nat. Acad. Sci.*, **35**, 495 (1948).

(*b*) *Mechanical Properties of Polymerized Sulfur*

Threads of freshly prepared amorphous sulfur may be stretched to about ten times their original length; the modulus of elasticity is small, as in the case of rubber. Stretched and cooled threads solidify and crystallize, and in this condition their tensile strength is remarkably high (Trillat and Forestier[21]). According to Sakurada and Erbring[4] the tensile strength may amount to 11.2 kg. per sq. mm. This result is in agreement with the view that strong linkages are present in the direction of the fiber axis. From the dissociation of sulfur vapor at equilibrium, the energy required for the rupture of an S—S linkage is calculated to be 15,000 to 17,000 calories; if similar linkages are present in elastic sulfur, this high value for the energy of the linkage would be compatible with the high tensile strength in the direction of the fiber axis.

If warmed to 70°C., the stretched threads contract and regain their initial rubberlike character. Similar behavior is shown by unvulcanized rubber. The theory of rubberlike bodies will be discussed later (Chapter C, sect. VI 2, and Chapter I, sect. I).

(*c*) *Formation and Breakdown of Polymerized Sulfur*

Polymerized sulfur is formed by heating normal sulfur, S_8, and at lower temperatures it gradually changes to normal sulfur again. Polymerized sulfur is, therefore, the form possessing the greater energy and the greater entropy. In spite of their fixation within the chain, and in spite of the considerable diminution in volume during polymerization, the atoms must possess greater possibilities of movement in this form than in unpolymerized sulfur; this follows from the relationship between entropy and thermodynamic probability. The chains must, accordingly, be flexible, while the atoms of the eight-membered ring (present, according to Warren,[5] in rhombic sulfur) are rigidly bound to one another by the ring structure. We shall meet the phenomenon of reversible transformation of cyclic compounds into high polymeric chains again when we discuss polyphosphonitrilic chloride and organic lactones and lactims (see Chap. D, sect. VI).

If small quantities of halogens are added, the viscosity of the melt is reduced. Meyer and Go suggest this is due to chain rupture with the formation of terminal —S—S—Cl groups; according to Bacon and Fanelli, hydrogen persulfides have a similar action: chains are formed with the terminal group —S—S—H.

[4] K. Sakurada and H. Erbring, *Kolloid-Z.*, **72**, 129 (1935).

[5] B. E. Warren and J. D. Burwell, *J. Chem. Phys.*, **3**, 6 (1935).

The molecular weight can be estimated from the effect of halogens on the viscosity. Even as little as 0.02% of iodine (that is, one molecule of iodine to 40,000 atoms of sulfur) reduces the viscosity to a tenth of its normal value, according to Rotinjanz.[6] From the reduction in viscosity one may conclude that each chain breaks into several fragments ($S_{40000}I_2$) and, hence, that the original chain must have contained a multiple of 40,000 sulfur atoms; that is, the molecular weight must have been more than one and a half million. The figure of 12,000 sulfur atoms to a chain, suggested by Kauzmann and Eyring[7] on the basis of a comparison with the viscosity of organic chain polymers, is certainly too low.

Concerning the mechanism of polymerization it seems reasonable to suppose that the reaction proceeds as a chain reaction. Individual rings are split open by heating, and these activated S_8 molecules catalyze the splitting of other rings. A number of simple experiments, such as the initiation of polymerization in a superheated melt of unpolymerized sulfur by nuclei of the polymerizing product, lend support to this view (Meyer[8]).

The conversion of polymeric sulfur on cooling into S_8 is most rapid with pure sulfur. It is accelerated by light and slowed down or even stopped by traces of halogen. The chain degradation probably starts at the ends of the chains where free sulfur radicals —S• are present; these ends act as nuclei. The chains are broken and new nuclei are formed by absorption of light; halogen saturates the ends to —SCl and destroys the nuclei.

(*d*) *Amorphous Sulfur*: S_μ

If sulfur is liberated from its compounds in the cold, as for example in the decomposition of hydrogen persulfide, thiosulfate, or sulfur monochloride, or by the action of sulfur dioxide on hydrogen sulfide, the insoluble form is the first to be formed. The atoms set free do not immediately associate to form rings but form chains. According to the initial material the ends of the chains consist of —SH, —SCl, or —SO_3H residues. Depending on previous conditions, the chains may be of various lengths, so that here as with other polymers, a great variety of products may be formed. It has been known for a long time that amorphous, finely divided, or colloidal sulfur, prepared in various ways, exhibits great variation in its properties. A polymer of relatively low molecular weight is S_π, formed by the prolonged action of sulfur on sulfur monochloride, *e.g.*:

$$S_2Cl_2 + 6\,S \rightarrow Cl—S—S—S—S—S—S—S—S—Cl$$

[6] L. Rotinjanz, *Z. physik. Chem.*, **62**, 609 (1908).

[7] W. J. Kauzmann and H. Eyring, *J. Am. Chem. Soc.*, **62**, 3113 (1940).

[8] K. H. Meyer, *Trans. Faraday Soc.*, **32**, 148 (1936).

2. Polymerized Selenium

—Se—Se—Se—Se—Se—Se—

Selenium crystallizes in the hexagonal system, $a = 4.34$, $c = 4.95$ Å., three atoms in the unit cell. According to Bradley,[9] the selenium atoms in the lattice of grey selenium are distributed in the following way: at a distance of 2.32 Å. from each atom two other atoms occur, and at a distance of 3.41 Å. four other atoms. The former are arranged in spiral fashion around the trigonal axis along *c*. Since it is possible to deduce, from differences in interatomic distances, differences in the strength of the bonds uniting the atoms, it follows that chains of selenium atoms, in which the atoms are united more firmly with each other than with atoms in neighboring chains, extend through the crystal. The length of these chains is the same as the length of the crystals, and selenium may accordingly be classed as a chain polymer.

Molten grey selenium (melting point 217°C.) is readily supercooled; below 70°C. the supercooled product is solid and rather brittle, while between 70° and 73°C. it is elastic, like rubber (Meyer and Sievers[10]). At this temperature it may be drawn out into threads (most easily under water) which contract when the tension is removed. If stretched threads are cooled, they solidify and, on warming, such solidified fibers contract once more.

It is probable, *a priori*, that during the melting of the chain lattice, the residual valences (spacing 3.4 Å.) are weakened first, while the more solid linkages within the chains remain unaffected. This supposition is confirmed by the characteristic rubberlike behavior of the supercooled melt (*cf.* Chapter C, sect. VI 2, on the state of aggregation of rubberlike bodies).

It is as yet impossible to reach any decision about the length of the chains in the melt. It may well be that the mean chain length is dependent on the temperature. The density of vitreous, amorphous selenium is 4.28, that of crystalline selenium 4.79. The very high viscosity of molten selenium has been measured by Dobinski and Wesolowski.[11] According to Jenckel[12] the viscosity of vitreous selenium at 30°C. is 10^{13} c.g.s. units.

In stretched threads of selenium, oriented crystallites of grey selenium soon form, the chain axis lying in the direction of stretching, as has been shown from the x-ray diagrams of Prins and Dekeyser.[13] At 73°C. the

[9] A. J. Bradley, *Phil. Mag.*, **48**, 477 (1924).

[10] K. H. Meyer and J. F. Sievers, *Naturwissenschaften*, **25**, 171 (1937).

[11] S. Dobinski and J. Wesolowski, *Chem. Zentr.*, **1937, II**, 2655.

[12] E. Jenckel, *Kolloid-Z.*, **84**, 266 (1938).

[13] J. A. Prins and W. Dekeyser, *Physica*, **9**, 900 (1937).

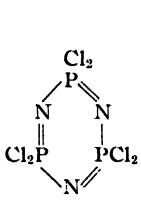
melt gradually becomes covered by a very thin crystalline surface film. Complete crystallization of the melt only takes place rapidly at temperatures in the region of the melting point (217°C.) of the crystallized form. The rate of crystallization is therefore much increased by the orientation of the chains in the same direction, whether by stretching or by surface tension. The same phenomenon is encountered in rubber, and in silk fibroin and other proteins.

3. Polyphosphonitrilic Chloride

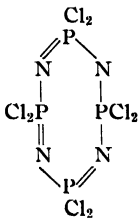
$$-PCl_2{=}N-\,-PCl_2{=}N-\,PCl_2{=}N-$$

(*a*) *Preparation, Properties, and Constitution*

By the action of phosphorus pentachloride on ammonium chloride, a mixture of compounds $(PNCl_2)_n$, is formed, from which the ring compounds:



and

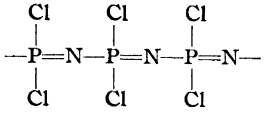


(I)

are easily isolated. Their structure has been determined by x-ray analysis (Jaeger[14]). If the mixture or one of the pure compounds is heated for several hours at 300°C., the liquid melt becomes more viscous and diminishes in volume, as Stokes showed.[15] According to Renaud,[16] the mean molecular weight of the mixture increases during the process. Finally a rubberlike mass results. This substance is deceptively similar in its mechanical properties to rubber, and is, therefore, often referred to as inorganic rubber. If the preparation has been properly carried out, the substance contains no constituents soluble in benzene (lower polymers, tri- and tetramers); it swells in benzene without passing into solution (Schenck and Römer[17]). That the substance is a high polymer has been realized by all these workers. The presence of primary valence chains was demonstrated

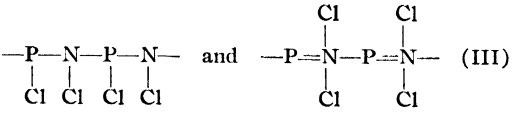
[14] F. M. Jaeger and J. Beintema, *Proc. Acad. Sci. Amsterdam*, **35**, 756 (1932).
[15] H. N. Stokes, *J. Am. Chem. Soc.*, **17**, 275 (1895); **18**, 629 (1896); **19**, 782 (1897).
[16] P. Renaud, *Compt. rend.*, **194**, 2054 (1932).
[17] R. Schenck and G. Römer, *Ber.*, **57**, 1343 (1924)

by the study of mechanical properties and by x-ray analysis. The latter showed, moreover, that the two Cl atoms are attached to the phosphorus atoms, so that the formula must be:



(II)

which is chemically much more satisfactory than the chain formulas:



(III)

(b) X-Ray Analysis and Three-Dimensional Model

If suitable fragments of polyphosphonitrilic chloride are stretched, they become opalescent and yield an x-ray pattern which disappears on contrac-

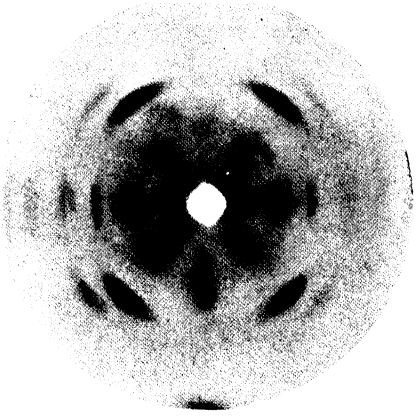


Fig. 3. Fiber diagram of polyphosphonitrilic chloride. Preparation showing higher orientation, oscillating about 020.

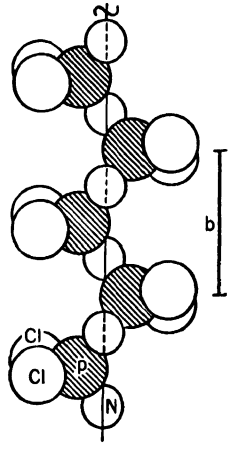


Fig. 4. Molecular model of crystalline polyphosphonitrilic chloride. b = identity period.

tion (Meyer, Lotmar, and Pankow[18]). The amorphous unstretched material gives a diagram with three indistinct rings corresponding to the spacings 13.5 Å., 6.15 Å. (the strongest ring), and 3.35 Å.

[18] K. H. Meyer, W. Lotmar, and G. W. Pankow, *Helv. Chim. Acta*, **19**, 930 (1936).

The evaluation of diagrams from higher orientated preparations (one of which is shown in Figure 3) gave a rhombic unit cell with the dimensions:

$$a = 11.07 \pm 0.1 \text{ Å.}$$
$$b = 4.92 \pm 0.05 \text{ Å.}$$
$$c = 17.72 \pm 0.1 \text{ Å.}$$

with eight $PNCl_2$ residues in the cell and a calculated density of 2.21. The density of one sample was found to be 1.91, that of another 1.70 (both in the amorphous state). The density of the first sample in the crystalline condition was found to be 1.98 at 0°C. A consideration of the absent reflections led to the space group $Pna2_1$. Parallel to the fiber axis, three sets of digonal screw axes and two sets of glide reflection planes (at right angles to each other and between these axes) traverse the unit cell. Considerations of the shape of the chains (derived from interatomic distances and valence angles in the ring-shaped trimers) lead to the result shown in figure 4. In order to make a quantitative determination of the position of the atoms, it would be necessary to have more accurate data on the intensities of the various reflections. An exact determination of the structure is certainly possible in the present state of our technique.

(*c*) *Mechanical Properties*

In its mechanical properties, polyphosphonitrilic chloride bears an extraordinary resemblance to rubber, not only in general character but in detailed behavior. It affords so typical an example of a rubberlike polymer that it will be discussed in some detail without, however, anticipating the general treatment of high polymers showing rubberlike elasticity, which will be given later.

Different preparations may show considerable quantitative differences in behavior. If polymerization is incomplete, the product contains lower cyclic polymers which may be removed by benzene. Such products have been examined by Renaud and by de Ficquelmont.[19] But even when conversion is complete, differences may be encountered. Two products will be described: (*1*) obtained by heating the pure trimer; and (*2*) formed from a mixture of trimers, tetramers, and liquid products, by heating for many hours at 300°C. The stress–strain curve of both preparations is shown in figure 5. The points determining the curve were obtained very slowly, so that the values for the tension are practically equilibrium values. It is apparent that (*1*) is much less extensible than (*2*). If the experiment

[19] A. M. de Ficquelmont, *Compt. rend.*, **202**, 423 (1936).

is performed more rapidly, the difference is less. The difference is due mainly, therefore, to the greater relaxation of product (*2*). This product also swells much more markedly in benzene. Product (*2*) doubtless consists of shorter chains than (*1*), since relaxation is due to the presence of shorter chains.

At ordinary temperatures (*2*) gradually becomes hard and shows the same reflections in the powder diagram as the stretched product does. The shorter chains in the unstretched amorphous state associate to form crystallites with greater ease than the longer chains of (*1*), and it is therefore understandable that (*2*) crystallizes relatively rapidly at room temperature while

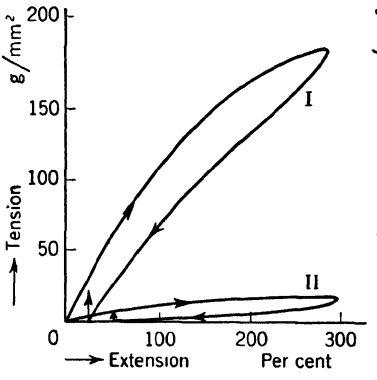


Fig. 5. Stress–strain curves for polyphosphonitrilic chloride.

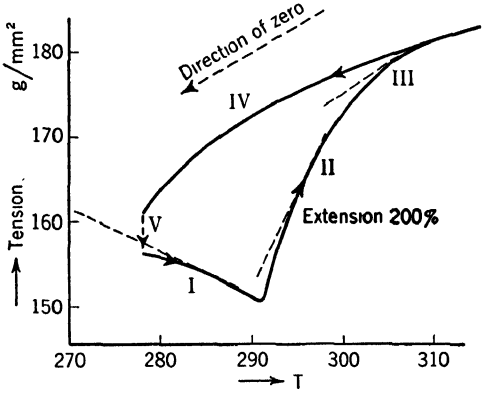


Fig. 6. Hysteresis of polyphosphonitrilic chloride (tension–temperature diagram).

(*1*) remains amorphous for weeks. The slowness of crystallization is also shown by the hysteresis loop which is obtained if the product, stretched at constant length, is warmed and then cooled and the force exerted is plotted as a function of temperature. If a sample is used in which the tension has reached a constant value at the highest temperature (the relaxation has therefore come to a standstill), the course of the change in tension is as shown in figure 6. The descending curve applies to the supercooled preparation, the tension of which is higher as the temperature falls than is that of the partially crystallized sample as the temperature rises. The tension returns very slowly to the equilibrium value as crystallization progresses.

The mechanical strength of the stretched product is markedly anisotropic, the material being strong in the direction of stretching and weaker in the directions at right angles to this. If pulverized in the stretched con-

dition at very low temperatures, the substance breaks up into fibers. This behavior shows the presence of strong linkages (primary valences) in the direction of stretching, as compared with weaker linkages (van der Waals' forces) at right angles to this direction.

4. Polyphosphonitrilic Fluoride

Schmitz-Dumont[20] reports that phosphonitrilic fluorides of low molecular weight yield a rubberlike polymer on heating in the autoclave to 260°; the product decomposes at relatively low temperatures. This substance has not as yet been further investigated.

5. Equilibrium between Polymeric Forms and Forms of Lower Molecular Weight in the Case of Sulfur and Phosphonitrilic Chloride

It is well known that high polymers are generally decomposed at high temperatures; examples are provided by the changes which occur in polystyrene, rubber, cellulose, etc., on heating. In the case of sulfur and polyphosphonitrilic chloride, on the other hand, we find that at lower temperatures, octomeric sulfur, and tri- and tetrameric $PNCl_2$ are the stable forms; the polymeric form is more stable at higher temperatures. From this it is clear that the polymeric forms have a higher entropy; this signifies that the atoms in the primary valence chain molecule have greater possibilities of motion and arrangement than those in the ring molecule. The rings appear to be more rigidly built than the flexible chains, in which rotation about the single linkages is not as limited as it is in the rings. In the case of selenium, the chain polymeric form of grey selenium is stable even at room temperature while the red form, Se_8, with a ring structure, is unstable.

A transition from ring forms to chain polymers is known in many other polymers, for example, in metaphosphates, and also in organic polyesters and polyamides of which commercial use is made in the form of artificial fibers.

6. Other Inorganic Chain Polymers

(*a*) *Polysilene and Polygermanene*

$$-SiH_2-SiH_2-SiH_2-SiH_2-\text{ and }-GeH_2-GeH_2-GeH_2-GeH_2-$$

If silicon is fused with an excess of calcium, monocalcium silicide[21] is obtained. With other proportions of the constituents the product is cal-

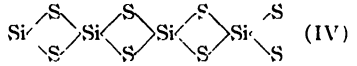
[20] O. Schmitz-Dumont, *Angew. Chem.*, **50**, 415 (1937).

[21] L. Wöhler and F. Müller, *Z. anorg. u. allgem. Chem.*, **120**, 149 (1921).

cium disilicide; this will be discussed later. According to Schwarz[22] the cautious addition of glacial acetic acid to monocalcium silicide results in the formation of a high polymeric compound of the approximate composition SiH_2, which he regards as a chain of SiH_2 groups (polysilene). On heating the compound is decomposed with the formation of lower silanes. The polymeric hydride $(GeH_2)_n$, polygermanene, may be prepared in the same way from calcium germanide.[23]

(*b*) *Silicon Disulfide*

Silicon disulfide is obtained by heating a mixture of finely powdered aluminum sulfide with powdered quartz in a stream of nitrogen at 1200°C. The product takes the form of asbestos-like flexible fibers.[24] The chain structure corresponding to the structural formula:



(IV)

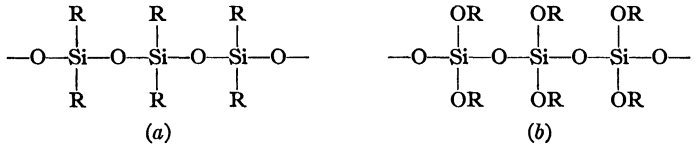
was predicted by Zintl and Loosen[25] and confirmed by x-ray analysis. They found a rhombic unit cell with the dimensions:

a = 5 60 Å.	c = 9.55 Å.
b = 5.53 Å. (fiber axis)	Observed density = 2.02

the unit cell contains 4 silicon and 8 sulfur atoms. The space group is probably $I_b^2\frac{2}{a}\frac{2}{m}$. Each silicon atom is surrounded by 4 sulfur atoms at a distance of 2.14 Å. The latter form a somewhat distorted tetrahedron, the length of the greatest side of which, S—S, is 3.62 Å. Each sulfur atom is shared by two silicon atoms which succeed one another at a distance of $b/2$ in the direction of the fiber axis. These primary valence chains traverse the unit cell parallel to the fiber axis. The smallest distance from a silicon atom to a sulfur atom of a neighboring chain is 4.18 Å. and the smallest S—S spacing between two chains is 3.75 Å. The primary valence chains are united with each other by van der Waal's forces. The fibrous structure, the tendency to split along the fiber axis, and the high tensile strength (to which the authors draw particular attention) are all compatible with the picture of three-dimensional structure given here. Nothing is known of the properties of molten SiS_2 which may, perhaps, possess elastic properties. No solvents for this compound have as yet been discovered.

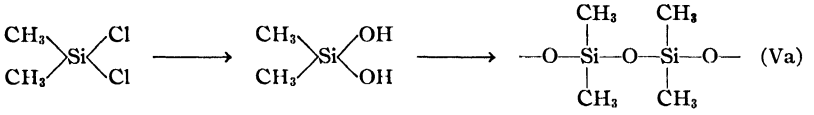
[22] R. Schwarz and P. Heinrich, *Z. anorg. u. allgem. Chem.*, **221**, 277 (1935).
[23] P. Royen and R. Schwarz, *Z. anorg. u. allgem. Chem.*, **215**, 295 (1933).
[24] E. Tiede and M. Thimann, *Ber.*, **59**, 1703 (1926).
[25] E. Zintl and K. Loosen, *Z. physik. Chem.*, **A174**, 301 (1935).

(c) *Polymeric Alkyl Silicon Oxides and Polymeric Esters of Silicic Acid*



(a) (b) (V)

Kipping[25a] was the first to recognize that dialkyl silicones are polymeric compounds. They are formed by saponifying alkyl silicochlorides such as dimethyldichlorosilane. The silandiol obtained in this way condenses to a rubberlike gel on heating to 200°C. and on further heating is converted to a brittle mass:



(Va)

The dialkyl silandiols are unstable no matter how carefully they may be handled. When prepared by saponification with water in the cold, they first yield polymers of lower molecular weight in the form of more or less viscous oils; these give rise to longer chains on warming. This explains the change in the properties of the product on heating. Starting from the CH_3SiCl_3, or from mixtures of this with $(CH_3)_2SiCl_2$, one obtains (on heating the products of hydrolysis) infusible masses, which withstand temperatures of 500° and which must be regarded as net polymers. The corresponding ethyl derivatives, investigated by Alfrey, Honn, and Mark,[26] behave in a very similar way. Other derivatives have been prepared by Hyde.[27]

Stable chain polymers of definite length and definite physical properties, i.e., oils of low and high viscosity and elastomers may be obtained by adding small but accurately measured amounts of trimethyl chlorosilane to dimethyl dichlorosilane. The trimethylsilanol molecules which are formed by hydrolysis constitute the ends of the chain molecules.

Diphenylsilandiol, $(C_6H_5)_2Si(OH)_2$, can be isolated in a pure condition. Above the melting point it polymerizes slowly with loss of water to form a brittle glass. Both the velocity of polymerization and the physical properties of the finished polysilicone can be influenced by suitable admixture of alkyl and aryl groups.

[25a] F. S. Kipping, *J. Chem. Soc.* (*London*), **91**, 218 (1907); **107**, 459 (1915).
[26] T. Alfrey, Jr., F. J. Honn, and H. Mark, *J. Polymer Sci.*, **1**, 102 (1946).
[27] J. F. Hyde and R. C. DeLong, *J. Am. Chem. Soc.*, **63**, 1194 (1941).

Because of their heat resistance the polymeric silicones are attracting increased interest in industry. The products made from polymeric dialkyl silicones include highly hydrophobic varnishes, resins, and silicone rubber. By making use of silicone insulation it has been possible to raise the working temperature of electric motors. Silicone rubbers have exceptional heat resistance and are flexible over a wide range of temperature.

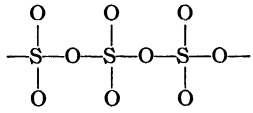
A mixture of methyl chlorosilanes is used to form a water-repelling surface coating on textile fibers,[28] on steatite, or on other insulating materials. The surface is exposed to the vapor of the mixture which reacts rapidly with moisture adsorbed on the surface. Hydrochloric acid is split off, and a thin, water-repelling film is formed. The surface resistance of the insulating material is thus increased considerably.

Polymeric esters of silicic acid are formed by partial hydrolysis of monomeric tetraalkyl silicate, for example, by cautious addition of a little water to solutions of tetramethyl or tetraethyl silicate in organic solvents. The viscous solutions obtained in this way dry in air to form clear varnishes and are of considerable commercial interest, as color-bonding agents in varnishes, in the preservation of stone surfaces, and in the preparation of heat-resistant coatings.

According to Konrad, Bächle, and Signer,[29] the viscous solutions contain polymers with two to ten silicon atoms, while on addition of more water gelatinous or solid net polymers are formed.

(*d*) *Asbestos-like Sulfur Trioxide*

It is well known that sulfur trioxide may occur in two asbestos-like modifications. From considerations of the possible nature of the linkage between O and S, Hägg[30] has suggested the chain formula:



This assumption is supported (at least in the case of the asbestos-like form with the low melting point, 31.5°C.) by the study of the Raman spectrum (Gerding and Moerman[31]). These investigators made the interesting observation[32] that sulfur trioxide, dissolved in sulfur dioxide, polymerizes; a

[28] E. G. Rochow, *Introduction to the Chemistry of the Silicones.* Wiley, New York, 1946.
[29] E. Konrad, O. Bächle, and R. Signer, *Ann.*, **474,** 276 (1929).
[30] G. Hägg, *Z. physik. Chem.*, **18,** 206 (1932).
[31] H. Gerding and N. F. Moerman, *ibid.*, **B35,** 216 (1937).
[32] H. Gerding, *Naturwissenschaften*, **25,** 251 (1937).

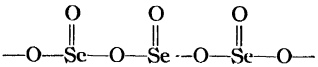
firm and elastically extensible surface skin is formed. If this film is torn, it breaks up into fibrous fragments consisting of the high polymeric modification of higher melting point. It seems probable that the skin consists of amorphous and, perhaps, swollen polymeric sulfur trioxide, which may be caused to crystallize as a result of tension, as is the case with rubber.

(*e*) *Sulfur Monoxide*

It seems probable that an unstable, polymeric sulfur monoxide, —S—O—S—O—S—O—, is formed as a readily decomposed intermediate product in the condensation of gaseous sulfur monoxide. Sulfur is precipitated out when the compound decomposes, and it has not been possible as yet to prepare this substance in a pure condition.[33]

(*f*) *Selenium Dioxide*

According to McCullough,[34] crystalline selenium dioxide has a chain lattice in which the chains have the following structure:

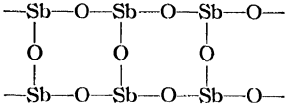


(VI)

The distance from a selenium atom to a bridging oxygen atom is 1.78 Å.; while that from a selenium atom to one of the "oxide" oxygens is 1.73 Å. The substance sublimes at atmospheric pressure without melting. In the gaseous condition the distance between selenium and oxygen atoms is reduced to 1.61 Å., showing that the oxygen atoms must be bound in a different way.

(*g*) *Antimony Trioxide*

According to Buerger and Hendricks,[35] chains of the following constitution are present in valentinite, a modification of antimony trioxide:



(VII)

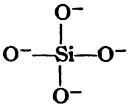
7. Chain Polymers with Extended Anions

In the chain polymers hitherto considered, neutral atoms are united in one direction by homopolar primary valences to form chains which associate as a result of van der Waal's forces. In contrast to these, there is another group of compounds in which chains, consisting of anions united by primary

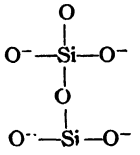
[33] P. W. Schenk, *Z. anorg. u. allgem. Chem.*, **248**, 297 (1941).
[34] J. D. McCullough, *J. Am. Chem. Soc.*, **59**, 789 (1937).
[35] M. J. Buerger and S. B. Hendricks, *Z. Krist.*, **98**, 1 (1937).

valences, are held together by the electrostatic forces exerted by cations lying between them. To this group belong the silicates of the pyroxene and amphibole types.[36] Before discussing these, a few introductory remarks about the structure of silicates will be made.

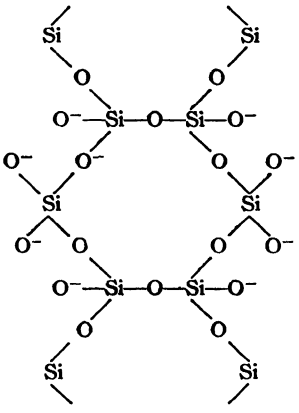
In all silicates, and in silicon dioxide, the silicon atoms are each surrounded by four oxygen atoms placed at the corner of a tetrahedron. The oxygen atoms may be united either through a second silicon atom, forming a bridge Si—O—Si, or through a metallic ion (by means of a nonhomopolar valence). We have therefore two extreme cases: that of silicon dioxide in which only Si—O—Si linkages occur, and that of the orthosilicates in which quadrivalent SiO_4 anions are associated with an equivalent number of metallic cations. Between these forms—that is, between the ideal three-dimensional network and the quadrivalent anion—are the silicates consisting of chains or possessing two- or three-dimensional net structures, as the following formulas show:



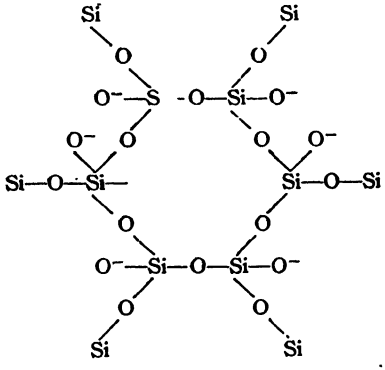
Orthosilicate



Pyroxene chain



Amphibole chain



Net-structure

[36] Here and subsequently the silicates will only be considered in so far as this appears justified from the standpoint of a general treatment of high polymers. Comprehensive accounts are given by W. H. and W. L. Bragg, *The Crystalline State*, Bell, London, 1933; F. Machatschki, *Naturwissenschaften*, **26**, 67 (1938); *Strukturbericht* with supplementary volumes (special numbers of *Z. Krist.*), Akadem. Verlagsgesellschaft, Leipzig.

(*a*) *Pyroxene Group*

By systematic x-ray analysis Warren and Bragg[37] obtained the following structure for diopside, which may be taken as an example of a pyroxene, $Ca_nMg_n(SiO_3)_{2n}$. The unit cell is monoclinic and of the following dimensions:

$$a = 9.7 \text{ Å}.$$
$$b = 8.89 \text{ Å}.$$
$$c = 5.24 \text{ Å}.$$
$$\beta = 74° 10'$$

Observed density = 3.20–3.38
Number of structural units in the unit cell = 4.

The unit cell contains eight silicon atoms. The crystal cleaves readily along *c*.

Four oxygen atoms are distributed at the corners of a regular tetrahedron at the center of which lies a silicon atom. Two oxygen atoms of each tetra-

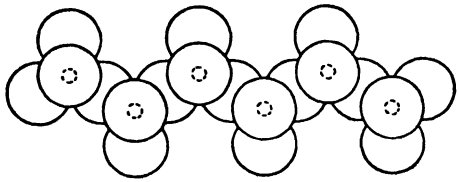


Fig. 7. Diagram of a pyroxene chain (Bragg).

hedron participate in two neighboring tetrahedra so that endless chains are formed, the spatial arrangement of which is shown in figure 7.[38] These chains lie parallel to the *c* axis of the crystal. Each silicon atom forms a complex bivalent anion with oxygen; the formula of the endless linear anion is therefore $(SiO_3^-)_n$. The chains are held together laterally by Mg and Ca ions which neutralize the negative charges of the anionic chains. The high tensile strength in the *c* direction and the ease of splitting along *c* are explained by the fact that the Si—O—Si linkages are stronger than the electrostatic forces of attraction which hold the chains together in directions at right angles to *c*.

(*b*) *Amphibole Group*

In this type, double chains (bands) traverse the crystal, instead of single chains. These arise as a result of the sharing of an oxygen atom from every

[37] B. E. Warren and W. L. Bragg, *Z. Krist.*, **69**, 168 (1928).
[38] From the paper by B. E. Warren, *Ind. Eng. Chem.*, **24**, 420 (1932).

second tetrahedron with a tetrahedron of another chain (Fig. 8). The formula of the extended hexavalent anion is $(Si_4O_{11})_n$. The amphiboles are closely related to the pyroxenes, the b axis being twice as large as in the corresponding pyroxenes, since the chain is double. Tremolite, $(Ca)_{2n}$-$Mg_{5n}(Si_4O_{11})_{2n}(OH)_{2n}$, occurring in the form of single crystals and as a form of asbestos, may be mentioned as an example. The dimensions of the

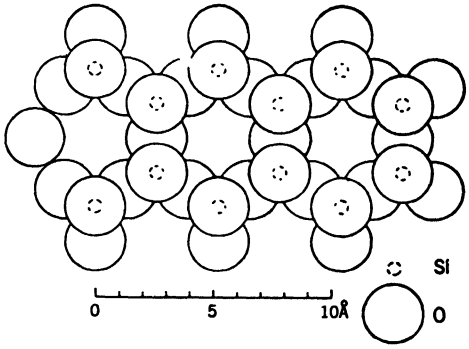


Fig. 8. Amphibole chain (Bragg).

unit cell of tremolite and the corresponding pyroxene, diopside, are as follows.

Tremolite	Diopside
a = 9.78 Å.	a = 9.71 Å.
b = 17.8 Å.	b = 8.89 Å.
c = 5.26 Å.	c = 5.24 Å.
β = 73° 58′	β = 74° 10′
Density = 2.9–3.2	Density = 3.20–3.33

The unit cell of tremolite contains two molecules of the composition indicated above. The silicon atoms form double chains lying along c and held together laterally by Mg and Ca cations. The tendency to fibrous splitting is much more marked here than in the case of pyroxene type; this means that the double chain is particularly strong.

(*c*) *Chrysotile*

A particularly interesting case is provided by chrysotile, $(OH)_6Mg_6$-$(Si_4O_{11})H_2O$, the structure of which was established by Warren and Bragg.[39] Figure 9 shows the position of the atoms in the unit cell of chrysotile pro-

[39] B. E. Warren and W. L. Bragg, *Z. Krist.*, **76**, 201 (1930).

jected on the a,b plane; the fiber axis (c axis) is perpendicular to the plane of the paper. The lateral union of the chains is weaker than in the amphiboles, because, along the sections indicated by arrows, secondary valence

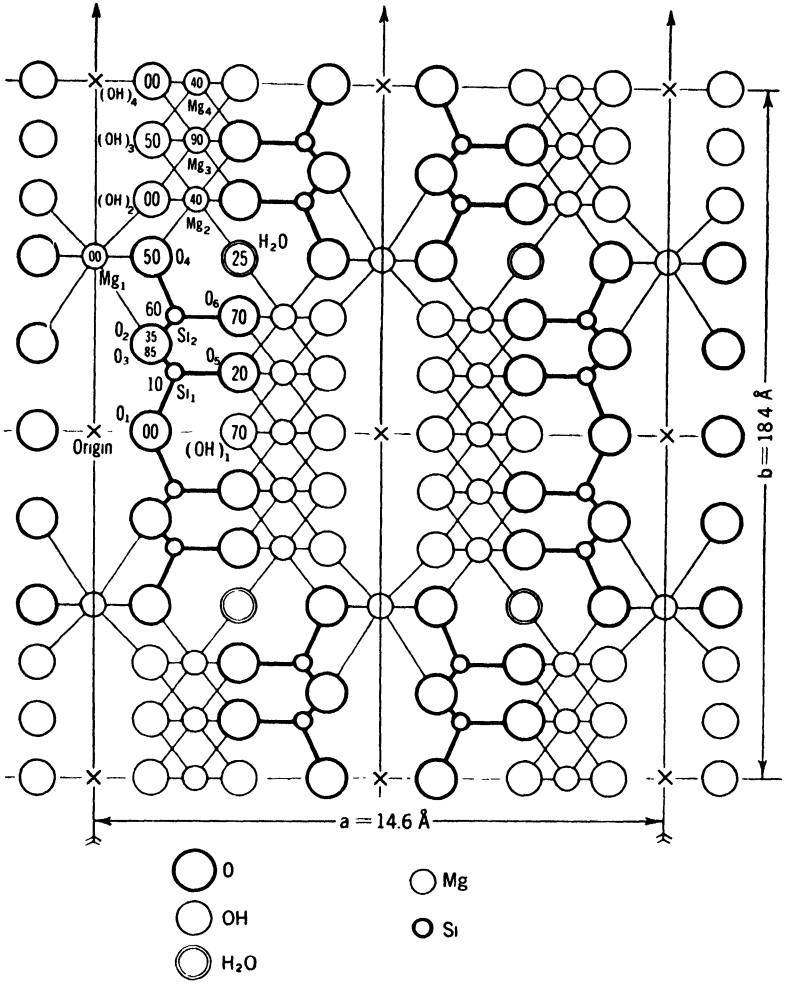


Fig. 9. Arrangement of atoms in the unit cell of chrysotile. Projection on the a,b-plane (Warren and Bragg).

forces alone are acting. The mechanical properties correspond to this three-dimensional model; the substance is fibrous and the fibers can easily be split up into still finer fibrils. The fibers are flexible, since the atoms are

able to slide over each other in the planes across which only secondary valences are acting.

The substance occurs as fibers, the crystallites of which lie parallel to the *c* axis while the *a* and *b* axes are oriented in all directions in the plane at right angles to *c*. It gives, therefore, a typical fiber diagram (Fig. 10) when irradiated at right angles to the fiber axis.

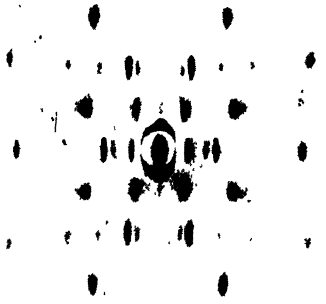


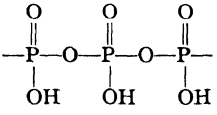
Fig. 10. Fiber diagram of chrysotile.

(*d*) *Alkali Silicates*

Since the presence of primary valence chains in certain crystalline silicates has been demonstrated with certainty, it may be asked whether such chains can also occur in the amorphous and dissolved conditions. The answer cannot as yet be given with certainty. Commercial sodium silicate presumably contains chains of the pyroxene type; the viscosity (among other things) of solutions of water glass indicates the presence of relatively long chains. The striking elastic properties of concentrated solutions of water glass are in agreement with this interpretation.

Silicic acid, liberated from water glass by the addition of acid, also consists at first of soluble chains which are rapidly converted by the elimination of water into extended networks; this leads to gelatinization.

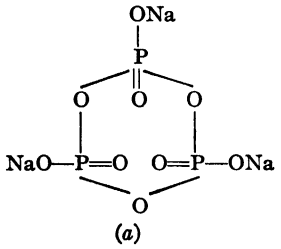
8. Polyphosphoric Acids[40]



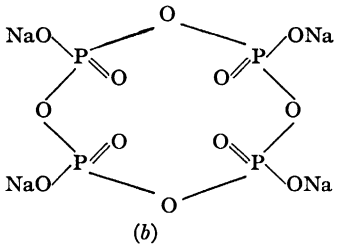
(IX)

[40] For a review see K. Karbe and G. Jander, *Kolloid-Beihefte*, **54**, 1 (1943).

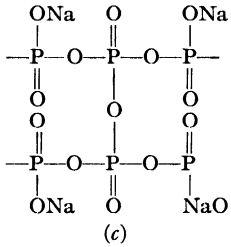
Compounds of composition $MePO_3$, known as metaphosphates, are obtained when primary alkali phosphates are heated; according to the conditions of preparation these may have very various properties. A monomeric metaphosphate does not exist; on the other hand, by dehydration at 600° and slow cooling to 350°, a crystalline substance of low molecular weight, probably trimeric metaphosphate, may be prepared. Following Treadwell[41] we shall regard this as a ring compound (*a*).



(*a*)



(*b*) (IXa)



(*c*)

On heating it is transformed, like sulfur or trimeric phosphonitrilic chloride, into a high polymeric form. This is a salt of polyphosphoric acid; it is vitreous, and forms highly viscous solutions in water.

On prolonged heating of potassium monophosphate to 660°, a high polymeric polyphosphate is obtained, which dissolves in solutions of many salts to give highly viscous solutions. The viscosity is lowest in 0.4 *N* solutions of sodium thiocyanate. The molecular weights of potassium polyphosphates prepared at temperatures from 260° to 665° were found by Malmgren and Lamm,[42] by ultracentrifuge measurements in 0.4 *N* thiocyanate solution, to lie between 150,000 and 2,500,000. So-called tetra- and hexaphosphates have been described, but these undoubtedly belong to the group of homologous polyphosphates, and also insoluble metaphosphates, which swell in water to form rubberlike elastic masses.

Conditions for the preparation of the various metaphosphates have not

[41] W. D. Treadwell and F. Leutwyler, *Helv. Chim. Acta*, **21**, 1450 (1938).

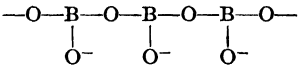
[42] H. Malmgren and O. Lamm, *Z. anorg. Chem.*, **245**, 103 (1940); **252**, 256 (1944).

been well defined, and the information given in the literature is often contradictory. In the insoluble compounds it may be assumed that on heating cross linkage occurs according to the scheme shown in (*c*). It is easy to see how contradictory results may have been obtained: cross linkage will occur if there is a quite small alkali deficit, whereas a slight excess will reduce the molecular weight of the polymers, as the number of oxygen bridges which can be formed is limited. Ring-shaped metaphosphates, particularly the trimeric form (*a*), and perhaps also the tetrameric form (*b*), may occur in the melt in proportions depending on the temperature, the nature of the cation, and the previous treatment (sudden cooling or tempering). It is worth noting that in the swelling high polymeric alkali phosphates, sodium is not easily replaced by other cations and seems in some way to be bound, although potassium can easily be removed by dialysis against hydrochloric acid (Malmgren and Lamm).

All metaphosphates gradually take up water, with subsequent hydrolysis and the formation of monomeric orthophosphates. The kinetics of this reaction are very incompletely known, which, considering how little we know about the metaphosphates, is scarcely remarkable.

Free polyphosphoric acid, obtained by liberation from polyphosphates or directly by the hydration of phosphorus pentoxide, precipitates protein and is used for removing proteins from various preparations. Experiments on the application of true high polymer polyphosphoric acid in organic chemistry seem to be lacking.

9. Polyborates



(X)

The so-called "metaborates" contain salts of polyboric acid. The structure of calcium metaborate is better known; according to Zachariasen and Ziegler[43] this consists of a chain lattice with anions of unlimited length. Free polyboric acid (metaboric acid) is unstable; in water it is broken down at once to fragments of low molecular weight or to monomeric boric acid.

II. INORGANIC NET POLYMERS

1. Graphite and Its Derivatives

(*a*) *Graphite*

The most familiar example of inorganic polymers with two-dimensional nets is provided by graphite, the structure of which has been revealed by

[43] W. H. Zachariasen and G. E. Ziegler, *Z. Krist.*, **83**, 354 (1932).

the work of Debye and Scherrer,[44] Hull and Bernal,[45] and Hassel and Mark.[46] Graphite (density 2.09–2.25) consists of plane sheets of carbon atoms in hexagonal arrays linked by homopolar bonds to form two-dimensional nets (see Fig. 11). The spacing of the C atoms in these nets amounts to 1.44 Å. and is therefore practically the same as the C—C spacing in benzene. The distance between layers amounts to 3.38 Å. The nets themselves are solidly built, whereas the great spacing between the nets corresponds to a less resistant linkage. On this depend the familiar mechanical properties of graphite, its ready cleavage, softness (degree of hardness 1–2), and suitability for use in pencils and as a lubricant. These are

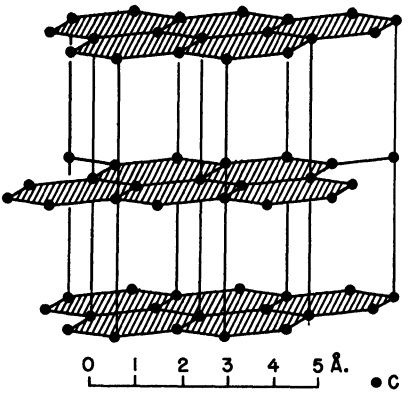


Fig. 11. Crystal structure of graphite (Hassel and Mark).

all explained by the fact that the nets are able to slide over each other and that entire nets can be rubbed off.

While the linkages between the carbon atoms of a particular net are similar to those between the atoms in benzene, the weak linkage between the layers is fundamentally different. The fact that only every other atom has two neighbors in the nearest layer shows that the linkage is not to be regarded as a chemical valence, since in that case one would have to assume that the C atoms were alternately ter- and quinque-valent. The linkages in question appear to be nondirectional; in fact, a sort of metallic linkage seems to exist between the layers, produced by free electrons, the presence of which is indicated by metallic conductivity and by the absorption of

[44] P. Debye and P. Scherrer, *Physik. Z.*, **17**, 277 (1916); **18**, 291 (1917).
[45] A. W. Hull, *Phys. Rev.*, **10**, 661 (1917).
[46] O. Hassel and H. Mark, *Physik. Z.*, **25**, 317 (1924).

light. A rhombohedral modification of graphite has been described by Lipson and Stokes.[47]

Graphite exhibits a series of remarkable reactions which may be understood on the basis of a model consisting of superimposed primary valence nets. It is able to take up various substances between the nets, the structure of the nets remaining unchanged while the spacing between the netlike lamellae increases.

(*b*) *Graphite Oxide* (*Graphitic Acid*)

Of the substances to be mentioned here the so-called graphitic acid, obtained by the treatment of graphite with a mixture of nitric and sulfuric acids in the presence of chlorate, has been known for the longest time. In contrast to graphite the oxidation product is hydrophilic and is not a metal-

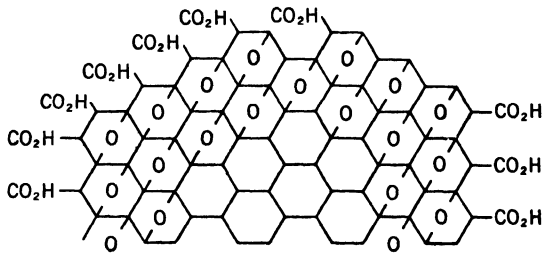


Fig. 12. Provisional formula for graphitic acid (Meyer).

lic conductor. In aqueous alkalis it swells, and a cloudy solution is formed from finely divided preparations. The analytical composition varies; in well-dried preparations, the ratio of oxygen to hydrogen is greater than two, the ratio of oxygen to hydrogen in the carboxyl group, showing that most of the oxygen must be bound to carbon, with only a small proportion in hydroxyl groups. The reaction leading to the formation of graphitic acid was interpreted by Meyer[48] as a permutoid reaction of the graphite network, which remains essentially unchanged during the reaction; the formula suggested is shown in figure 12. Later investigations by Hofmann and König[49] confirmed the main features of this interpretation, suggested a modification, and, above all, widened and deepened our knowledge of the permutoid reactions of net polymers by abundant experimental results.

[47] H. Lipson and A. R. Stokes, *Nature*, **149**, 328 (1942).

[48] K. H. Meyer, *Z. angew. Chem.*, **41**, 944 (1928).

[49] U. Hofmann and E. König, *Z. anorg. u. allgem. Chem.*, **234**, 311 (1937).

The x-ray analysis by Hofmann showed that the lamellae are separated up to 6 Å. as compared with 3.55 Å. in graphite; the C–C distance in the nets is very little increased (by about 0.01 Å.). The oxygen atoms are placed, as in ethylene oxide, between neighboring C atoms and lie above and below the nets, distributed at random (see Fig. 13). Carboxyl groups may

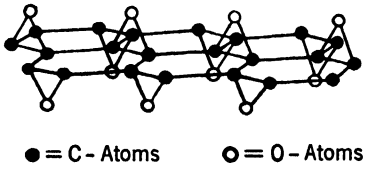


Fig. 13. Graphitic acid (Hofmann).

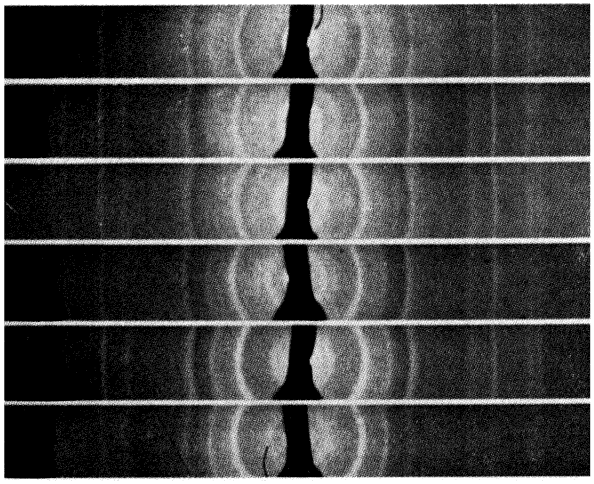


Fig. 14. X-ray diagrams of graphitic oxide of increasing water content; top, free from water (Hofmann).

be present at the edges.[50] Graphite oxide swells in water. In this reaction water is adsorbed between the nets, and the spacing of the nets increases up to 11 Å.; this unidimensional swelling is macroscopically visible in single crystals. The crystal finally breaks down into scales; swelling is reversible. According to Hofmann, about 10% of the water is firmly bound as water of crystallization at a spacing of 2 Å. from the plane of the layers.

[50] U. Hofmann, A. Frenzel, and E. Csalán, *Ann.*, **510**, 1 (1934).

With increasing aqueous vapor tension a further 25% is taken up continuously.

Figure 14 shows the change in the x-ray powder diagram during swelling. It can be seen that the inner ring decreases in diameter during swelling, while the spacing of the planes (001) remains unchanged. In place of water, lower alcohols, nitric acid, acetone, and other polar liquids may penetrate between the layers of graphite oxide (see also Derksen and Katz[51])

On slow heating, graphite oxide is converted into graphite. In this process the oxygen atoms react with the carbon atoms on the edge of the nets as they are split off, oxidizing these to CO_2, so that the nets become smaller. The spacing of the lamellae changes continuously as the oxygen content diminishes.

(*c*) *Other Compounds of Graphite*

According to Ruff,[52] a graphite fluoride $(CF)_x$ exists in which fluorine atoms are united to the unchanged nets like the oxygen atoms in graphite oxide. Another fluoride (C_4F) has been described by Rüdorff.[53]

Graphite also forms compounds resembling alloys with alkali metals.

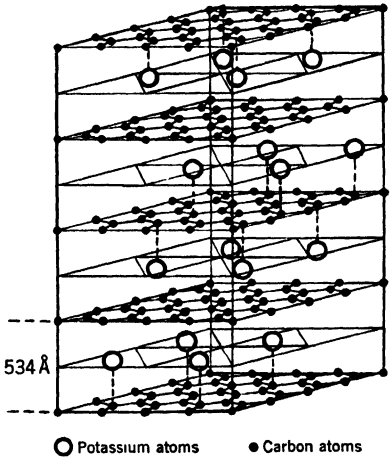


Fig. 15. Potassium graphite (Hofmann and Frenzel).

[51] J. C. Derksen and J. R. Katz, *Rec. trav. chim.*, **53**, 652 (1934).
[52] O. Ruff, *Z. Elektrochem.*, **44**, 333 (1938).
[53] W. and G. Rüdorff, *Chem. Ber.*, **80**, 417 (1947).

These were discovered by Fredenhagen[54] and have been investigated by him as well as by Schleede and Wellmann[55] and by Hofmann and Frenzel.[56] The composition is C_8K, and the alkali atoms are disposed in layers between the nets of carbon atoms (see Fig. 15). The spacing of the graphite nets, 3.4 Å., is increased by the interpolation of K to 5.3 Å., of rubidium to 5.7 Å., and of caesium to 5.7 Å.

Graphite also undergoes change in a mixture of sulfuric acid and potassium nitrate; it becomes brown by transmitted light, blue by reflected light. According to Hofmann and Frenzel, the spacing of the laminae is greater in blue graphite, while the primary valence nets themselves remain unchanged. Rüdorff and Hofmann[57] discovered later that, during this reaction (as also during reaction with perchloric acid, selenic acid, and nitric acid, together with oxidizing reagents), salts of graphite are formed, in which the graphite nets form cations, while HSO_4^-, ClO_4^-, etc., have entered as anions between the graphite laminae. The spacing of the nets is 7.98 Å. in the case of the sulfuric acid compound, 7.94 Å. in the perchloric acid compound, 7.84 Å. in the nitric acid compound, and 8.25 Å. in the selenic acid compound. One HSO_4^- ion and two molecules of adsorbed H_2SO_4 are associated with about 24 carbon atoms. The sulfuric acid molecules and ions can be exchanged reversibly for ions and molecules of other strong inorganic acids. If oxidation is incomplete, intermediate products are obtained in which only every second, third, or fourth interlaminar space is occupied by acid ions and molecules.[58]

As far as one can see, the chemical compounds formed as a result of all these reactions are not very well defined stoichiometrically. The graphite crystal does not react as a solid phase in the sense of the phase rule, and the system graphitic acid–water follows the mass action law of homogeneous reactions, rather than the isotherms of heterogeneous reactions. The lamellae react to a certain extent as if they are able to enter into reaction over their whole surface. We shall meet this behavior again in considering clays and zeolites, as well as in cellulose and its derivatives.

(*d*) *"Amorphous" Carbon*

Soot, charcoal, and coke are usually referred to as "amorphous" carbon, but a continuous series of intermediate forms exists between well-crystal-

[54] K. Fredenhagen and G. Cadenbach, *Z. anorg. u. allgem. Chem.*, **158,** 249 (1926). K. Fredenhagen and H. Suck, *ibid.*, **178,** 353 (1929).

[55] A. Schleede and M. Wellmann, *Z. physik. Chem.*, **B18,** 1 (1932).

[56] U. Hofmann and A. Frenzel, *Kolloid-Z.*, **58,** 9 (1932).

[57] W. Rüdorff and U. Hofmann, *Z. anorg. u. allgem. Chem.*, **238,** 1 (1938).

[58] W. Rüdorff, *Z. physik. Chem.*, **B45,** 42 (1939).

lized, conducting, soft graphite and amorphous, nonconducting carbon. X-ray analysis shows that, as the amorphous condition is approached, the interference spots become broader and finally most of them disappear. The first observation indicates the presence of very small crystallites; the second shows that the lattice is less well developed. The discussion of the x-ray data by Debye,[59] Berl and his collaborators,[60] Wesselowski,[61] Hofmann,[62] and Warren[63] indicates that amorphous carbon consists of small graphite nets superimposed to form small bundles; the slip planes, in which the transport of electricity also occurs, are so much interrupted that for practical purposes they have no influence. Ruff[64] has expressed the opinion that amorphous carbon contains no plane nets but has an entirely irregular net structure. It seems probable, however, that the nets themselves are regularly built, but that their regular superposition in the crystal is disturbed.

The surface of such carbon particles contains atoms which are unsaturated and which may take up or adsorb organic materials, becoming partially saturated in the process. Thus, finely divided gas carbon unites firmly with rubber; the particles of carbon mixed in with the rubber act as points of linkage of the rubber chains. As a result of the presence of carbon, rubber becomes less extensible. It is assumed by various workers that the brownish gas black suitable for rubber compounding may really be a hydrogen-containing compound of carbon (see, for example, Esch[65]).

In the various forms of amorphous carbon (better described as microcrystalline) the individual crystallites are closely packed together to form secondary aggregates. The differences in chemical and physical properties between the different forms depend not only on the size of the crystallites but also on that of the secondary aggregates the surface of which determines the adsorption capacity. Electron microscope studies by Ardenne and Hofmann[66] showed that gas carbon black, suitable for rubber compounding, consists of secondary particles about 300 Å. in diameter, while soot consists of particles from 1000 to 3000 Å. in diameter. At higher tem-

[59] P. Debye and P. Scherrer, *Physik. Z.*, **18**, 291 (1917).

[60] E. Berl, K. Andress, L. Reinhardt, and W. Herbert, *Z. physik. Chem.*, **A158**, 273 (1932).

[61] W. S. Wesselowski and K. W. Wassiliew, *Z. Krist.*, **89**, 156 (1934).

[62] U. Hofmann and D. Wilm, *Z. physik. Chem.*, **B18**, 401 (1932). U. Hofmann and W. Lemcke, *Z. anorg. u. allgem. Chem.*, **208**, 194 (1932).

[63] B. E. Warren, *J. Chem. Phys.*, **2**, 551 (1934).

[64] O. Ruff, *Z. Elektrochem.*, **44**, 333 (1938).

[65] W. Esch, *Kautschuk*, **14**, 30 (1938).

[66] M. v. Ardenne and U. Hofmann, *Z. physik. Chem.*, **B50**, 1 (1941).

peratures each secondary particle is converted into a graphite crystal; the larger the secondary particles, and the more closely packed the initial material, the more suitable it is for graphitization, and the less suitable as an adsorption medium.

The familiar adsorptive powers of active charcoal depend on the particularly porous structure of this type of carbon, in which the porous structure of the initial material is retained. According to Bachmann,[67] charcoal from coconut shells contains pores 10–40 mμ in diameter together with still finer pores 5–6 mμ in diameter. Active charcoal also contains inorganic constituents, such as salts, which may lead to exchange adsorption.

2. Boron Nitride

Boron nitride is similar in structure to graphite[68]: it consists of hexagonal networks of atoms lying one on top of the other, in which the ions B^-N^+, analogous to the carbon atoms, are arranged at a distance of 1.45 Å. from each other to form regular hexagons. As in graphite, the distance between the nets is 3.35 Å.; in the interstices there is an electron density[69] corresponding to one electron per C atom or per 0.5 ($B^- + N^+$). While in graphite these electrons can move freely between the uniformly charged layers, the ionic structure of the B^-N^+ nets determines the formation of potential wells between the layers in which the electrons are fixed. It is for this reason that, despite the similarity of their structures, boron nitride differs from graphite in that it is transparent and does not conduct electricity.

3. Black Phosphorus

Whereas yellow phosphorus consists of P_6 molecules, black phosphorus contains extended net layers in which each phosphorus atom is united with three others spaced at 2.10 Å. (Fig. 16).[71] This structure appears to be present in red phosphorus also, which differs from black phosphorus in the small size of the crystals. The relationship between black and red phosphorus is probably comparable, therefore, to that between graphite and amorphous carbon. Red phosphorus may be obtained in different modifications: as amorphous, triclinic, hexagonal, and tetragonal phosphorus.[70]

[67] W. Bachmann, *Z. anorg. u. allgem. Chem.*, **100**, 32 (1917).
[68] V. M. Goldschmidt and O. Hassel, *Norsk Geol. Tids.*, **9**, 266 (1926).
[69] A. K. Breger and G. Zhdanow, *Compt. rend. acad. sci. U.R.S.S.*, **28**, 629 (1940).
[70] W. L. Roth, T. W. De Witt, and A. J. Smith, *J. Am. Chem. Soc.*, **69**, 2881 (1947).
[71] R. Hultgren, N. S. Gingrich, and B. E. Warren, *J. Chem. Phys.*, **3**, 351 (1935).

Phosphorus forms an insoluble and infusible compound with nitrogen, having the composition PN. In view of the pronouncedly high polymeric character of this compound, it seems not improbable that its structure is similar to that of black phosphorus.

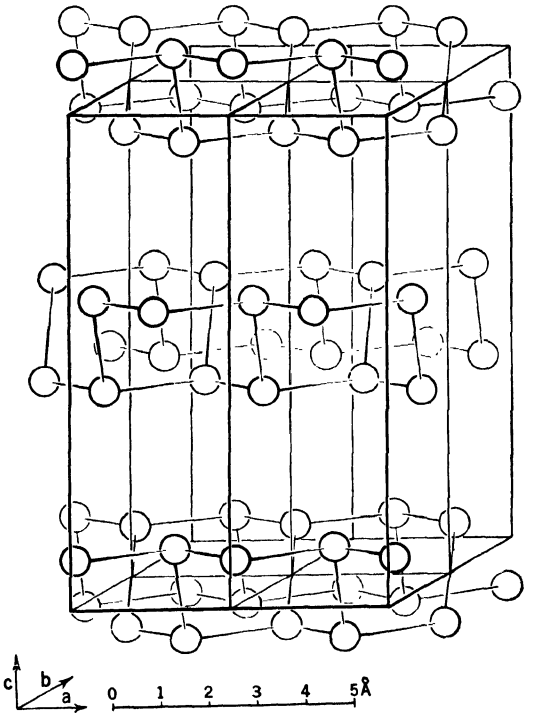


Fig. 16. Crystal structure of black phosphorus (Hultgren, Gingrich, and Warren).

4. Calcium Silicide and Siloxene

By fusing together silicon and calcium oxide at 1300°C., the compound $CaSi_2$ is obtained. The product is formed as six-sided tablets cleaving readily at right angles to the hexagonal axis. Their behavior in this respect recalls that of graphite and boron nitride. The x-ray analysis of Boehm and Hassel[72] has shown that the structure is similar to that of the metallic compounds of graphite; the silicon atoms form hexagonal nets between which the calcium atoms are distributed in hexagonal layers.

[72] B. Boehm and O. Hassel, *Z. anorg. u. allgem. Chem.*, **160**, 152 (1927).

If calcium silicide is decomposed with acids, siloxene is obtained. This compound has been investigated by Kautsky[73] and, according to him, has the composition $Si_6O_3H_6$. It occurs as lamellae, which react in a permutoid way and are insoluble in all solvents. It is to be assumed, therefore, that the primary valence nets of silicon have persisted and that the three OH groups and the three H atoms lie between the nets. Chemical substitutions of the hydrogen atoms (but not of the OH groups) lying between the nets may take place; they may, for example, be replaced by halogens, and the halogen siloxenes formed in this way may be hydrolyzed to oxysiloxenes. These compounds are solid, insoluble, and intensely colored. Many of them show luminescence.

Still other compounds of silicon are high polymeric in character; among these is the so-called silicoformic acid anhydride ($Si_2O_3H_2$). It is described as a voluminous, colorless, insoluble substance. Polysilicon monochloride (SiCl) and polysilicon monoiodide have been obtained by Schwarz[74] by thermal decomposition of halogenides of lower molecular weight ($Si_{10}Cl_{22}$ and Si_2I_6); they form yellow or orange-red scaly masses and are infusible and insoluble.

5. Silicates with Layer Lattices: Talc, Clays, and Mica

One of the most attractive chapters in the investigation of mineral structure is that concerned with talc, clays, and micas. The structural picture, derived chiefly from the results of x-ray analysis, makes comprehensible the striking and characteristic physical properties of these minerals—the softness of talc, the cleavage of mica, and the swelling powers and plasticity of clays. Without considering in detail the methods employed, it is desirable to summarize here the results of recent investigations by Bragg,[75] Bradley, Grim, and Clark,[76] Edelman and Favejee,[85] Gruner,[77] Hofmann,[78] Megaw,[79] Nagelschmidt,[80] Pauling,[81] and Hendricks.[82]

The three minerals have a laminated structure; all possess layers con-

[73] H. Kautsky and G. Herzberg, *Z. anorg. u. allgem. Chem.*, **147**, 81 (1925).

[74] R. Schwarz and R. Thiel, *Z. anorg. Chem.*, **235**, 249 (1938). R. Schwarz and A. Pflugmacher, *Ber.*, **75**, 1062 (1942).

[75] W. H. and W. L. Bragg, *The Crystalline State.* Bell, London, 1933.

[76] W. F. Bradley, R. E. Grim, and G. L. Clark, *Z. Krist.*, **97**, 216 (1937).

[77] J. W. Gruner, *ibid.*, **A88**, 412 (1934); *Am. Mineral.*, **20**, 699 (1935).

[78] U. Hofmann, K. Endell, and D. Wilm, *Z. Krist.*, **A86**, 340 (1933).

[79] H. D. Megaw, *ibid.*, **87**, 185 (1934).

[80] G. Nagelschmidt, *ibid.*, **A87**, 120 (1934); **93**, 481 (1936).

[81] L. Pauling, *Proc. Natl. Acad. Sci. U. S.*, **16**, 578 (1930).

[82] St. B. Hendricks and W. H. Fry, *Soil Sci.*, **29**, 457 (1930); *Am. Mineral.*, **23**, 863 (1938).

sisting of silicon atoms united by oxygen bridges to form nets in which the small silicon atoms are surrounded by four oxygen atoms. Three of these (the oxygen atoms forming links with other silicon atoms) lie in the plane of the lamina; the fourth oxygen atom forms a hydroxyl group with hydrogen. The hydroxyl group may be represented to a first approximation by a sphere of diameter 2.93 Å., as compared with one of 2.55 Å. representing the linking oxygen atom, and one of 0.8 Å. for silicon. The hydroxyl groups

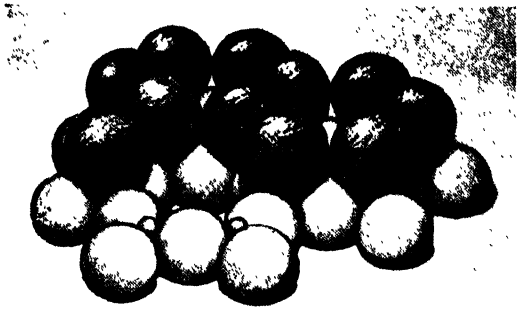


Fig. 17. Arrangement of the atoms in a hydrated silicon dioxide sheet. Lower layers, oxygen atoms; upper layers, hydroxyls. The small silicon atoms occupy the cavities in the oxygen tetrahedra (Bragg).

lie vertically above the silicon atoms; the arrangement of such a double layer possessing the elementary formula $Si_2O_5H_2$ [that is, for each layer $Si(\frac{1}{2}\ O)_3$ surmounted by OH] is shown in figure 17. A crystalline mineral consisting of superimposed nets of polysilicic acid, $Si_2O_5H_2$, is unknown; the acid is found only in combination with nets consisting of other elements.

(*a*) *Talc*

In talc, two such silicic acid layers alternate with one layer of magnesium hydroxide. The structure of this latter sheet is as follows: The small magnesium ions lie in one plane; each magnesium ion is bounded by six hydroxyl ions, of which three lie in a layer below and three others in a layer above the plane of the magnesium ions. Each hydroxyl ion is associated with three magnesium ions, so that only $^1/_3$ of each OH group belongs to any one Mg ion; hence, $^1/_3$ of the 6 OH ions lying above and below each Mg ion, *i.e.*, 2 OH ions in all, belong to that Mg ion; consequently the fundamental composition is given by $Mg(OH)_2$. The investigation of these

complex substances has been much facilitated by the discovery that brucite is a form of magnesium hydroxide consisting entirely of such layers. In brucite the layers are 4.73 Å. thick. In talc, each brucite layer is enclosed by two polysilicic acid layers. The three layers are closely united with one another as pairs of contiguous hydroxyl layers fuse together with elimination of water to form a single layer consisting of oxygen atoms and hydroxyl groups. The total thickness of the layers is determined mainly by the oxygens and hydroxyls; each oxygen layer has a thickness of 2.4 to 2.6 Å. We have, therefore, the following arrangement of layers and the following atoms in the unit cell of talc:

		Talc $Si_8O_{20}Mg_6(OH)_4$
Polysilicic acid	6O	6O
	4Si	4Si
	4OH	4O + 2OH
Brucite	6OH	
	6Mg	6Mg
	6OH	4O + 2OH
Polysilicic acid	4OH	4Si
	4Si	6O
	6O	
		Thickness 9.4 Å.

Such relatively solidly built layers are packed one on top of another and are held together only by weak forces of attraction, that is, by the residual valences of the layers of oxygen atoms. From this fact follows the ease of cleavage, the occurrence in lamellae, the pliability, the softness (degree of hardness 1.0–1.5), and the greasy feel of talc, all of which recall the similar properties of graphite and are to be referred in both cases to the same structural peculiarities. The softness is a consequence of the ease of cleavage, since the thin lamellae are easily broken.

It is of interest to note that entirely similar properties are encountered among the higher paraffins, and that in this case also a pronounced lamellar structure is responsible for these properties. The paraffin chains arrange themselves parallel to each other like matches packed together at right angles to a given plane, and thus form monomolecular layers which are in themselves substantially built by comparison. In the crystal the individual lamellae lie one on top of another but are held together only by very weak forces.

(*b*) *Clay Minerals*

Clay minerals are the chief constituents of clays, and themselves exhibit the properties of clays. They include kaolinite, montmorillonite, and hal-

loysite. With these are associated the micalike clay minerals[83] which though close to muscovite and biotite in structure and chemical composition, differ from all known types of mica and are to be distinguished from

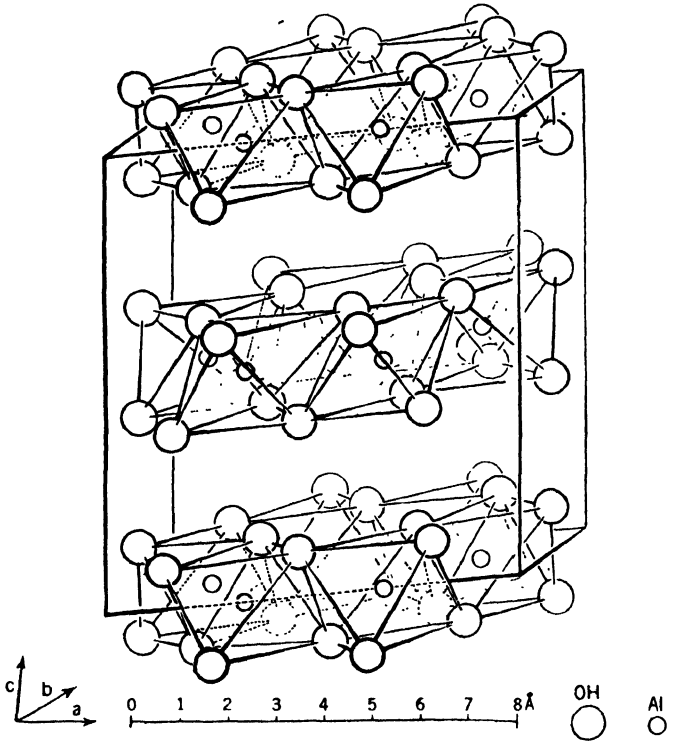


Fig. 18. Unit cell of hydrargillite (polyaluminum hydroxide) (Megaw).

true mica in their greater powers of water absorption and base exchange. The lattice of kaolinite, montmorillonite, and halloysite is composed of nets of silicic acid and of aluminum hydroxide.

A mineral consisting only of layers of the composition $Al(OH)_3$ is hydrargillite. As in brucite, each metallic ion is surrounded by six hydroxyls—three above and three below—but each hydroxyl is associated

[83] E. Maegdefrau and U. Hofmann, *Z. Krist.*, **98**, 31 (1937). E. Maegdefrau, *Sprechsaal*, **74**, 369, 381, 393, 399 (1941). R. E. Grim, R. H. Bray, and W. F. Bradley, *Am. Mineral.*, **22**, 813 (1937). R. E. Grim and W. F. Bradley, *J. Am. Ceram. Soc.*, **23**, 242 (1940).

with only two aluminum atoms. The number of metallic atoms per unit cell is related to the number present in brucite as two is to three. Figure 18 shows the arrangement. In clay minerals hydrargillite layers of polyaluminum hydroxide alternate with polysilicic acid layers. Two classes may be distinguished: the kaolinite and the montmorillonite groups, containing, respectively, one or two polysilicic acid layers in association with each hydrargillite layer.

The first group includes kaolinite and halloysite; the second, apart from montmorillonite, contains several rarer minerals in which the aluminum of montmorillonite is replaced by iron (nontronite) or magnesium.

Hallyosite	Kaolinite	Pyrophyllite	Montmorillonite
OH	O; OH	O	O; OH
Al	Al	Si	Si
OH	O; OH	O; OH	O; OH
OH	Si	Al	Al
Si	O	O; OH	O; OH
O; OH		Si	Si
		O	O; OH
			Layers of water
Thickness 10.25 Å.	Thickness 6.2 Å.	Thickness 9.4 Å.	Thickness 9.2–21 Å.

In vacuo at 50°C. halloysite loses water irreversibly and is transformed into metahalloysite. The structure of halloysite is still a matter of dispute. According to Hendricks[84] it consists of layers of kaolin separated by layers of water; on the basis of this view, metahalloysite should be identical with kaolin. Edelman and Favejee[85] postulate the structure shown in figure 19a in which the boundary layer contains hydroxyl groups, in contrast to the structure of kaolinite in which the silicic acid layers are bounded externally only by bridging oxygen atoms.

According to Hofmann,[86] the structure of montmorillonite corresponds to that of pyrophyllite[87] except for the water molecules reversibly absorbed in varying quantities between the polysilicic acid layers. The fact that montmorillonite, in contrast to pyrophyllite, has this property of absorbing water seems to indicate that in montmorillonite the boundary layer contains hydroxyl groups with which water molecules tend to associate (see Fig. 19) as in halloysite.

The clays of the ceramics industry are usually very finely divided forms

[84] S. B. Hendricks, *Am. Mineral.*, **23**, 295 (1938).
[85] C. H. Edelman and J. C. L. Favejee, *Z. Krist.*, **102**, 417 (1940).
[86] E. Maegdefrau and U. Hofmann, *Z. Krist.*, **98**, 299 (1937).
[87] J. W. Gruner, *Z. Krist.*, **88**, 412 (1934).

of kaolinite containing feldspar, quartz, and similar substances as impurities, and associated with a small quantity of montmorillonite. The purest form of kaolinite is kaolin itself. On the other hand, montmorillonite is the chief component of the bentonites, of Fuller's earth, bleaching earths, and similar materials. The laminated structure of kaolinite and montmorillonite corresponds to that of the kaolin and bentonite particles. Electron micrographs show that the kaolin laminae are less than 20–100 $\mu\mu$ thick and about 100–500 $\mu\mu$ in diameter. According to von Ardenne, Endel, and Hofmann,[88] the laminae may be dissociated down to single laminae in mont-

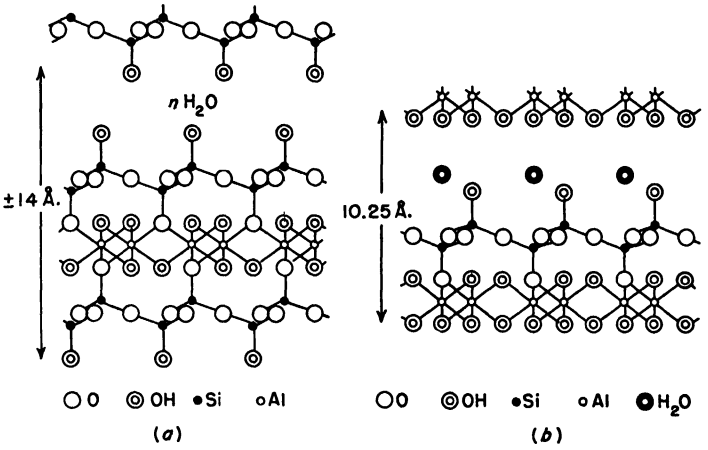


Fig. 19. Structure of: (*a*) halloysite; (*b*) montmorillonite.

morillonite. The enormous particle surface of the montmorillonite earths is undoubtedly connected with their use as bleaching agents for fats. The catalytic activities of bentonites and bleaching earths in certain reactions may be due, not only to the enormous particle surface, but also to the polar nature of the boundary surfaces, to certain structural peculiarities, and to the nature of the metallic atoms attached to the hydroxyl groups in the boundary layer.

Clays and bentonites always contain a certain number of alkali and alkaline earth ions built into the lattice, and some of these—the alkali ions in particular—dissociate from the fabric in aqueous suspensions and can be exchanged for other ions; this is the explanation of the base exchange

[88] M. v. Ardenne, K. Endell, and U. Hofmann, *Ber. deut. keram. Ges.*, **21**, 209 (1940).

capacity of clays. Hofmann[89] has suggested that the exchangeable ions neutralize the negative charges on the margins of the silicic acid nets carrying terminal oxygen atoms, as shown in figure 20. More recent observations suggest, however, that the exchangeable ions in bentonite are not situated on the edges of the nets, but are associated with the hydroxyl groups between the layers of the montmorillonite lattice. In this connection Gieseking[90] has shown that organic cations can be taken up by base exchange between the laminae with an increase in the (001) spacing up to

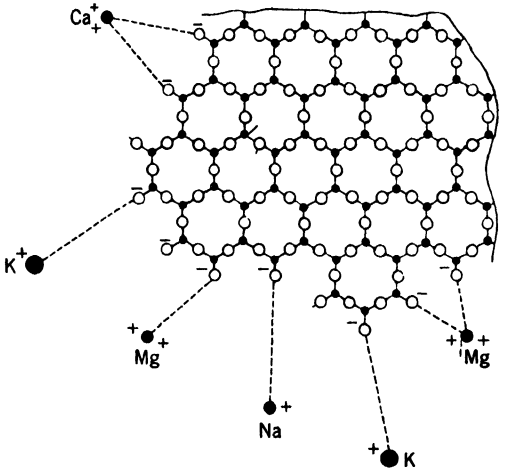


Fig. 20. Diagram showing the binding of cations by kaolin (Hofmann).

0 Å. in the case of Magdala red. It is not yet certain whether kaolinite itself has any capacity for base exchange, or whether the slight observed base exchange in kaolinite clays is due to the presence of small amounts of other clay minerals. The observation of Kelley and Jenny[91] that the base exchange capacity of kaolin is considerably increased by careful grinding certainly suggests that kaolinite, like other clay minerals, has its own capacity for base exchange. Obviously, the softness of kaolin (hardness 2.0–2.5) makes it possible so to reduce the size of the particles that the number of atoms at the surface is no longer inconsiderable in relation to the number in the interior of the lattice. The properties of the surface under these conditions become conspicuous.

[89] U. Hofmann and W. Bilke, *Kolloid-Z.*, **77**, 238 (1936).
[90] J. E. Gieseking, *Soil Sci.*, **47**, 1 (1939).
[91] W. P. Kelley and H. Jenny, *Soil Sci.*, **41**, 367 (1936).

Bentonites containing heavy metallic ions act catalytically on benzidine, which is adsorbed from water and converted into blue or green oxidation products. In a study of this catalytic activity, Krüger and Oberlies[92] made the interesting observation that copper bentonite not only has the highest capacity for effecting oxygen transfer, but that it is also able to induce condensation reactions. Conceivably, this enhancement of the catalytic activity of the metallic ion is similar in principle to that which occurs in enzymes containing metallic ions. It has often been observed that their catalytic activity exceeds by many powers of ten that of the pure metallic ion.

Particles of clay or bentonite, which have lost alkali ions by dissociation behave as polyvalent anions, and their mutual repulsion in aqueous suspensions is responsible for the strikingly large sedimentation volumes of finely divided clays and bentonites. On the addition of acid undissociated hydroxyls are formed from the negative SiO ions of the surface and the H ions of the acid, so that coagulation takes place. The charge on the particles, or rather the shell of hydrophilic cations by which they are surrounded is the reason for the water-binding properties of such clays. Plasticity and water uptake are functions of the movable cation content (Hofmann).[93] The water-binding power and capacity for base exchange of montmorillonite, which is present in loamy and crop soils in various degrees of purity and containing various exchangeable cations, is of great significance for the water and base content of soils.

With increasing pressure of water vapor, a succession of hydrates is formed containing 6, 12, 18, and 24 adsorbed water molecules per unit cell. The water forms molecular layers from 1 to 4 molecules thick and in this condition is considerably more dense than in the normal liquid state or in ice. The existence of these well-defined hydrates has been detected by x-ray analysis; the observed thickness of the water layers[94] may amount to 3, 6, 9, and 12 Å. They cannot be detected, however, by study of the water-uptake isotherms, which show no steps corresponding to the formation of these compounds; the curves are continuous and S-shaped. This shape arises from the superposition of two curves. At first, water is laid down within the crystallites (in sheets from 1 to 4 molecules thick) and the surface of the crystallites is covered with water. At 20% of the total water uptake, saturation is reached and, even if the external aqueous vapor

[92] D. Krüger and F. Oberlies, *Naturwissenschaften*, **31**, 92 (1943).

[93] U. Hofmann, *Kolloid-Z.*, **69**, 531 (1934).

[94] According to W. F. Bradley, R. S. Grim, and G. L. Clark, *Z. Krist.*, **97**, 216 (1937), the thickness of the first water layer amounts only to 2.8 Å.

pressure is increased, no further hydration of the surfaces is possible. Water which is now taken up is absorbed by capillarity in the spaces between the crystallites. In the process the smaller cavities are filled first and subsequently the larger spaces. This leads to the inflection of the curve (see Fig. 21).

The one-dimensional swelling of bentonite is strongly reminiscent of the analogous phenomenon shown by graphite oxide, which also has a lamellar structure. The S-shaped water-uptake isotherms, which occur in both are found in all cases in which a finely porous material can first bind water by specific attractive forces and subsequently absorb it. An example

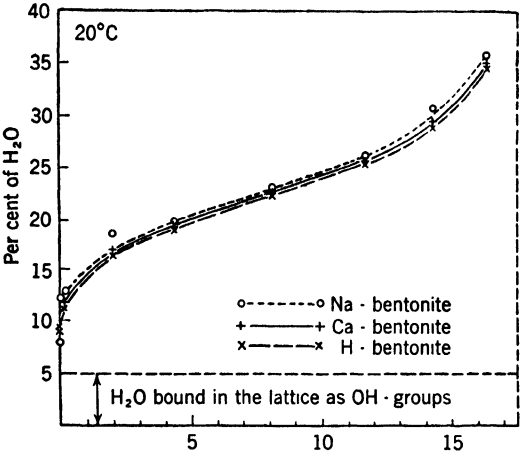


Fig. 21. Swelling isotherm of bentonite (impure montmorillonite) (Hofmann).

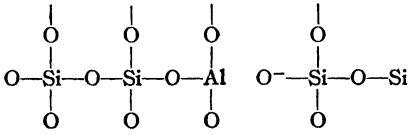
is provided by cellulose. Montmorillonite also takes up water at the surface of the crystallites; this phenomenon becomes more marked the smaller the particles. In this case, just as in that of kaolin, differences between the behavior of individual clays are dependent upon the cations which neutralize the negative charges on the particle. This explains how it is that up to 25% of the water uptake is independent of the nature of the cations, since this uptake is intracrystalline. Further water uptake (which takes place at the surface of the crystallites and may be measured by determination of the sedimentation volume) is influenced, however, by the nature of the cations present. Thus, for example, the water uptake of sodium bentonite is higher than that of calcium bentonite.

Bentonites are strikingly thixotropic; suspensions which are allowed to

remain undisturbed for a time solidify, but become liquid again on shaking. If allowed to stand, such suspensions solidify once more. This phenomenon (which has been studied by Freundlich[95]) occurs in many suspensions of finely crystalline and electrically charged particles, whether needle-shaped or platelike. It is supposed that the particles associate at equilibrium to form a loose structure (as a result of charges localized on individual groups or atoms of the particles). The phenomenon is by no means fully understood as yet. In view of its great practical importance in the question of the consistency of soils, for example, further work on this subject is desirable. Pastelike suspensions of bentonite form films consisting of fibrous felted crystalline aggregates[96] if poured out or spread on a smooth surface. The sensitivity of such films to the presence of water can be reduced by treatment with lead salts (ionic exchange) and they are of commercial interest as a substitute for mica.[97]

(*c*) *Mica*

As our third example we shall take mica and, in particular, the form muscovite. It is necessary to consider first a property characteristic of many silicates, namely, the formation of aluminosilicates. Silicon atoms in two-dimensional nets and, as we shall see later, in three-dimensional lattices of polysilicic acids, may be replaced by aluminum atoms. Since the aluminum ion possesses one positive charge less than the silicon ion, a net containing aluminum atoms possesses one negative charge more per aluminum atom than an otherwise equivalent aluminum-free polysilicic acid net. A bridging oxygen atom is replaced, in fact, by an oxygen anion, as shown in the following scheme:



(XI)

These charges are neutralized by adsorbed cations. Such aluminum atoms may be built up irregularly into the polysilicic acid net, and certainly occur in the nets of clays, where the extra negative charges (due to the presence of the aluminum atoms) are neutralized by cations, thus increasing the basic content of the clay. They may also occur in stoichiometrical proportion

[95] H. Freundlich, *Thixotropy* (Actualités Scientifiques et Industrielles). Hermann, Paris, 1935.

[96] E. A. Hauser and D. S. Le Beau, *J. Phys. Chem.*, **43**, 1037 (1939).

[97] See *Ind. Eng. Chem., News Ed.*, **18**, 300 (1940).

to the silicon atoms. In this case one speaks of aluminosilicates, and in the formulas of such minerals the alumino-aluminum is placed together with the silicon in brackets. Fluorine may also take the place of oxygen anions or hydroxyl groups, that is, Si—F may replace Si—O^- or Si—OH.

Muscovite, a typical mica, is an aluminosilicate with the formula $(OH)_2KAl_2(AlS_3O_{10})$. Its structure is similar to that of pyrophyllite, as is shown by the number of layers per unit cell:

Muscovite
10O
3Si + Al
6O + 4OH
6Al
6O + 4OH
3Si + Al
10O
4K
Thickness 10.0 Å.

Whereas in pyrophyllite (density 2.7–2.9) the layers are held together by the residual valences of the oxygen atoms and therefore slip readily over one another, in muscovite the negatively charged layers are held together by potassium ions, so that the structure is somewhat more rigid (density 2.8–3.0). The ready cleavage shown by mica disappears when, as in the case of margarite, the place of potassium is taken by smaller calcium ions the strong interionic forces of which hold the lamellae more strongly together.

Mica may be synthesized by slowly cooling a suitable melt. Large crystals are formed if a magnetic field is laid at right angles to the vertical axis of the crucible.

Before closing this section, it should be emphasized that the modern attitude toward the problems raised by the study of clays and soil colloids differs profoundly from the earlier, generally accepted, "colloid chemical" point of view. In place of surface forces and diffuse charges on colloidal particles we now have a much more precise picture of the structure and the forces involved. It is important to note that electrical charges are localized in ionized atoms or groups of atoms, just as in the case of electrolytes of lower molecular weight. This conception has been developed by Pauli.[98] Generalizing, we can say that the same kinds of forces determine the behavior of colloids as determine the behavior of atoms and small molecules.

[98] W. Pauli and E. Valkó, *Elektrochemie der Kolloide*. Springer, Wien, 1929.

III. INORGANIC POLYMERS WITH THREE-DIMENSIONAL PRIMARY VALENCE LATTICES

Both neutral atoms or groups of atoms and negatively charged complexes may be united with one another in all three dimensions by strong valences such as the homopolar bond. The meshes of such an anion lattice will contain the cations necessary for neutralization. High polymeric inorganic cationic lattices are unknown.

1. Neutral Space Network Polymers

(*a*) *Diamond, Silicon, Boron*

The classical example of a neutral three-dimensional polymer is the diamond. In this lattice each carbon atom is united with four others. Neighboring carbon atoms are spaced at 1.52 Å. From the point of view of the interatomic distance and the energy necessary for rupture, this bond corresponds entirely to the homopolar linkage between neighboring carbon atoms in aliphatic compounds. The whole diamond may, therefore, be regarded as a single molecule, if the molecule is defined as a group of atoms held together by primary valences. The density (3.52) and the degree of hardness (10), together with the other physical properties, are in satisfactory agreement with this picture of the structure.

Silicon, silicon carbide, grey tin, and germanium possess similar structures. SiC crystallizes in various forms, which, however, are similar in that each carbon atom is surrounded by four silicon atoms and each silicon atom by four carbon atoms. The element boron also belongs to this class of substances. Its great hardness (9), high specific gravity (2.45), and high melting point (2300°C.) are properties which suggest that it possesses a structure related to that of diamond; boron carbide, the complex structure of which has been revealed by x-ray analysis,[99] also belongs to this class. On account of its hardness, which exceeds that of diamond, it is prepared commercially and is used for cutting stone drills. A fibrous, feltlike silicon oxycarbide has been obtained by Tone[100] by the action of carbon monoxide on silicon vapor. It is flexible and very resistant to heat; its density (1.9–2.3) depends on the content of silicon carbide. The material may be obtained as a spongelike mass suitable for thermal insulation.

[99] G. S. Zhdanow and N. G. Sevast'yanow, *Compt. rend. acad. sci. U.R.S.S.*, **32**, 432 (1941). H. K. Clark and J. L. Hoard, *J. Am. Chem. Soc.*, **65**, 2115 (1943).

[100] F. J. Tone, *Ind. Eng. Chem.*, **23**, 1312 (1931).

(*b*) *Silicon Dioxide*

Silicon dioxide (silica) in its various modifications provides a picture of a typical primary valence three-dimensional lattice. Each silicon atom is surrounded by four oxygen atoms placed at the corners of a tetrahedron; each of these oxygen atoms is shared by two tetrahedra. The silicon atoms, therefore, are all united by ether-like oxygen bridges. The distance Si–O is 1.59 to 1.63 Å. The same arrangement is found in amorphous quartz glass. Apart from water-free, amorphous silicon dioxide, a number of amorphous water-containing forms are known, such as opal and diatomaceous earth (the siliceous skeletons of diatoms). Hydrated silicic acids may be obtained artificially by the liberation of silicic acids from water soluble alkali silicates. The water-soluble silicates may be supposed to consist of short-chain anions. If, by the addition of acid to a solution of an alkali silicate, the corresponding silicic acid of low molecular weight is liberated, the unstable acid unites rapidly (with elimination of water) with other silicic acid molecules, in which process ether bridges appear (—Si—O—Si—). This process of polymerization may be followed by determining the increasing viscosity of the liquid. Finally, the solution solidifies, forming a transparent jelly consisting of a loose three-dimensional network. In this reaction an irreversible gelatinization takes place, which is due to the formation of chemical linkages and is not to be interpreted (as is the reversible gelatinization of other polymers) as a phenomenon of crystallization. By prolonged dehydration, completed at a high temperature, it is possible to obtain all transition stages from hydrated to completely dehydrated silicon dioxide. It may be supposed that in the natural, hydrated, amorphous silicic acids (opal) the water present is chemically bound and the development of the network is not as complete as in silicon dioxide.

During dehydration, the mesh of the net is gradually reduced by the formation of more and more cross linkages. If dehydration is interrupted at a suitable moment, porous masses may be obtained in the canals of which water and other polar substances may be adsorbed. These silica gels find commercial application as drying agents, in the dehydration of mineral oils, etc. The water-adsorption isotherms of such preparations have been investigated in various ways by van Bemmelen[101] and later by Zsigmondy.[102] The dehydration curve shows a marked hysteresis loop; that is to say, at a given water content, equilibrium is approached infinitely slowly from either side.

[101] J. M. van Bemmelen, *Z. anorg. u. allgem. Chem.*, **66**, 322 (1910).
[102] R. Zsigmondy, *Ber.*, **59**, 467 (1926).

In the interpretation of these measurements it must, however, be remembered that new bridges may form during dehydration, and that such irreversible changes may perhaps be concerned in the observed "hysteresis" phenomenon.

Esters of silicic acid readily polymerize to form chains and nets with elimination of alcohol.

B_2O_3, GeO_2, TiO_2, and other oxides and sulfides are related to silicon dioxide. They form a transition group to substances with saltlike linkages.

2. Three-Dimensional Polymers with Extended Anions

(*a*) *Crystalline Silicates: Feldspars, Ultramarines, and Zeolites*

If aluminum atoms are built into the neutral, three-dimensional net of silicic acid (as in the aluminosilicates), extended anions with one negative

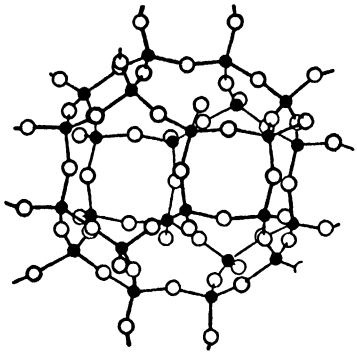


Fig. 22. Portion of the 3-dimensional network of ultramarine (F. M. Jaeger). Solid circles, silicon; open circles, oxygen.

charge per aluminum atom may be formed. Up to half of the silicon atoms may be replaced by aluminum. As the number of alumino-aluminum atoms increases, the number of neutralizing cations occupying the meshes of the space lattice also increases.

The following simple types may be distinguished:

(*a*) Feldspars (for example, orthoclase) · $K_n(AlSi_3O_8)_n$
(*b*) Feldspatoids (for example, leucite) $K_n(AlSi_2O_6)_n$
(*c*) Nepheline group $Na_n(AlSiO_4)_n$

In many silicates of this type the three-dimensional lattice is so open in construction that various salts or water may be taken up. To this cate-

gory belong the ultramarines, the structure of which has been worked out by Jaeger.[103] Figure 22 represents the three-dimensional lattice of ultramarine. Examples are:

Nozeane $Na_6Al_6(SiO_4)_6$, $2Na_2SO_4$
Hauyne $Na_6Al_6(SiO_4)_6$, $2(Ma_2, Ca)SO_4$
Lazurite $Na_6Al_6(SiO_4)_6$, $2Na_2S_3$

The sodium ions which neutralize the aluminosilicates can be replaced by other ions; the salts taken up within the lattice may also be varied considerably.

Of particular interest is the group of the zeolites, hydrated aluminosilicates which lose their water of hydration at relatively low temperatures and regain it when the aqueous vapor tension rises again. The zeolites possess a three-dimensional network similar in composition to that of the feldspars; the net is much more loosely constructed, however, so that the spaces are larger and contain water molecules, as well as cations (mostly Na and Ca) which neutralize the charge on the anionic network. Examples are:

Analcime $(AlSi_2O_6)_nNa_n(H_2O)_n$
Heulandite $(Al_2Si_6O_{16})_nCa_n(5H_2O)_n$
Desmine $(Al_2Si_6O_{16})_nCa_n(6H_2O)_n$
Natrolite $(Al_2Si_3O_{10})_n2Na_n(2H_2O)_n$
Chabasite $(Al_2Si_4O_{12})_nCa_n(6H_2O)_n$

The zeolites are able to lose a large part of their water without undergoing change in the crystal lattice. Thus, chabasite is able to lose up to 16.4% of water. In this process the specific gravity diminishes. If the aqueous vapor pressure is increased, water is taken up once more. The water-adsorption isotherms of several zeolites (for example, chabasite) are continuous curves, but in natrolite the stepped character of the isotherm indicates the presence of definite hydrates (Milligan and Weiser[104]). Dehydrated zeolites are able to absorb considerable quantities of H_2, He, N_2, CO_2, NH_3, and H_2O (Rabinowitch[105]). The adsorption of mercury on dehydrated chabasite resulted in no change in the x-ray diffraction spectra of the chabasite, according to Wyart.[106] The bases may also be exchanged without destroying the lattice: for example, 0.7 of the Ca of chabasite may

[103] F. M. Jaeger, *Verlag Akad. Wetenschappen Amsterdam*, **32** (1929); *Trans. Faraday Soc.*, **25**, 320 (1929). See also E. Podschus, U. Hofmann, and E. Leschewski, *Z. anorg. u. allgem. Chem.*, **228**, 305 (1936).

[104] W. O. Milligan and H. B. Weiser, *J. Phys. Chem.*, **41**, 1029 (1937).

[105] E. Rabinowitch, *Z. physik. Chem.*, **B16**, 43 (1933).

[106] I. Wyart, *Bull. soc. franç. minéral.*, **56**, 81–187 (1933).

be replaced by Ba. It is even possible to exchange one Ca for two K. All these facts indicate that the $(Al_mSi_{n-m})O_{2n}$ space lattice is extremely stable and contains canals through which ions may wander in and out.

(*b*) *Structure of Glasses*

The term glasses in the widest sense denotes all amorphous, transparent or translucent, solid masses; they are generally regarded as supercooled liquids. They resemble liquids in structure; both show local structure over short ranges but no large-scale, long range regularity; starting from the position of a given atom the position of its immediate neighbors may be fixed approximately, but not that of more remote atoms. On the other hand, in specific heat and mechanical strength, glasses resemble crystals. It is true that glasses have no sharp melting point, but the transition from the vitreous state to the liquid state, and the associated fall in viscosity from 10^{12} poise to lower values, take place over a relatively narrow temperature range,[107] so that one may speak legitimately of a softening temperature characteristic of each glass.

The forces which hold the atoms or molecules in glass together may, as in a crystal or in a liquid, be of various kinds: in vitreous glycerol (obtained by the rapid supercooling of liquid glycerol) they are hydrogen bonds; in the glass-forming oxides, B_2O_3, P_2O_3, As_2O_3, Sb_2O_3, SiO_2, GeO_2, P_2O_5, As_2O_5, Sb_2O_5, V_2O_5, Nb_2O_5, and Ta_2O_5, they are forces of attraction between the oxygen atoms and the element concerned, forces intermediate in character between electrostatic forces of attraction and those of homopolar linkages. They are of the same kind as the forces holding together the lattice of crystalline compounds.

Glasses whose cohesion depends on secondary valences have a relatively low softening temperature and are soluble in suitable solvents. Examples are provided by copal, Canada balsam, and other glasslike resins, some of which are of practical importance not as glasses in the narrow sense but as varnishes or transparent layers binding together true glasses.

Glasses formed by chain polymers such as polystyrene, polymethacrylate, or acety cellulose have special properties. At the softening temperature, which is low as in the preceding class, they pass into the rubberlike state. This is associated with the presence of very long chains; with shorter chains the rubberlike elastic properties are masked by plasticity and often, as with selenium glass, they can only be observed over a small range of temperature. Glasses, used in the limited sense—those formed from the oxides

[107] See, however, E. Jenckel, *Z. Elektrochem.*, **45**, 202 (1939).

already mentioned—exhibit a net structure like that of the crystals of chemically related compounds.

Amorphous quartz glass, SiO_2, may be regarded as an agglomerate of fused and distorted quartz crystals. It consists of a three-dimensional, aperiodic, primary valence net in which, as in quartz, each silicon atom is surrounded by four oxygen atoms, and each oxygen is bounded by two silicon atoms. But the valence angles are not constant and the structure is therefore not strictly regular. As the temperature rises, the glass is transformed

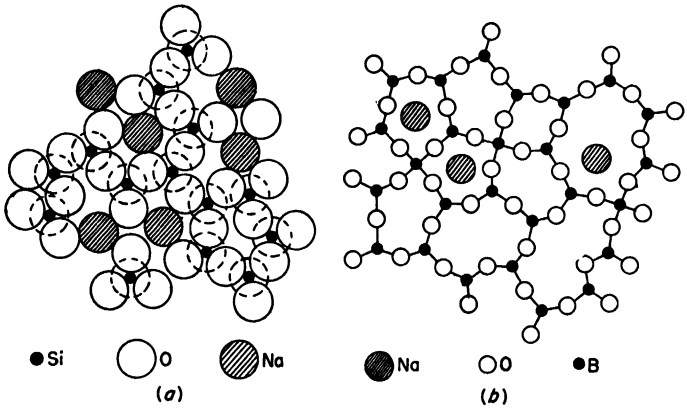


Fig. 23. Structure of: (*a*) sodium silicate glass (Warren and Loring); (*b*) boric acid-sodium oxide glass (Biscoe and Warren).

gradually into a viscous liquid. It is possible to introduce oxides into the glass and so to obtain net structures like those of the crystalline silicates. The common glasses consist of SiO_2 mixed with an alkali oxide and an oxide of a bivalent metal (Ca, Ba, Pb, etc.). In all these glasses, each silicon atom is surrounded by four oxygen atoms lying approximately at the corners of a tetrahedron and spaced at about the same distance (1.6 Å.) from the silicon atom, as in a crystal. Each oxygen atom is associated with two silicon atoms or with a silicon atom and at least one metallic atom. For special purposes borate glasses from B_2O_3 and various other oxides are used. Figure 23*a* shows diagrammatically the structure of a sodium silicate glass according to Warren and Loring.[108] In pure boric acid glass the boron atoms are surrounded by three oxygen atoms in a triangle, each oxygen shared by two boron atoms. A two-dimensional diagram of the quasi-

[108] B. E. Warren and A. D. Loring, *J. Am. Ceram. Soc.*, **18**, 269 (1935).

crystalline arrangement in a Na_2O–B_2O_3 glass with a low Na_2O content is shown in figure 23*b*, taken from Biscoe and Warren.[109] From this it is clear that if Na_2O is added to B_2O_3, certain boron atoms are surrounded by four oxygen atoms.

On warming certain glasses, such as fused quartz, the arrangement becomes more regular and the "amorphous" or much distorted lattice is gradually transformed into the crystalline condition of cristobalite without any sharp boundary between "amorphous" and "crystalline" networks. Solids in the vitreous condition may present advantages as compared with crystals for certain commercial purposes owing to their complete isotropy and the absence of regions of less compact texture.

Threads or fibers of glass or of molten quartz (glass wool, quartz wool) are prepared by stretching the melt as it issues from a jet, or by blowing bubbles from the melt, down to a thickness of 3 μ. They are used for insulation and as filter materials. The finer threads (glass silk) are also used for textile purposes. As the diameter of the threads diminishes, the strength increases very rapidly and reaches a value of 120–150 kg. per sq. mm. for threads 3–5 μ in diameter.

[109] J. Biscoe and B. E. Warren, *J. Am. Ceram Soc.*, **21**, 287 (1938).

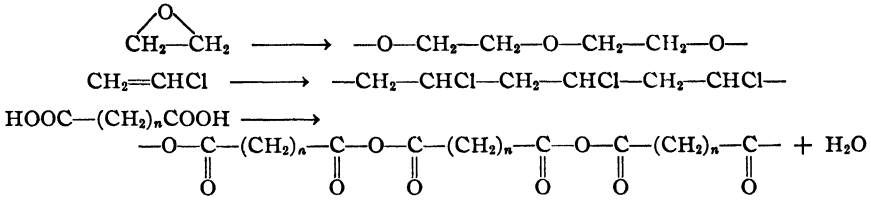
C. HIGH POLYMERIC HYDROCARBONS AND THEIR DERIVATIVES

I. INTRODUCTION

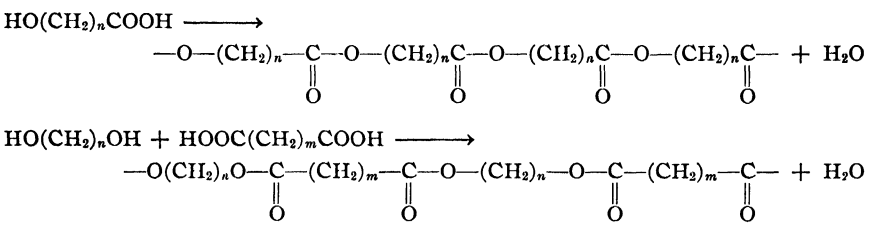
In addition to cellulose, rubber, proteins, and certain other natural products, organic high polymers include a number of substances which may be synthesized from materials of low molecular weight. These substances are important: (*1*) because the study of their formation and properties may throw light on the formation and properties of natural products; and (*2*) because of their commercial applications. Such substances have already found use in many branches of industry where formerly only natural products and their derivatives were used. We shall begin our survey with some general remarks on the synthesis of substances of this kind and on the various types of polymerization.

In synthesizing high polymeric substances, it is not practicable to employ the usual methods of synthesis of organic chemistry in which groups are added to the molecules one by one; the reaction must be allowed to proceed in such a way that very large molecules are formed in a single operation.

The initial substances must consist of molecules capable of uniting with at least two other molecules. Such substances are described by Carothers[1] as *bifunctional.* Many are capable of reacting without undergoing any change in composition; in this case we speak of *polymerization.* In others, combination is accompanied by the elimination of water or other substances. This type of polymerization is known as *polycondensation.* The reacting groups of the molecule may be similar or different; in other cases different kinds of molecules enter into combination. The following formulas illustrate various types of polymerization and polycondensation reactions:



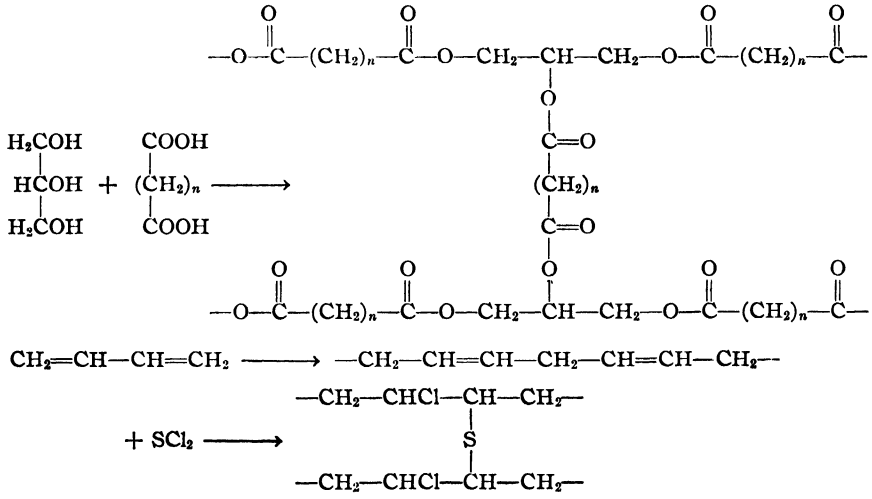
[1] W. H. Carothers, *J. Am. Chem. Soc.*, **51**, 2548 (1929); *reprinted in this series*, Vol. I, p. 4.



It is evident that bifunctional molecules can only form chain molecules (linear polymers). All artificial thermoplastic products which do not harden on heating consist of chain polymers.

If, on the other hand, the molecules used are able to react with more than two molecules (tri-, tetra-, and polyfunctional molecules), the polymerizing molecule is capable of linkage and growth in more than one dimension; in this way cross linkings and three-dimensional networks are formed. Even when the networks are extremely loose, that is, when the chains are united with one another at relatively few points, the properties of such compounds differ from those of chain polymers. The product is no longer an assemblage of long molecular threads which may be separated by solvents or caused to slip over each other by mechanical deformation. (Such a structure is indicated macroscopically by solubility and plasticity.) A cross-linked polymer may be regarded as a single giant molecule.

Linkage may also be brought about after polymerization by means of a second reaction independent of the polymerization process. The following schemes indicate several types of cross-linking reactions:



A low degree of cross linkage results in products showing limited swelling and reversible extensibility. As the number of linkages in three dimensions increases, the power of swelling and the deformability are reduced more and more until, finally, hard masses of very considerable strength are obtained. This category of highly cross-linked materials includes natural horn, synthetic phenolic plastics, and all other artificial materials which harden on heating. They are known as thermosetting plastics.

In the following pages we shall consider natural and artificial polymers side by side, and adopt the classification customary in the chemistry of organic substances of low molecular wight.

II. POLYMERIZATION[2]

1. Polymerization Reaction

(a) *Kinetics*

The group of polymers characterized by a succession of primary valence linkages between carbon atoms includes, besides rubber and gutta-percha, synthetic products formed by the polymerization of olefins and their derivatives. It has been known for a long time that some ethylene derivatives (vinyl chloride, styrene, acrylic acid) may be transformed without change in chemical composition into compounds of high molecular weight which are either insoluble or only slightly soluble and are destroyed by distillation. Such compounds (for example, polyvinyl chloride) were described as polymers; expressions such as metastyrene were also used. Until 1924 the constitution of these polymers received little attention; an exception is provided by the work of Stobbe,[3] who suggested that polystyrenes were ring compounds, formed by the union (by chemical valences) of styrene molecules which had lost their double bonds. It was Staudinger who, as a result of his investigations on polyoxymethylene, first introduced chemical valence formulas for all such polymers and established their chain structure.[4] The polymerization reaction is represented accordingly in the following way:

$$n(H_2C{=}CHR) \longrightarrow -H_2C-CHR-CH_2-CHR-CH_2-CHR-CH_2-$$

[2] See the Faraday Society Symposium 1935 on Polymerization and Condensation, *Trans. Faraday Soc.*, **32**, 1 (1936). R. E. Burk, *Polymerization*, Reinhold, New York, 1937; this Series, Vol. III.

[3] H. Stobbe and G. Posnjak, *Ann.*, **371**, 259 (1909).

[4] H. Staudinger and M. Lüthy, *Helv. Chim. Acta*, **8**, 41 (1925). H. Staudinger, *Ber.*, **59**, 3019 (1926).

Polymerization always proceeds with the evolution of heat, and at room temperature the concentration of the monomer in thermodynamic equilibrium with the polymer is negligibly small. Most of the olefins are, however, too stable to react spontaneously at room temperature; it is therefore necessary for one molecule to be activated in such a way that it in turn can react readily with other monomeric molecules. The reaction is, consequently, a typical chain reaction. The first observer to recognize the nature of the reaction to the extent possible at that time was Stobbe,[5] who came to the conclusion, after very careful investigation, that "nuclei of polymerization" are formed in styrene and that these accelerate polymerization in a remarkable way. Such nuclei are formed under the influence of light or heat.

Insight into the polymerization reaction was only possible after the constitution of vinyl polymers had been clarified and after Bodenstein had discovered the "chain reaction" in systems of substances of low molecular weight. Starkweather and Taylor,[6] and simultaneously Chalmers,[7] were the first to recognize that vinyl polymerization is a chain reaction. A year later, Staudinger and Kohlschütter[8] expressed the same opinion, and in 1934 Chalmers[7] presented the theory in the form in which it is still accepted. In the light of chain reaction theory, polymerization has been investigated in detail from many different points of view, so that it is now possible to review the various processes participating in polymerization.

(*1*) *Chain Initiation.* An olefin molecule may be activated by absorbing a quantum of light or, thermally, by a collision, and may thus be converted into an active molecule capable of reaction. In order to raise the double bond to the next higher energy level, about 35,000 cal. per mole are probably necessary for ethylene, while for substituted ethylenes, styrene, or vinyl chloride, for example, the activation energy amounts to about 25,000 cal. According to Eyring[9] the catalysts BF_3, $AlCl_3$, and $SnCl_4$ polarize the double bond by pulling over the two electrons of the double bond to one of the carbon atoms thus giving rise to active centers of the carbonium ion type with reduced activation energy. The catalyst is not incorporated in the chain. On the other hand, carbanion centers are probably formed by addition of alkali metals as catalysts.

[5] H. Stobbe and G. Posnjak, *Ann.*, **371**, 259 (1909). H. Stobbe, *ibid.*, **409**, 1 (1915).

[6] H. W. Starkweather and G. B. Taylor, *J. Am. Chem. Soc.*, **52**, 4708 (1930).

[7] W. Chalmers, *Can. J. Research*, **1**, 32 (1930); **7**, 113 (1932); *J. Am. Chem. Soc.*, **56**, 912 (1934).

[8] H. Staudinger and H. W. Kohlschütter, *Ber.*, **B64**, 2091 (1931).

[9] J. L. Magee, W. Shand, Jr., and H. Eyring, *J. Am. Chem. Soc.*, **63**, 677 (1941).

The action of the most widely used catalysts, however, is not true catalysis. It consists in a chemical reaction between a peroxide and a monomer as a result of which a nucleus is formed and continues to grow. The peroxide is used up in this process and is found as an ester in the finished chains. One molecule of peroxide is built into each chain, as can be shown by using those which are easily recognized, such as bromobenzoyl peroxide. Peroxides and other substances initiating polymerization should therefore be called initiators rather than catalysts.

The incorporated ester groups can also be demonstrated by their absorption in the infrared.[10] The peroxide decomposes into radicals:

$$(C_6H_5COO)_2 \longrightarrow 2\ C_6H_5COO\cdot$$

having only a very short life and which rapidly combine with olefins, to form a new radical which reacts with more olefin:

$$C_6H_5COO\cdot + CH_2{=}CXH \longrightarrow C_6H_5COOCH_2CXH\cdot$$

$$C_6H_5COOCH_2CXH\cdot + CH_2{=}CXH \longrightarrow C_6H_5COOCH_2CXHCH_2CXH\cdot$$

where X = Cl or phenyl, etc. If hydrogen peroxide is used free hydroxyls are formed. The formation of free radicals from peroxides is in many cases accelerated by reducing agents. In fact the best catalysts are "redox systems," *i.e.*, peroxides together with suitable reducing agents. By means of these systems the temperature of polymerization can be kept low and the time required can be reduced. An example is provided by a mixture of hydrogen peroxide and ferrous salt; according to Evans[11] it reacts in the following way:

$$Fe^{++} + H_2O_2 \longrightarrow Fe^{+++} + OH^- + HO\cdot$$

Other redox systems have been applied successfully in the manufacture of synthetic rubbers of the GR-S type (Buna S, butadiene-styrene copolymer), *e.g.*, the system: one part of benzoyl peroxide, one part of sorbose, 0.1 part of Fe^{++} in the form of a salt of a higher fatty acid, a trace of ferrous ammonium sulfate, and sodium pyrophosphate.[12] Similar mixtures are being applied in the U. S. for low-temperature polymerization of GR-S.[13]

A very effective initiator is formed by cymene hydroperoxide in the presence of ferrous salt and a mercaptan:

[10] F. Pfann, V. Z. Williams, and H. Mark, *J. Polymer Sci.*, **1**, 14 (1946).

[11] J. H. Baxendale, M. G. Evans, and K. Kilham, *Trans. Faraday Soc.*, **62**, 668 (1946).

[12] E. R. Weidlein, Jr., *Chem. Eng. News*, **24**, 771 (1946).

[13] W. H. Shearon, Jr., J. P. McKenzie, and M. E. Samuels, *Ind. Eng. Chem.*, **40**, 769 (1948). See also *Ind. Eng. Chem.*, **41**, 1553 (1949).

$$R{-}OOH + Fe^{++} \longrightarrow RO\cdot + Fe^{+++} + OH^-$$

$$RO\cdot + R_1SH \longrightarrow ROH + R_1S\cdot$$

$$R_1S\cdot + CH_2{=}CHR_2 \longrightarrow R_1S{-}CH_2{-}CHR_2\cdot$$

According to Hagger[13a] sulfinic acids can initiate olefinic polymerization. They probably form redox systems with olefinic peroxides produced by autooxidation.

(*2*) *Chain Growth.* The activated molecules act as nuclei of polymerization, since they react rapidly with neighboring molecules containing a double bond. The larger radicals formed in this way unite with more monomer, and so on.

The heat of activation of the growth reaction is very small in the case of the radical chain mechanism brought about by peroxides (*ca.* 4000 to 5000 cal. per mole of the monomer); in the case of chain growth by activation of the double bond the heat of activation is rather greater (6000 to 10,000 cal.).[14] In each step the heat of activation is furnished directly from the energy set free in the reaction. Table I gives several values for quantity of heat set free during polymerization.

TABLE I
Heats of Polymerization[a]

Monomer	Product	Heat, cal./mole of monomer
Methacrylic acid methyl ester	Polymer	21,400[15]
Butadiene	Polymer	37,000[16]
Styrene	Polymer	16,680[17]
Butadiene + styrene	Copolymer	41,900[18]

[a] Calculated from heats of combustion of monomers and polymers. A comprehensive list is wiven by D. E. Roberts.[17]

Direct measurements on the heat of reaction gave lower values: methacrylic acid methyl ester → polymethacrylate resin + 13,000 cal.[19]

(*3*) *Chain Termination.* The chain grows with considerable velocity

[13a] O. Hagger, *Helv. Chim. Acta*, **31**, 1624 (1948).

[14] H. Mark and H. Raff, *High Polymeric Reactions* (*High Polymers*, Vol. III). Interscience, New York, 1941.

[15] W. A. Roth, *unpublished.*

[16] L. Hock and H. Leber, *Kautschuk*, **16**, 76 (1940).

[17] D. E. Roberts, W. W. Walton, and R. S. Jessup, *J. Research Natl. Bur. Standards*, **38**, 627 (1947). D. E. Roberts, *ibid.*, **44**, 221 (1950).

[18] W. A. Roth, G. Wirths, and H. Berendt, *Kautschuk*, **17**, 31 (1941).

[19] L. K. L. Tong and W. O. Kenyon, *J. Am. Chem. Soc.*, **68**, 1355 (1946).

until it is interrupted. This may occur as result of various factors, but chiefly as a result of the collision of the activated molecule with the wall of the vessel or with a molecule of another species. Hydroquinone and phenol are particularly effective in interrupting the process of chain growth; anthracene has a similar action. It is for this reason that traces of hydroquinone are used to stabilize monomeric preparations (such as styrene).

This type of chain termination probably depends on a chemical reaction, like that of halogens on polymerizing sulfur. Thus, if nitrobenzene is used to interrupt the growth of chains in styrene polymerization, one molecule of nitrobenzene is found at the end of each chain.[20] Termination of the chain may also occur as a result of two activated chains coming into contact.

(*4*) *Chain Transfer.* As Flory[21] has shown, the growth of a particular chain may be interrupted if it withdraws a hydrogen atom from a monomer or from another molecule, thus forming a new radical which may act as a nucleus for the formation of a new chain.

Certain substances when added in small quantities bring about chain transfer of this kind. It was found by Price,[22] for example, that tetrachlorobenzoquinone (chloranil) attaches itself to the end of the growing chain, for example, a chain of styrene, while a chlorine atom is split off. The separated chlorine atom serves to initiate a new chain. It is probable that *modifiers*, that is, substances added during polymerization in order to avoid too great a chain length, act in the same way: their effect is important for some technical purposes, as for example, in the production of synthetic rubbers. The effect (discovered entirely empirically) of dodecyl mercaptan in improving the workability of the synthetic rubber GR-S obtained by polymerization is interpreted in the following way:[23]

$$\underset{\text{growing chain}}{\ldots CH_2{-}CRH\cdot} + \underset{\text{modifier}}{HSR_1} \longrightarrow \underset{\substack{\text{interrupted}\\ \text{chain}}}{\ldots CH_2{-}CRH_2} + \underset{\substack{\text{new}\\ \text{nucleus}}}{R_1S\cdot}$$

$$\underset{\text{nucleus}}{R_1S\cdot} + \underset{\text{monomer}}{CH_2{=}CRH} \longrightarrow \underset{\text{new growing chain}}{R_1S{-}CH_2{-}CRH\cdot}$$

The course of polymerization differs according to the various factors in operation: the presence of many nuclei may lead to the formation of short

[20] C. C. Price and D. A. Durham, *J. Am. Chem. Soc.*, **65**, 757 (1943).

[21] P. J. Flory, *J. Am. Chem. Soc.*, **58**, 1877 (1936).

[22] C. C. Price, *J. Am. Chem. Soc.*, **65**, 2380 (1943); C. C. Price and D. H. Read, *J. Polymer Sci.*, **1**, 44 (1946).

[23] H. R. Snyder, J. M. Stewart, R. E. Allen, and R. J. Dearborn, *J. Am. Chem. Soc.*, **68**, 1422 (1946).

chains; few nuclei and the absence of all impurities leading to the interruption of the chains tend to produce longer chains, that is, a more highly polymerized product. Since lowering of the temperature of reaction slows down the rate of formation of nuclei (which has a high heat of activation) much more than the rate of chain growth, low temperatures favor the formation of long chains, that is, of high polymeric products.

Schulz and Husemann[24] have attempted to give a kinetic interpretation of measurements made on pure styrene; Schulz, Dinglinger, and Husemann claim to be able to derive the mechanism of chain start from measurements made on dilute solutions of styrene. The conclusions reached in these papers have been rejected by Breitenbach,[25] who draws attention to contradictions and mistakes in the equations used.

Stockmayer[26] has rejected calculations by Mark and his collaborators[27] on the different constants of styrene polymerization and has also criticized formulas given by Ginell and Simha.[28] Among experimental results it may be mentioned that in the purely thermal polymerization of styrene the velocity of polymerization is proportional to the square of the thermodynamic activity of monomeric styrene.[29] In polymerization by peroxides, on the other hand, the velocity of polymerization both of styrene and of other vinyl derivatives is proportional to the expression:

$$\sqrt{C}\, c \sqrt{\frac{Kc}{1 + Kc}}$$

where K is a constant, C is the concentration of the peroxide and c that of the monomer.[30] This remarkable observation has been interpreted in various ways.

By means of different ingenious methods using, for example, intermittent illumination to initiate photopolymerization, rate constants of the various stages have been determined.[30a] In general, radicals have life

[24] G. V. Schulz and E. Husemann, *Z. physik. Chem.*, **B34**, 187 (1936). G. V. Schulz, A. Dinglinger, and E. Husemann, *ibid.*, **B43**, 385 (1939); **B45**, 105 (1939).

[25] J. W. Breitenbach, *Monatsh.*, **71**, 275 (1938); *Z. physik. Chem.*, **B45**, 101 (1939).

[26] W. H. Stockmayer, *J. Chem. Phys.*, **12**, 143 (1944).

[27] J. Abere, G. Goldfinger, H. Mark, and H. Naidus, *Ann. N. Y. Acad. Sci.*, **44**, 267 (1943).

[28] R. Ginell and R. Simha, *J. Am. Chem. Soc.*, **65**, 706 (1943).

[29] C. Walling, E. R. Briggs, and F. R. Mayo, *J. Am. Chem. Soc.*, **68**, 1145 (1946).

[30] G. V. Schulz and E. Husemann, *Z. physik. Chem.*, **B39**, 246 (1938). G. V. Schulz and F. Blaschke, *ibid.*, **B51**, 75 (1942). H. Mark and D. Josefowitz, *Thesis*, Brooklyn Polytechnic Institute, 1945. M. S. Matheson, *J. Chem. Phys.*, **13**, 584 (1945).

[30a] G. M. Burnett and H. W. Melville, *Proc. Roy. Soc.* (London), **A189**, 456 (1947); C. H. Bamford and M. G. S. Dewar, *ibid.*, **A192**, 309 (1948); M. S. Matheson *et al.*, *J. Am. Chem. Soc.*, **71**, 497, 2610 (1949).

times of the order of seconds, during which time many thousand monomers are added to the growing chain. Radical concentrations lie in the range of 10^{-8}–10^{-9} molar.

The constitution of the monomers has a great influence on the rate of polymerization. Olefins in which one of the following groups is bound to an olefinic carbon atom: —Cl, —Br, —I, —$OCOCH_3$, —COOH, or C_6H_5, polymerize rapidly. Ethylene and its homologues polymerize less readily, as do allyl alcohol, CH_2=CH—CH_2OH, and symmetrically substituted compounds of the type CHR=CHR. For this reason substances which can be obtained without difficulty by polymerization, *e.g.*, polyvinyl chloride, polystyrene, and polyvinyl acetate, were the first to be used on a technical scale. At present, however, technical methods are available for polymerization of ethylene and other much less readily polymerizing olefins.

(*b*) *Methods of Polymerization*

Polymerization may take place in a pure, undiluted liquid phase, in solution, in suspended droplets, or in emulsion.

(*1*) *Bulk Polymerization.* If polymerization occurs in the absence of a diluting medium the entire contents of the vessel gradually polymerize and form a solid mass at room temperature. Only those substances which yield a polymer that melts without decomposition are suitable for bulk polymerization on a commercial scale.

(*2*) *Solution Polymerization.* Polymers of a medium grade of polymerization are often formed since many solvents bring about termination of the chains or chain transfer.[31] If the monomer is soluble but the higher polymers insoluble, the originally homogeneous solution finally separates into a swollen gel and a supernatant liquid solution. An example is provided by the polymerization of acrylonitrile in water.

(*3*) *Bead Polymerization.* The monomer is mechanically dispersed in a liquid (usually water) in which it is only very slightly soluble. Each globule of monomer is gradually transformed into a bead of high polymer. Polymerization takes place partly within the droplet (this is the same as bulk polymerization) and partly in the aqueous phase in which the monomer is always soluble to some extent. The polymer which is practically insoluble in water is precipitated onto the droplets (Fikentscher[32]). As

[31] F. R. Mayo, *J. Am. Chem. Soc.*, **65**, 2324 (1943).

[32] H. Fikentscher, *Angew. Chem.*, **51**, 433 (1938).

a rule it is of much lower molecular weight than the fraction formed within the droplet. The products obtained by *bead polymerization* (droplet suspensions) are therefore often very inhomogeneous. In many cases the molecular weight distribution curve shows two distinct peaks, the smaller of which corresponds to the fraction of lower molecular weight.[33]

(*4*) *Emulsion Polymerization.* The most important process is that of emulsion polymerization. In this the monomer is emulsified in water to which an emulsifying agent has been added. At the end of the reaction, an emulsion of the polymer is obtained and may be used as an artificial latex for certain technical purposes. The polymer may be coagulated by the addition of acids or salts, or by centrifuging.

It is difficult to obtain any general picture of the processes which take place in the complex heterogeneous system of a polymerizing emulsion, and the most favorable conditions can only be determined empirically. The variables include, for example, the choice of a suitable emulgent; either a soap, such as ammonium oleate, or the salt of a long-chain aliphatic sulfonic acid may be used, in both cases at a concentration of 0.2–2% of the monomer present. The proportion of monomer or, in copolymerization, of the mixture of monomers, to water may be varied between one fourth and one seventh. Use is made of peroxides, such as hydrogen peroxide, urea peroxide, benzoyl peroxide, and sodium persulfate, as initiators. Higher aliphatic mercaptans and several other compounds have a favorable influence on the regular course of polymerization. An appropriate *p*H is fixed by phosphate buffer and the mixture is emulsified by vigorous stirring. At 40–60°C. polymerization takes about 24 hours. By using redox systems, *i.e.*, suitable reducing agents in the presence of peroxides, the polymerization temperature may be lowered below 0°C.

The reaction may take place in various ways and various processes may be superimposed. The monomer, for example, may be relatively soluble in water so that polymerization takes place for the most part in the aqueous phase, yielding an emulsion of insoluble polymer; or the monomer may be dispersed in the form of droplets stabilized by an adsorbed layer of soap molecules and it may happen that the polymer is embedded in the soap micelles.

The distribution of the catalyst in the phases present—aqueous solution, droplets, or micelles—is of considerable significance for the course of the reaction. In some cases it has been shown that the actual polymerization—

[33] W. P. Hohenstein and H. Mark, *J. Polymer Sci.*, **1**, 127, 549 (1946). Review on polymerization in emulsion and suspension.

activation, propagation, and termination—starts and takes place preponderantly inside the soap micelles.[33] Larger droplets do not react at all, whereas the monomer dissolved in water also forms nuclei.

2. Homogeneity and Constitution of Polymers

For most industrial purposes it is desirable that the polymerized product should be as homogeneous as possible, with the chains all the same length. For some purposes long chains are required, for example, for artificial fibers; for others, such as varnishes, a shorter chain length is preferred. There is still no exact theory by which the form of the distribution curve for different sizes of molecules may be calculated, so that the conditions giving the most homogeneous product have to be found by trial and error. Homogeneity is estimated by fractionation followed by measurement of the viscosity of individual fractions.

In vinyl polymers, one would expect only unbranched paraffin chains to result from progressive addition of olefin molecules to the end of the activated chain. This is in fact true for all vinyl polymers except styrene, which branches when polymerized by heating, and is the more branched the higher the temperature (see Chapter C, section III 8). The mechanism

TABLE II[34]

Free Energy of Formation of Isomeric Octanes

Octane	Free energy of formation cal./mole
n-Octane	−16,740
2- or 4-Methylheptane or 3-Ethylhexane	−19,700
2,3-, 2,4-, 2,5-, or 3,4-Dimethylhexane or 2-Methyl-3-ethylpentane	−22,700
2,2- or 3,3-Dimethylhexane or 3-methyl-3-ethylpentane	−24,700
2,3,4-Trimethylpentane	−25,700
2,2,3- or 2,2,4-Trimethylpentane	−28,700
2,2,3,3-Tetramethylbutane	−32,700

of this abnormal polymerization is still not clear. In this connection it may be mentioned that the free energy of formation of unbranched paraffins is greater (less negative) than that of branched paraffins. Since data for paraffins of really high molecular weight are not available Table II gives values for the free energy of formation of several octanes at 298.1°K. = 25°C. in calories per mole of gas at 1 atmosphere.

[34] Calculated from the results of R. H. Ewell, *Ind. Eng. Chem.*, **32**, 778 (1940).

If equilibrium between all isomers could be established, it is to be expected that branched structures would predominate even among substituted higher paraffins. It seems not impossible, therefore, that branching occurs as a result of secondary reactions.

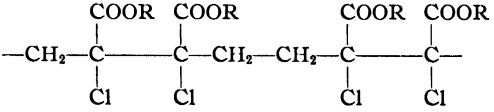
Vinyl monomers usually attach themselves to the growing chain in the same sense (head-to-tail) so that a paraffin derivative of the following type is formed:

$$—CH_2—CHR—CH_2—CHR—CH_2—CHR—CH_2—CHR—CH_2—CHR—$$

In a few instances, however, the monomers are joined together head-to-head:

$$—CH_2—CHR—CHR—CH_2—CH_2—CHR—$$

as in polychloracrylic ester, which has the following constitution:[35]



Polyacrylic esters either have the same structure or are irregularly built.

In order to explain these observations we can regard the process of chain propagation as the result of four reactions with different velocities. Let the olefin be composed of the groups A and B (for example, CH_2CHCl). Then a chain ending in A can react with A or with B of the monomer and conversely:

Chain A·	—AB (K_1)	gives	—AAB·	(head-to-head)
	—BA (K_2)	gives	—ABA·	(head-to-tail)
Chain B·	—BA (K_3)	gives	—BBA·	(head-to-head)
	—AB (K_4)	gives	—BAB·	(head-to-tail)

If K_1/K_2 and K_3/K_4 are very small then only head-to-tail association will occur.

Nothing is known of the configuration of the carbon atoms, that is, of the arrangement of the substituents in space. In the first stage of polymerization of monosubstituted vinyl derivatives optically active molecules appear: ($CHCl{=}CH—CHCl—CH_3$ with an asymmetrical C atom). If only atoms of the same rotation are added as the chain grows, the end

[35] C. S. Marvel and J. C. Cowan, *J. Am. Chem. Soc.*, **61**, 3156 (1939). C. S. Marvel and C. L. Levesque, *ibid.*, **61**, 3244 (1939).

product will be a racemate of regularly built chains. Furthermore, the formation of regularly built but inactive chain molecules is also conceivable in which *l* and *d* atoms alternate; finally, one can envisage a statistical arrangement of *l* and *d* atoms, which would also lead to the formation of inactive chains. The last possibility seems to be the most probable.

3. Copolymers

Copolymerization, that is, the union of various vinyl derivatives to form high polymeric saturated molecules according to the following scheme:

$$—CH_2—CHCl—CH_2—CHCl—CH_2—CH(OCOCH_3)—CH_2—CHCl—$$

is of great industrial importance. The process was discovered by Klatte,[36] who observed the polymerization of a mixture of vinyl esters. Later Voss and Dickhäuser[37] described the incorporation of maleic acid into polymerizing styrene. Wagner-Jauregg[38] recognized that copolymerization is not limited to a few special cases, but is of a general nature.

Mixtures of monomers as well as pure monomers may be caused to polymerize in bulk, in solution, or in aqueous suspension. The method of polymerization frequently determines the course of copolymerization; thus, many monomers which form copolymers in bulk do not do so if polymerized as emulsions. The converse may also occur (Hopff[39]).

Not all vinyl compounds give rise to copolymers; a mixture of styrene and vinyl acetate, for example forms only polystyrene—the polymerization of the vinyl acetate is inhibited. Conversely, monomers which alone do not polymerize may be built up into other readily polymerizing compounds forming true copolymers. Thus, maleic anhydride, which does not polymerize alone, polymerizes with styrene to form styrene–maleic acid and crotonic acid, similarly, can be built into polymerizing methacrylic esters (Hopff).

Different monomers are incorporated at different velocities; for example, allyl chloride is incorporated into polymerizing styrene only 0.03 times as quickly as styrene[40]; *p*-chlorostyrene and methyl methacrylate polymerize together in the ratio of 1.46:1.[41]

On account of its great industrial importance, copolymerization has been

[36] F. Klatte, Austrian Pat. 70,348 (1914), Griesheim Electron Chem. Works.

[37] German Pat. 540,101 (1930), I. G. Farbenindustrie A.-G.

[38] T. Wagner-Jauregg, *Ber.*, **63**, 3213 (1930).

[39] H. Hopff, *Angew. Chem.*, **51**, 432 (1938). H. Hopff, W. Stark, and K. Billig, U. S. Pat. 2,228,270 (1938).

[40] T. Alfrey, Jr., and J. G. Harrison, Jr., *J. Am. Chem. Soc.*, **68**, 299 (1946).

[41] C. S. Marvel and G. L. Schertz, *J. Am. Chem. Soc.*, **65**, 2054 (1943).

investigated intensively, both theoretically and in quantitative experimental studies. It was pointed out by Norrish and Brookman[42] that chain propagation in the formation of a copolymer involves four different reactions; the growing chain may have a radical unit derived from either of the two monomers at the end of the chain, to which molecules of either of the two monomers may be added:

$$\begin{aligned}
&\text{—A}^{\bullet} + \text{A} = \text{—AA}^{\bullet} && \text{(rate constant, } K_{AA}\text{)}\\
&\text{—A}^{\bullet} + \text{B} = \text{—BA}^{\bullet} && \text{(rate constant, } K_{AB}\text{)}\\
&\text{—B}^{\bullet} + \text{A} = \text{—BA}^{\bullet} && \text{(rate constant, } K_{BA}\text{)}\\
&\text{—B}^{\bullet} + \text{B} = \text{—BB}^{\bullet} && \text{(rate constant, } K_{BB}\text{)}
\end{aligned}$$

On the assumption of a steady state with respect to free radical concentration Mayo and Lewis[43] and Alfrey and Goldfinger[44] have shown that:

$$d\text{A}/d\text{B} = \frac{\text{A}}{\text{B}} \times \frac{\alpha\text{A} + \text{B}}{\beta\text{B} + \text{A}}$$

where A and B are the concentrations of the two monomers, $\alpha = K_{AA}/K_{AB}$ and $\beta = K_{BB}/K_{BA}$.

Wall[45] has made the simplifying assumption that two rate constants only determine the reaction, the rates of addition of one monomer to either end of the growing chain being equal. In this case $\beta = 1/\alpha$ and:

$$\frac{d\text{A}}{d\text{B}} = \alpha \times \frac{\text{A}}{\text{B}}$$

It is stated, however, that this "ideal copolymerization" is not observed in the majority of copolymerizing systems. α and β can be calculated from the values of $d\text{A}/d\text{B}$ obtained at different ratios of A to B. It emerges from the work of Mayo and Lewis[43] and of Alfrey, Merz, and Mark[46] that

TABLE III
Relative Rates of Copolymerization

Monomer A	Monomer B	k_{AA}/k_{AB}	k_{BB}/k_{BA}
Styrene	Methyl methacrylate	0.5	0.5
Styrene	Dichlorostyrene	0.2	0.8
Styrene	Methyl acrylate	0.75	0.2
Dichlorostyrene	Methyl acrylate	1.0	0.05
Styrene	Maleic anhydride	0.04	0.0
Styrene	Diethyl maleate	5.0	0.0
Styrene	Diethyl chloromaleate	2.5	0.0

[42] R. G. W. Norrish and E. F. Brookman, *Proc. Roy. Soc. London*, **A171**, 147 (1939).
[43] F. R. Mayo and F. M. Lewis, *J. Am. Chem. Soc.*, **66**, 1594 (1944).
[44] T. Alfrey, Jr., and G. Goldfinger, *J. Chem. Phys.*, **12**, 205 (1944).
[45] F. T. Wall, *J. Am. Chem. Soc.*, **63**, 1862 (1941); **66**, 2050 (1944).
[46] T. Alfrey, Jr., E. Merz, and H. Mark, *J. Polymer Sci.*, **1**, 37 (1946).

the ratios α and β are less than unity in substances which polymerize quickly. This means that alternation of residues is favored. α and β only become larger if a monomer which does not polymerize easily is incorporated. Table III gives figures[47] for reaction velocities.

Each of the parameters $\alpha = K_{AA}/K_{AB}$ and $\beta = K_{BB}/K_{BA}$ depends on both monomers. In many cases, however, one can calculate parameters characteristic solely of each monomer. Price[47a] and Alfrey[47b] deduced the following equations:

$$\alpha = \frac{Q_1}{Q_2} l^{-l_1(l_1 - l_2)}$$

$$\beta = \frac{Q_1}{Q_2} l^{-l_2(l_1 - l_2)}$$

in which Q_1 and l_1 depend only on monomer 1, and Q_2 and l_2 on 2. Q_1 and Q_2 are connected with the reactivity of the monomers and l_1 and l_2 with the distribution of the electrical charge between the substituent and the double bond.

The rate constants for the copolymerization initiated by metal halides (carbonium ion center formation) and by alkali metals (carbanion centers) differ from the constants for radical chain polymerization. In an equimolecular mixture of styrene and methyl methacrylate an initial copolymer containing 51 mole per cent styrene is formed, whereas stannic chloride leads to a polymer consisting of almost pure polystyrene, and metallic sodium to practically pure polymethyl methacrylate.[47c]

The composition of copolymers, particularly the sequence of the residues, has in a few cases been determined by chemical methods.[48] Cases are also known where K_{AA} and K_{BB} are very small, that is, in which the pure monomers show scarcely any tendency to polymerize, while K_{AB} and K_{BA} are considerably larger, for example, 100 times. A copolymer is formed with a regular alternation of residues. This occurs with allyl acetate and maleic

[47] From C. C. Price, *J. Polymer Sci.*, **1**, 83 (1946).

[47a] C. C. Price, *J. Polymer Sci.*, **3**, 772 (1948).

[47b] T. Alfrey, Jr., Copolymerization. Interscience, New York, *in preparation.*

[47c] C. Walling, E. R. Briggs, W. Cummings, and F. R. Mayo, *J. Am. Chem. Soc.*, **72**, 48 (1950).

[48] See the theoretical treatment by: P. Flory, *J. Am. Chem. Soc.*, **61**, 1518 (1939); **64**, 177 (1942); F. Wall, *ibid.*, **66**, 2050 (1944); as well as E. Merz, T. Alfrey, Jr., and G. Goldfinger, *J. Polymer Sci.*, **1**, 75 (1946).

acid anhydride.[49] In polymerization in solution, as in bulk polymerization, α and β were found to be "constant," that is, independent of the composition of the mixture of monomers and of the nature of the catalyst. In emulsion polymerization, on the other hand, α and β are to some extent dependent on the composition of the mixture, as was shown by Meehan[50] in the copolymerization of butadiene and styrene which results in the synthetic rubber known as Buna S (GR-S).

Since as a rule one monomer is incorporated more rapidly than the other, the remaining mixture of monomers becomes deficient in the more quickly reacting component during the course of copolymerization, and the composition of the polymeric molecules being formed changes accordingly. If all the original material is polymerized, the over-all composition of the copolymer corresponds to the mixture of monomers, but the copolymer is relatively heterogeneous—the molecules first formed contain more of the more quickly polymerizing monomer (for example, of butadiene in the copolymerization of butadiene with styrene); in the molecules formed subsequently the styrene content increases, since the remaining mixture of monomers is richer in styrene.

The instantaneous styrene content, f_s (that is, the styrene content of the newly forming polymer after a particular conversion, C, has been reached), is related to the over-all styrene content, F_s (that is, the styrene content of the polymer already formed after the same conversion, C), by the following equation:[51]

$$f_s = F_s + C(dF_s/dC)$$

dF_s/dC can be determined graphically from the slope of the curve obtained by plotting F_s against C. Table IV gives the values for the over-all styrene

TABLE IV

Emulsion Polymerization of Butadiene (75%) and Styrene (25%)

Conversion	Over-all styrene content	Instantaneous styrene content
0	---	17.2
20	17.9	18.8
40	18.7	20.6
60	19.7	23.3
80	21.2	29.5
90	22.5	36.4
100	25.0	100

[49] P. D. Bartlett and K. Nozaki, *J. Am. Chem. Soc.*, **68**, 1495 (1946).

[50] E. J. Meehan, *J. Polymer Sci.*, **1**, 318 (1946).

[51] F. T. Wall, *J. Am. Chem. Soc.*, **67**, 1929 (1945).

content and the instantaneous styrene content found by Meehan for a mixture of 75% butadiene and 25% styrene polymerized in emulsion.

The solubility of the product formed in copolymerization is determined by the sum of the effects of all the groups present, and it is therefore possible to influence the solubility by the choice of constituents in the mixture. Of some technical importance is the fact that mixed polymers are not crystalline, and that their softening temperature is generally lower than that of homopolymers consisting of the same kind of monomeric unit. By copolymerization, rubberlike as well as thermoplastic masses may be obtained. The commercially important copolymers will be discussed in connection with the homopolymers to which they are related. The industrial possibilities of copolymers of the majority of commercially available olefin and diolefin derivatives are by no means exhausted as yet. The intensity of research in this field is indicated by the publication of a *Safety Manual for Copolymerization—Plant Laboratories* by the American Office of Rubber Reserve.

4. Technically Important Properties of Polymers in Relation to Constitution

Synthetic vinyl polymers and the polyesters and polyamides to be discussed later find application as synthetic fibers, plastics, artificial rubbers, lacquers and coatings, etc. A brief review will therefore be given of the effects of constitution and degree of polymerization on the technologically important properties.

Behavior toward solvents and swelling agents, *e.g.*, stability toward water and oil, is determined primarily by chemical constitution and is therefore dependent on the choice of monomer. For example, a high polymeric alcohol like polyvinyl alcohol is soluble or swells in water but not in benzene; on the other hand, a high polymeric paraffin such as polyethylene, is hydrophobic. Crystallinity also has its effect; crystalline high polymers are less soluble than those of similar structure but which are amorphous. Finally, the degree of polymerization plays its part; easily soluble products are only obtainable if a known limit of molecular weight is not exceeded. The permeability is also determined chiefly by structure; for instance, hydrophilic polymers are permeable to moisture. Here too crystallinity is a factor, crystalline polymers being more permeable than amorphous polymers owing to the possibility of fissures.

Electrical properties likewise depend to the greatest extent on structure, and so on the monomer. Unsubstituted high polymer hydrocarbons like

polyethylene and polystyrene are the best insulators and have the lowest dielectric losses.

With regard to mechanical properties the position is considerably more complicated. In the main these are determined by intermolecular forces, and they in their turn depend chiefly upon the monomer; thus, groups which can form hydrogen bridges with others in neighboring chains will confer a specially high degree of cohesion. In the second place, these forces depend on the degree of polymerization; they increase with it just as the boiling point of low molecular compounds rises with the molecular weight. Since the molecular weight is always increased by cross linkings, the cohesion also increases on that account. Furthermore, the mechanical properties are affected by the formation of crystalline regions. Many polymers crystallize spontaneously or due to mechanical deformation. In this category are included polyethylene, polyvinyl chloride, polydichloroethylene, and polyvinyl alcohol; rubber and gutta-percha which will be discussed later also being in this class. In contrast, polyvinyl acetate, polyacrylates, and polystyrene, as well as most of the synthetic rubbers, have not yet been crystallized; this is also the case for all copolymers. As will be seen for copolymers of vinyl chloride and vinyl acetate, the inclusion of relatively small amounts of the acetate sufficies to prevent crystallization of the vinyl chloride elements. The introduction of such components into the structure is also referred to as *internal softening*. Irregularities in structure, such as are found in the acryl derivatives render crystallization impossible. This is of technical importance as the presence of crystallites has a very considerable effect on the mechanical properties of the solid body. On the one hand, the possibility of cleavage of the crystallites is appreciably greater than that of the amorphous parts, and so for purposes where equal solidity in all directions is desirable noncrystallizable polymers are generally selected. On the other hand, for use in fibrous form crystallizable polymers are advantageous because, in general, a completely amorphous fiber would flow under stress, whereas crystalline regions in which the chains are immovably bound to each other prevent flow.

Amorphous, low-grade polymers may be inflexible and brittle or soft and sticky, depending on their structure, but they are never hard. Heating or the addition of suitable swelling agents (softeners) can convert brittle products to a soft and sticky state. The higher amorphous polymers, especially the copolymers, are either glasslike solids or rubberlike; glassy amorphous polymers become *elastomers*, that is, elastic like rubber, on addition of softeners or increase of temperature. The temperature at which change from the solid to the rubbery state occurs is independent of the molecular weight but depends on the structure.

Crystalline high polymers also become elastic at their *"melting" points*; this property is also dependent on the structure but not on molecular weight. Softeners can depress the melting point. For elastomers tensile strength and extensibility depend on molecular weight and on the size of the meshes of the network because, in order to avoid plastic deformation in rubberlike materials, a three-dimensional network structure is induced (vulcanization). Only polymers in which cross linkages may be introduced in order to form a network can be utilized as technical elastomers. Elastomers with no structural network become plastic at a certain temperature. This temperature increases with molecular weight; for very high polymers plastification only occurs with simultaneous breakdown. In general, a molecular weight of 200,000 can be regarded as the upper limit for a plastically deformable polymer; actually most thermoplastic materials have a lower molecular weight.

As far as the mechanical properties of the finished state are concerned—film, fiber, or molded object—the method applied for confirming the form and the subsequent treatment (extrusion through an orifice, stretching, rolling, thermal after-treatment, etc.) are important. The disposition of the chain molecules, the proportions of crystalline and amorphous material, and the mechanical properties depend on both the induced form and any subsequent treatment. Objects intended for use at higher temperatures should not become plastic nor undergo any chemical change. The former requirement is achieved by effecting a three-dimensional structure (hardening), the latter by incorporation of silicon or fluorine atoms or by admixture of incombustible substances. Finally, it should be mentioned that for the preparation of glass-clear materials crystallizable substances are unsuitable since the small crystalline regions have a different refractive index from that of the amorphous surroundings and therefore scatter the light. Crystallizable polymers are therefore always opaque.

III. SATURATED HIGH POLYMERIC HYDROCARBONS AND THEIR DERIVATIVES

1. Polymeric Paraffins

(a) *Normal Paraffins (Polymethylenes, Polyethylenes)*

$$—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—$$

The formation of a polymethylene was observed by Bamberger and Tschirner[52] during the decomposition of diazomethane: $CH_2N_2 \rightarrow (CH_2)_n$

[52] E. Bamberger and F. Tschirner, *Ber.*, **33**, 955 (1900).

+ N_2. The product had a melting point of 128°C., from which a mean chain length of 170 carbon atoms is obtained, using van der Wyk's formula (see below). Polymers of similar chain length were obtained by Fischer[53] during the catalytic hydrogenation of carbon monoxide, and by Staudinger[54] by the reduction of polyvinyl bromide with hydriodic acid. Higher polymethylenes were obtained by Pichler[55] using Fischer's method. These polymers have a mean molecular weight of about 8000 and melt at 132°C. True high polymeric paraffins of molecular weight up to 40,000 are formed from ethylene by polymerization at high pressure in the presence of peroxides, according to data given in two British patents.[56] The reaction is allowed to proceed at a pressure above 1200 atmospheres and at a temperature of about 200° C. The products are translucent and very tough. The mean molecular weight varies from 3000 to 40,000 according to the conditions of preparation. At room temperature the products are insoluble in all solvents. If the temperature is raised, the solubility suddenly increases at 70°C. The melting point lies between 110° and 125°C. Above the melting point the products are either very viscous liquids or rubberlike solids (according to the degree of polymerization). The products are called Polythenes by the manufacturers, Imperial Chemical Industries, Ltd. and E. I. du Pont de Nemours.

The melting point of these substances is lower than one would expect. According to van der Wyk[57] the melting point of the paraffins, T_f (on the absolute scale), is given by the following equation:

$$\frac{1}{T_f} = a + \frac{b}{z}$$

where z is the number of carbon atoms, $a = 2.395 \times 10^{-3}$, and $b = 17.1 \times 10^{-3}$. A paraffin of very great chain length ($z \rightarrow \infty$) should therefore fuse at about 145°C. The fact that the observed melting point is lower than the equation predicts is undoubtedly connected with the anomalous phenomenon of fusion displayed by chain polymers of very high molecular weight; these effects are due to hampering of crystallization caused by the

[53] F. Fischer and H. Tropsch, *Ber.*, **60**, 1330 (1937).

[54] H. Staudinger, M. Brunner, and W. Feisst. *Helv. Chim. Acta*, **13**, 827 (1930).

[55] H. Pichler, *Angew. Chem.*, **51**, 412 (1938).

[56] Imperial Chemical Industries Ltd., *Chem. Zentr.*, **1937, II**, 3995 (Ind. Pat.). E. W. Fawcett, R. O. Gibson, M. W. Perrin, J. G. Paton, and E. G. Williams, and Imperial Chemical Industries, Brit. Pats. 471,590 and 474,426.

[57] K. H. Meyer and A. van der Wyk, *Helv. Chim. Acta*, **20**, 1313 (1937).

entanglement of the long chain molecules and to the formation of very small crystalline regions.

(*b*) *Crystalline Structure of Polyethylenes*

The investigations of Müller[58] and of Hengstenberg[59] have shown that the long molecules of normal crystalline paraffins are oriented parallel to one another and at right angles to the cleavage planes of the paraffin crystal.

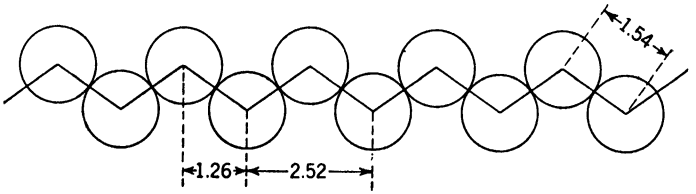


Fig. 1. Carbon atoms in a paraffin chain.

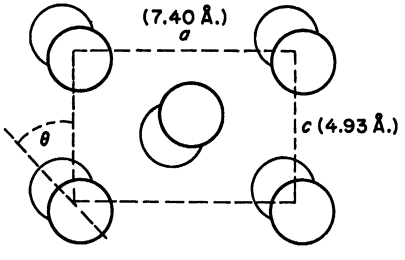


Fig. 2. Unit cell of polyethylene; projection on the *ac*-plane (Bunn).

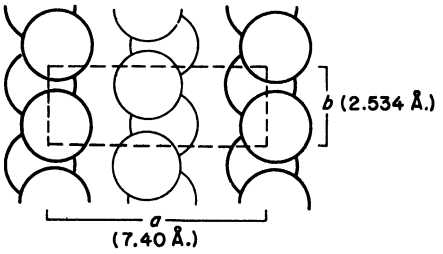


Fig. 3. Unit cell of polyethylene; projection on the *ab*-plane (Bunn).

The CH_2 groups of a paraffin molecule lie in a zigzag sequence in one plane. The unit cell is rhombic. The two dimensions at right angles to the chain axis are almost the same in all paraffins, whereas the identity period along the chain axis varies with the chain length. As the chain length increases, certain reflections of higher order, corresponding to the larger net plane spacings, become increasingly conspicuous as compared with those of lower order. Finally, to all intents and purposes, only the higher orders remain, indicating a small identity period along the chain

[58] A. Müller, *Proc. Roy. Soc. London*, **A120**, 437 (1928).

[59] W. Hengstenberg, *Z. Krist.*, **67**, 583 (1928). See also R. Kohlhaas and K. II Soremba, *ibid.*, **100**, 47 (1938).

axis. The transition from molecular lattice to primary valence lattice is then complete.

The unit cell of this primary valence lattice is given by Hengstenberg as a = 7.39 Å., b (fiber axis) = 2.53 Å., c = 4.93 Å. (in the case of paraffins of medium chain length). The same dimensions were found by Bunn[60] in a high polymeric paraffin with about 3000 CH_2 groups. This was determined on preparations of rolled paraffin. During rolling the chains come to lie in the direction of rolling, and the (101) planes lie in the plane of the rolled surface.

The structure of a paraffin molecule, according to the earlier investigations of Müller and Hengstenberg and the more recent work of Bunn,[60] is shown in Figure 1. Figures 2 and 3 represent projections along the b and c axes, respectively. This structure has been confirmed by a three-dimensional Fourier analysis.

Bunn has shown that polyethylene is incompletely crystalline at ordinary temperatures, and x-ray measurements have revealed the presence of amorphous material. The amorphous character of the substance becomes more pronounced as the temperature is raised, until at 120–125°C. no crystallinity can be detected. Heat capacity measurements[61] have shown that on cooling thermodynamic equilibrium between crystalline and amorphous regions is not attained instantaneously.

(c) *Technical Uses of Polyethylene*

Polyethylene has several unique characteristics which make its position among synthetic high polymers outstanding. It is flexible and tough over a wide temperature range—from below −45° to 100°C.; its resistance to water and moisture penetration is excellent; of all plastics it has the lowest specific gravity; and it is practically inert chemically and has very valuable electrical properties.

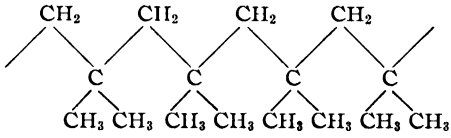
Polyethylene is readily molded by injection and compression techniques or it may be extruded as sheets, fibers, films, tubes, etc. Molding by compression methods is performed at a temperature between 121° and 160°C. The tensile strength may be increased to a considerable extent by cold-drawing which results in orientation of the crystallites.[62] Polyethylene may also be used in the preparation of water- and chemical-resistant coatings by applying hot solutions of the polymer in xylene, or dispersions

[60] C. W. Bunn, *Trans. Faraday Soc.*, **35**, 482 (1939).
[61] H. C. Raine, R. B. Richards, and H. Ryder, *Trans. Faraday Soc.*, **41**, 56 (1945).
[62] F. C. Hahn, M. L. Macht, and D. A. Fletcher, *Ind. Eng. Chem.*, **37**, 526 (1945).

in water or solvents, or by flame-spraying. After application, particularly from solution or by dispersion, the film should be dried at a temperature above 100° C. or fused after drying so that a continuous, adherent film is produced. Films of polyethylene may be joined by heat sealing, and the bulk plastic may be welded; for welding and patching, a mixture of polyisobutylene and polyethylene is more satisfactory than polyethylene alone.

Polyethylene has been used mainly for coatings, wire and cable insulation (for example, in high-frequency coaxial cables), films and sheets, flexible tubing, gaskets, battery parts containers of various types, and equipment to be used at low temperatures such as refrigerator trays. When compounded with other materials, especially waxes, polyethylene makes excellent adhesives.

(*d*) *Branched Paraffins* (*Polyisobutylene*)



The isobutylenes and other isoolefins polymerize in the presence of titanium tetrachloride, boron fluoride, or aluminum chloride.[63] If high polymeric products are desired, the temperature must be kept well below 0°C. In this way products with a molecular weight of ten million can be obtained.[64] In order to obtain a product of low molecular weight a little *n*-butylene is added, which stops further growth of the chains. The higher polymers are rubberlike and can be reversibly extended to fifteen times their initial length. They were first marketed under the names Oppanol (I. G.) and Vistanex (Standard Oil of N. J.)

Stretched, rubberlike polyisobutylene yields a fiber diagram, as shown by Brill and Halle,[65] and investigated by Fuller.[66] The unit cell is rhombic with $a = 6.94$, $b = 18.63$ (fiber axis), and $c = 11.96 \pm 0.05$ Å. Two chains traverse the cell; it is suggested that the chains cannot be plane zigzags, but that under the influence of the side chains they become helices.

[63] I. G. Farbenindustrie, *Chem. Zentr.*, **1934, II,** 547, 2158; **1936, I,** 1778. See also H. J. Waterman, J. Over, and A. J. Tulleners, *Rec. trav. chim.*, **53,** 699 (1934). P. K. Frolich, *Ind. Eng. Chem.*, **30,** 916 (1938). R. M. Thomas, W. J. Sparks, P. K. Frolich, M. Otto, and M. Mueller-Cunradi, *J. Am. Chem. Soc.*, **62,** 276 (1940).

[64] P. J. Flory, *J. Am. Chem. Soc.*, **65,** 372 (1943).

[65] R. Brill and F. Halle, *Naturwissenschaften*, **26,** 12 (1938).

[66] C. S. Fuller, C. J. Frosch, and N. R. Rape, *J. Am. Chem. Soc.*, **62,** 1905 (1940).

The identity period (8 × 2.3 Å.) is a little less than eight times the distance between identical CH_2— groups in a paraffin (2.5 Å.). This would imply that at each eighth CH_2— or $C(CH_3)_2$— group, identity is established once more, and that each $C(CH_3)_2$— group is rotated through 45° about the fiber axis with respect to the preceding group. Under these conditions the lateral CH_3— groups interfer little with one another. The constitution given above can be derived without any uncertainty from the x-ray diagram.

Polyisobutylene can be fractionally precipitated with acetone from dilute solution in cyclohexane and so separated into regularly uniform fractions which have often been used for scientific investigation. Molecular weight and intrinsic viscosity of the solutions in diisobutylene are related to each other according to the following equation:[64]

$$\log M = 5.378 + 1.56 \log [\eta]$$

Polyisobutylene is prepared on a commercial scale. It has excellent insulating properties and improves the viscosity of lubricating oils. Membranes resist dilute acids and alkalis and can be used for lining chemical-resistant vessels. Various grades of polymer are available commercially. The lower ones are plastic; the higher ones are elastic like rubber, down to temperatures of −50°C. The molecular weight of the more highly polymerized commercial products exceeds several hundred thousand; the specific gravity is 0.93, the dielectric constant is 2.2–2.3. Polyisobutylene cannot be vulcanized but a vulcanizable product (butyl rubber) is obtained by polymerizing in the presence of a small quantity of isoprene or butadiene. The double bonds present in the finished chains react with sulfur like the double bonds in natural rubber to form cross linkages. An extensive study on the influence of cross linkages on the physical properties of butyl rubber has been carried out by Flory.[67]

2. Halogenated Polyethylenes

(a) *Polyvinyl Halides*

$$—CH_2—CHCl—CH_2—CHCl—CH_2—CHCl—$$

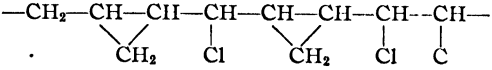
Vinyl chloride and vinyl bromide may be obtained under certain conditions from acetylene by the addition of HCl or HBr:

$$CH{\equiv}CH + HCl \longrightarrow CH_2{=}CHCl$$

While vinyl fluoride is inert, vinyl chloride and vinyl bromide slowly

[67] P. J. Flory, *J. Am. Chem. Soc.*, 65, 372 (1943).

polymerize in the dark (rapidly, in the light)[68] to form solid, slightly soluble polymers, which decompose on fusion. As Klatte and Rollett[69] showed in 1917, polymerization is accelerated by the presence of benzoyl peroxide. When polymerization is interrupted at an earlier stage, the products obtained are soluble in chlorobenzene. Drawn threads give a fiber diagram with an identity period along the fiber axis of 5.2 Å.; this corresponds to two residues, arranged zigzag.[70] The residues do not lie in one plane, otherwise the identity period would be 2.7 Å. The magnitude of the fiber period provides complete support for the formula given above; this has also received chemical confirmation. In dioxane, polyvinyl chloride does not give an iodine precipitate with sodium iodide, as it would if the halogen atoms were attached to neighboring carbon atoms. If, as was postulated in sect. II 2, the chains contain asymmetrical C atoms distributed statistically, unit cells would not have strictly identical atomic structures. Zinc splits off halogen from polyvinyl chloride without cross linkage occurring. Rings are formed in the chains, regarded by Marvel[71] as propane rings, and part of the halogen remains in the chain:



According to Staudinger and Schneiders,[72] polyvinyl chlorides from different sources but of the same molecular weight exhibit the same intrinsic viscosity in solution. From this we may conclude that the chains are unbranched, since otherwise samples with different degrees of branching (the molecular weight being the same) would exhibit different viscosities. According to measurements of osmotic pressure, the molecular weights of products used as synthetics lie between 60,000 and 150,000; the density is 1.38; the dielectric constant is 3.1–3.4. Polyvinyl chloride is insoluble in water, alcohol, and saturated hydrocarbons, but swells and may dissolve in benzene, chlorobenzene, esters, and ketones. At normal temperatures it is resistant to the action of acids (HCl at all concentrations, H_2SO_4 up to 50%, dilute HNO_3), alkalis (up to 20% caustic soda) and salts. Above 85°C. it softens, but cannot be melted without decomposing. It will

[68] V. Regnault, *Ann. chim. phys.*, **69**, 151 (1838).

[69] F. Klatte and A. Rollett, U. S. Pat. 1,241,738 (1914). F. Klatte and H. Müller, U. S. Pat. 1,920,403.

[70] R. Brill and F. Halle, *Naturwissenschaften*, **26**, 12 (1938).

[71] C. S. Marvel, J. H. Sample, and M. F. Roy, *J. Am. Chem. Soc.*, **61**, 3241 (1939). F. T. Wall, *ibid.*, **62**, 803 (1940).

[72] H. Staudinger and J. Schneiders, *Ann.*, **541**, 151 (1939).

withstand heating for a short period from 150° to 200°C. If polyvinyl chloride is compressed at 150–160°C., the particles fuse together and at ordinary temperatures the mass is tough and leathery. Polyvinyl chloride is used in the form of a very finely sieved powder for molding under pressure. It is also available as foil and in this form is used for waterproofing articles of clothing. The strength of these foils can be considerably increased by racking. Strengthened foils of this kind are used chiefly for electrical purposes. Polyvinyl chloride is also worked into the form of chemical-resistant tubes.

Polyvinyl chloride of low molecular weight obtained by emulsion polymerization is used for lacquering metals. A postchlorinated polyvinyl chloride containing 64% chlorine has a higher softening temperature, is readily soluble in acetone, and can be spun from acetone solutions to form nonrotting, chemical-resistant threads. This material can be made into acid- or alkali-resistant fabrics, such as filter cloths. It was produced and marketed by I. G. Farbenindustrie in 1934 under the name of W-K-silk (later Pe-Ce-fiber), which was the first fully synthetic fiber.

Objects molded from polyvinyl chloride can be stuck together with methylene chloride. On the addition of tricresyl phosphate or other plasticizers (liquid or low-melting, nonvolatile swelling agents), polyvinyl chloride becomes softer and rubberlike. It can then be worked into tubes and similar objects, whose resistance to the action of sunlight, water, and oxidizing agents is greater than that of objects made from rubber. Polyvinyl chloride powder may be mixed with cold plasticizers in which the resin is insoluble. It is then spread as a thick paste and heated until it congeals. Under the action of heat the resin becomes soluble in the plasticizer and forms a rubbery material used for shoe soles or acid-proof gloves and coated fibrous materials.

Vinyl chloride may be polymerized with other olefin derivatives to form copolymers, among which those formed with vinylidene chloride, vinyl acetate, and acrylic acid esters are the most important. These will be described in connection with polyvinyl acetate.

(*b*) *Polyvinylidene Chloride*

$$\left[\text{—}CCl_2\text{—}CH_2\text{—}CCl_2\text{—}CH_2\text{—}CCl_2\text{—}CH_2\text{—}\right]$$

Asymmetrical dichloroethylene (prepared from 1,1,2-trichloroethane by elimination of hydrochloric acid) can be transformed, like polyvinyl chloride, into polymers of commercial importance. They are manufactured by the Dow Chemical Company under the name *Saran* and are

used in the same way as polyvinyl chloride. Their water resistance and mechanical properties are recommended.

The material is amorphous but becomes crystalline on drawing; it gives a comparatively sharp fiber diagram with a fiber period[73] of 4.7 Å., indicating a deviation from the plane zigzag chain. The diagram shows that the residues are arranged head-to-tail. (Density 1.88; index of refraction 1.63; softening temperature 210–220°C.) Polyvinylidene chloride is insoluble in all organic solvents at temperatures below 100°C. Since pure polyvinylidene chloride cannot be worked with plasticizers its properties have been improved by copolymerization. Genuine copolymerization is difficult to obtain and most of the so-called copolymers are mixtures of two polymers. The mixture of the two monomers, for example, vinylidene chloride and vinyl chloride, polymerizes much more slowly than either monomer separately.[74]

Because of its resistance to the action of water and chemicals, as well as its good mechanical properties, polyvinylidene chloride and its copolymers are becoming of increasing industrial importance. In addition to being used for coatings, it is used particularly in the making of fabrics and bristles.

(*c*) *Polychlorotrifluoroethylene*

Probably —CClF—CF_2—CClF—CF_2—CClF

Among the polymers of ethylene containing both chlorine and fluorine substituents polymonochlorotrifluoroethylene is coming into prominence (*Fluorothene* of Union Carbide and Carbon Corp.) It can be molded by compression, injection, or extrusion into tubes, rods, and containers which are resistant to inorganic agents such as strong acids though not to molten alkali.[74a]

(*d*) *Polytetrafluoroethylene*

—CF_2—CF_2—CF_2—CF_2—CF_2—

Polytetrafluoroethylene (*Teflon*) developed by du Pont is worthy of notice because of its unique properties.[75] It is obtained by the polymerization of tetrafluoroethylene under pressure and in the presence of suitable catalysts. The considerable heat of polymerization (> 25,000 cal. per

[73] W. C. Goggin and R. D. Lowry, *Ind. Eng. Chem., Ind. Ed.*, 34, 327 (1942).

[74] R. C. Reinhardt, in J. Alexander, *Colloid Chemistry*. Vol. VI, Reinhold, New York, 1946, p. 1019.

[74a] G. M. Kline, *Ind. Eng. Chem.*, 41, 2132 (1949).

[75] M. M. Renfrew and E. E. Lewis, *Ind. Eng. Chem.*, 38, 870 (1946).

mole) must be carefully dissipated since otherwise the somewhat unstable monomer is decomposed.

Polytetrafluoroethylene, on the other hand, is very stable. (Density 2.2–2.3; index of refraction 1.37–1.38.) It is microcrystalline and only becomes transparent and somewhat softer above 320°C. Above 400°C. it begins to evaporate without melting. It neither swells nor is soluble in any solvent and is extremely resistant to oxidation or to strong acids; only molten alkali attacks it. This thermal and chemical stability is associated with very remarkable electrical properties: very low dielectric constant and very low dielectric loss (power factor). In those respects it surpasses polythene and polystyrene.

Polytetrafluoroethylene is admittedly very difficult to work but it may be assumed that it will find application as a heat-resistant insulating material of high quality for high-frequency work and that it will be used in the construction of tubes and pumps for chemically active substances. It can be obtained as strips, sheets, rods, and tubes.

3. Polyvinyl Esters

(a) *Polyvinyl Acetate*

$$—CH_2—CH(OCOCH_3)—CH_2—CH(OCOCH_3)—CH_2—$$

This important compound was found by Klatte[76] to be formed by the action of light upon vinyl acetate (also first prepared by him). Vinyl acetate is prepared by the addition of acetic acid to acetylene in the presence of a catalyst:

$$CH_3COOH + CH{\equiv}CH \longrightarrow CH_2{=}CHOCOCH_3$$

It polymerizes readily in light and in the presence of oxygen or peroxides. If polymerization takes place in solution, the solvent exerts a specific action. In chloroform, for example, viscous products having molecular weights of several thousand are obtained; in petroleum ether the products are more viscous. The most viscous products are obtained by polymerization in light in the absence of solvents. The effect of the solvent may be ascribed, according to Taylor and Vernon,[77] to the interruption of the chains by the solvent and the consequent prevention of the development of high polymeric chains. The validity of the head-to-tail formula is demon-

[76] Chemische Fabrik Griesheim-Electron, German Pat. 281,687 (1913) and Supplement (1914).

[77] H. S. Taylor and A. A. Vernon, *J. Am. Chem. Soc.*, **53**, 2527 (1931).

strated by the formation of polyvinyl alcohol on saponification. The structural formula of polyvinyl alcohol has been established beyond criticism.

Medwedew[78] has investigated the kinetics of the polymerization of vinyl acetate catalyzed by benzoyl peroxide. The heat of activation for polymerization is 25,500 cal. per mole.

As was shown by the investigations of Blaikie and Crozier[79] and later by Misch and Picken,[80] high polymeric polyvinyl acetate is incompletely soluble. A certain fraction is soluble, while the remainder swells to a limited extent in benzene and chloroform. Stretched polyvinyl acetate exhibits limited relaxation; this may be shown by following the change in tension at constant deformation. It is, therefore, presumably cross linked in some way. At 70° to 80°C. it melts and, in this condition, exhibits rubberlike elasticity. It may then be stretched reversibly to about twenty times its length, in which process no crystal structure is formed. In the stretched condition it yields an x-ray diagram showing an arc on the equator (corresponding to a net plane spacing of ~7 Å.). This indicates that a parallel orientation of the primary valence chains has occurred (Katz,[81] Misch and Picken[80]). The elastic force–temperature curve also shows that neither crystallization nor melting occurs during stretching and elastic return (Misch and Picken).

It is by no means clear why the substance is unable to crystallize. Staudinger[82] suggests that optical antipodes are developed irregularly along the chains; but against this explanation is the fact that polyvinyl acetate may be hydrolyzed to polyvinyl alcohol, which crystallizes on stretching. It is, however, possible that the irregularities are reduced and become irrelevant by substituting the small OH groups for the more voluminous $CH_3 \cdot COO$ groups.

(*b*) *Copolymers from Vinyl Chloride and Vinyl Acetate or Acrylic Acid Esters*

If comparatively small quantities (12–15%) of vinyl acetate are polymerized together with vinyl chloride, substances are obtained possessing the same chemical resistance as polyvinyl chloride but softening at a lower temperature and (as they do not crystallize) with higher mechanical resistance. They have been put on the market as pressure-molding powder

[78] S. Medwedew, *Acta Physicochim. U.R.S.S.*, **13**, 565 (1940).

[79] K. G. Blaikie and R. N. Crozier, *Ind. Eng. Chem.*, **28**, 1155 (1936).

[80] L. Misch and L. E. R. Picken, *Z. physik. Chem.*, **B36**, 398 (1937).

[81] J. R. Katz, *Kolloid-Z.*, **53**, 51 (1930).

[82] H. Staudinger, *Die hochmolekularen organischen Verbindungen. Kautschuk und Cellulose.* Springer, Berlin, 1932, p. 114.

under the name Vinylite[83] by the Union Carbide and Carbon Corporation. A similar product contains acrylic or maleic esters instead of vinyl acetate.

Copolymers may also be used for injection molding. In this process the substances are heated in a cylinder until they become plastic; they are then forced by means of a piston through a valve into the cold mold in which they solidify. On the addition of a plastizer, rubberlike mechanical properties may be conferred on the press moldings. In this way, copolymers may be used for the construction of lagging and sleeves.

Artificial fibers are also manufactured from Vinylite (Vinyarn, made by the Union Carbide and Carbon Corporation). They are recommended for the same purposes as fibers of polyvinyl chloride; they also find application as insulating material and in the form of flexible elastic sheets for use as packing, as windows, etc.

Polyvinyl acetates having a lower degree of polymerization, as well as copolymers, are used as varnish ingredients and as cements and adhesives. In the United States a polymer of this kind (Vinylite 80) is used for coating the interior of beer containers. Nonpolymerizing maleic ester may also be polymerized with vinyl ester. A copolymer of this type is used in the construction of pipes resistant to the commoner liquid fuels such as gasoline, benzene, and alcohol.

Many other copolymers have been described, for example, those with trifluorochloroethylene.

4. Polyvinyl Alcohol and Polyvinyl Ethers

(a) *Polyvinyl Alcohol*

$$—CH_2—CHOH—CH_2—CHOH—CH_2—CHOH—$$

Polyvinyl alcohol is obtained by the hydrolysis of polyvinyl acetate (Herrmann and Haehnel[84]). In contrast to polyvinyl acetate, it is soluble in water and insoluble in benzene. The alcohol is also said to be formed, under certain conditions, by the hydrolysis of polyvinyl halides.[85] Hydrolysis of polyvinyl acetates of various degrees of polymerization makes it possible to obtain alcohols of corresponding degrees of polymerization.

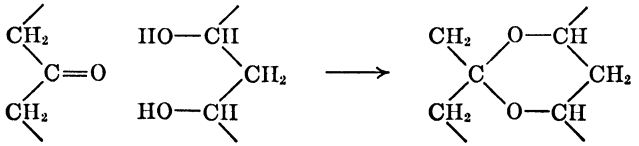
The most highly polymerized products yield viscous solutions in cold water; like starch, they give a blue coloration with iodine. On warming, the alcohol is precipitated. This behavior resembles that of butyl alcohol whose solubility is greater at low than at high temperatures. From the

[83] C. O. Curme and S. D. Douglas, *Ind. Eng. Chem.*, **28**, 1123 (1936).
[84] W. O. Herrmann and W. Haehnel, *Ber.*, **60**, 1658 (1927).
[85] W. Haehnel and W. O. Herrmann, German Pat. 516,996.

temperature coefficient of solubility it is clear that polyvinyl alcohol dissolves in water with evolution of heat. This behavior is also found in ethyl alcohol and in 1,3-butylene glycol, which it resembles in the proportion of —OH groups to hydrocarbon residues. Heat coagulation is not, therefore, associated with the "colloidal character" of polyvinyl alcohol.

At room temperature polyvinyl alcohol is solid. It softens on warming and may then be stretched like rubber, crystallizing in the process. The fiber diagram obtained by Halle and Hofmann,[86] and investigated in detail by Mooney,[87] reveals a pseudorhombic unit cell in which $a = 7.6$, $b = 2.52$ (fiber axis), and $c = 5.60$ Å. The unit cell is traversed by two chains, stretched like paraffin chains (see figures 1 and 2). Pairs of chains are held together by hydrogen bonds (spacing: —OH—OH = 2.7 Å.). Along the chain there is identity after a unit corresponding in length to the CH_2-CHOH residue; this excludes a head-to-head structure with adjacent hydroxyl groups and confirms the validity of the formula given at the beginning of this section. Correspondingly, polyvinyl alcohol reacts only to a very small extent with periodic acid—the reagent used for detecting the presence of 1,2-glycolic[88] linkages. The consumption of periodic acid permits, however, the conclusion that it contains about 1% of glycolic residues possibly formed by the combination of two growing chains[89] or by a chain transfer mechanism whereby a growing free radical of polyvinyl acetate becomes attached to a preformed chain. It is also possible that the rate of the head-to-head reaction is not negligibly small with respect to the head-to-tail reaction (compare section II 2).

Commercial polyvinyl alcohol also contains isolated ketone groups which may form acetal linkages with the hydroxyl groups of other chains. Branched molecules are formed by cross linkages of this kind:



[86] F. Halle and W. Hofmann, *Naturwissenschaften*, **23**, 770 (1935).

[87] R. C. L. Mooney, *Phys. Rev.*, **59**, 925 (1941); *J. Am. Chem. Soc.*, **63**, 2828 (1941). C. W. Bunn, *Nature*, **161**, 929 (1948).

[88] C. S. Marvel and C. E. Denoon, Jr., *J. Am. Chem. Soc.*, **60**, 1045 (1938). C. S. Marvel and E. H. Riddle, *ibid.*, **62**, 2666 (1940).

[89] A. D. McLaren and R. J. Davis, *J. Am. Chem. Soc.*, **68**, 1134 (1946). J. T. Clarke and E. R. Blout, *J. Polymer Sci.*, **1**, 419 (1946).

Polyvinyl alcohol is used in dressing artificial silk. It is also used for the manufacture of highly elastic tubing resistant to oil, benzene, and alcohol.

On partial hydrolysis of polyvinyl acetate, products may be obtained whose solubilities lie between those of the ester and the alcohol. The alcohol may be esterified by the usual methods, for example, with acid chlorides and pyridine. With aldehydes and ketones, acetals are formed whose solubility is about the same as that of polyvinyl acetate but which soften at a lower temperature than polyvinyl alcohol.[90] Such products (for example, the acetal with butyraldehyde) serve as cementing layers in the manufacture of shatterproof glass. By varying the aldehyde or ketone or the particular polyvinyl alcohol, acetals of different solubilities, softening temperatures, and viscosities can be prepared, which are suitable for various purposes.[91]

(*b*) *Polyvinyl Ethers*

$$—CH_2—CHOR—CH_2—CHOR—CH_2—CHOR—$$

Polyvinyl ethers can be obtained directly by the polymerization of vinyl ethers. Monomeric vinyl ethers, formerly prepared by the laborious method of heating the acetals with phosphorus pentoxide in the presence of quinoline, or from chloroacetals and sodium, are now readily prepared by a method developed by Reppe.[92] Acetylene is added to alcohols in the presence of sodium alkoxides (acting as catalysts) at a pressure of 15 to 30 atmospheres at 150°C.:

$$C_2H_5OH + CH{\equiv}CH \longrightarrow C_2H_5OCH{=}CH_2$$

Methyl vinyl ether is a gas, but higher homologues are mobile liquids or solids. According to Chalmers[93] they polymerize in the presence of iodine or on heating to form polymers of low molecular weight. By suitable means, especially with boron trifluoride as catalyst, high polymers are prepared on a commercial scale. According to the alkyl residue and the molecular weight the polymers may resemble fats, soft rubber, or hard wax. The methyl ether is soluble in water and benzene, insoluble in petroleum; the isobutyl ether is insoluble in water, soluble in petroleum, benzene, and similar solvents. (Density of polyvinyl methyl ether 1.045; of the ethyl ether 0.96.)

[90] A. Weihe, *Kunststoffe*, **31**, 52 (1941).

[91] H. Gibello, *Rev. gén. caoutchouc*, **18**, 198, 223 (1941).

[92] I. F. Farbenindustrie A.-G., Brit. Pat. 369,297, *Chem. Zentr.*, **1932**, II, 923. W. Reppe, U. S. Pat. 1,959,927 (1934).

[93] W. Chalmers, *Can. J. Research*, **7**, 472 (1932).

Vinyl ethers are also of commercial importance in copolymers, particularly in the form of latexlike emulsions for the preparation of artificial leather, varnishes on a tissue base, pressed cork plates, etc.[94]

5. Polymers from Allyl Alcohol and Allyl Esters

Allyl derivatives such as allyl chloride, allyl acetate, or allyl alcohol are only induced to polymerize with difficulty, for example, at high pressure and in the presence of a relatively large proportion of benzoyl peroxide. Allyl derivatives can be incorporated in other polymerizing olefins such as styrene. With maleic acid anhydride, which is also difficult to polymerize alone, allyl acetate polymerizes fairly readily[95] in the proportion of 1:1.

Allyl esters of polybasic acids, and also perhaps allyl ethers of polyvalent alcohols, *e.g.*, sorbitol, are used commercially in the "thermosetting plastics" made by the Columbia Chemical Division of the Pittsburgh Plate Glass Co.[96] Since the monomers contain several nonconjugated double bonds capable of polymerization, three-dimensional networks are formed and the higher polymers are therefore insoluble and infusible.

To bring about polymerization the monomer is heated with a certain amount of peroxide. Glass-clear, hard resins are formed, which are valuable on account of their transparency and resistance to abrasion. If mechanically resistant, fibrous substances such as a felt of glass fibers are impregnated with monomer, which is then polymerized, a very strong material is obtained.

6. Polyolefin Aldehydes and Ketones

(*a*) *Polyolefin Aldehydes*

Acrolein, CH_2=CH—CHO, can be induced to polymerize in what appear to be two entirely different ways, which we shall refer to as *vinyl* and *aldehyde* polymerization. Moureu and Dufraisse[97] have made a detailed study of the polymerization of liquid acrolein under the influence of light. It has been known for a long time that acrolein is converted in the light to diacryl, a substance insoluble in ordinary solvents. These workers found that the reaction was accelerated by traces of oxygen as well as by light, and it

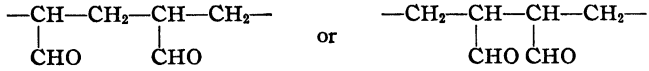
[94] C. Craemer, *Kautschuk*, **15**, 23 (1939).

[95] P. D. Barlett and K. Nozaki, *J. Am. Chem. Soc.*, **68**, 1495 (1946).

[96] A. Pechukas, in J. Alexander, *Colloid Chemistry*. Vol. VI, Reinhold, New York, 1946, p. 1063.

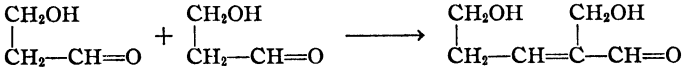
[97] C. Moureu and C. Dufraisse, *Bull. soc. chim.*, **31**, 1152 (1922); (4) **35**, 1564, 1572, 1591 (1924). C. Moureu, C. Dufraisse, and B. Badoche, *Compt. rend.*, **179**, 1229 (1924).

seems probable that acrolein peroxide is the effective catalyst. Hydroquinone and other polyphenols inhibit the reaction. This all points to the occurrence of true vinyl polymerization. It is not known whether the chains grow by head-to-tail or head-to-head additions:



Polymerization in aqueous solution in the presence of dilute alkali proceeds in an entirely different manner; the products first formed are viscous liquids which are gradually transformed into resinous masses. The reaction is not affected by oxygen, and Moureu and Dufraisse therefore regard it as totally different from the process in which diacryl is formed.

We may suppose that the reaction is primarily one of condensation involving the aldehyde group. Hydracrylic aldehyde may well be formed under the influence of the alkali present, and this may react with itself in the following way, the condensation reaction being continued:



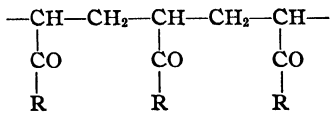
2-methylacrolein can also be caused to polymerize.

It is not clear whether crotonaldehyde, $CH_3—CH{=}CH—CHO$, can be caused to undergo pure vinyl polymerization. On the other hand, it readily forms resinous polymers in the presence of alkalis or amines which are known to catalyze aldehyde condensation. In this process polyenols are formed as intermediate products. These are intensively colored higher aldehydes with conjugated double bonds.

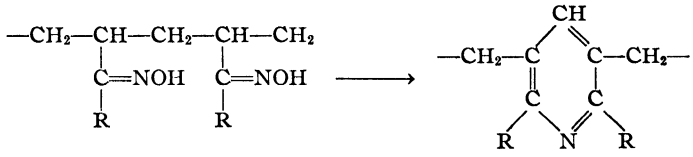
(*b*) *Aldehyde Resin*

The formation of aldehyde resin by the action of alkali on acetaldehyde depends in the first place on the self-condensation of acetaldehyde to aldol and crotonaldehyde. These two types of condensation may continue. Polymerization may also take place at the double bonds, so that larger molecules may be formed. Such polymerization products are used in the preparation of varnishes.

(*c*) *Polyalkyl Vinyl Ketones*



Methyl, ethyl, phenyl, and isopropyl vinyl ketones polymerize readily to yield elastic masses (Blaise and Maire[98]). Marvel and Levesque[99] showed that the head-to-tail formula given above is correct. They found that the compound is converted *via* the ketoxime on heating with alcoholic hydrochloric acid into a pyridine derivative:



7. Polyacrylic Acid and Its Derivatives

$$—CH_2—CH(COOH)—CH_2—CH(COOH)—CH_2—CH(COOH)—$$

or

$$—CH_2—CH(COOH)—CH(COOH)—CH_2—CH_2—CH(COOH)—$$

In the commercial preparation of acrylic acid and its derivatives, ethylene is first allowed to react with hypochlorous acid to form ethylene chlorohydrin, from which ethylene oxide is formed by the separation of hydrochloric acid. This compound adds on hydrocyanic acid and forms ethylene cyanohydrin which, by the elimination of water, forms acrylonitrile. By hydrolysis of the nitrile, acrylic acid is obtained:

$$CH_2{=}CH_2 + HOCl \longrightarrow CH_2ClCH_2OH \longrightarrow \underset{\diagdown O \diagup}{CH_2—CH_2} + HCN \longrightarrow$$

$$CH_2OHCH_2CN \longrightarrow CH_2{=}CHCN \longrightarrow CH_2{=}CHCOOH$$

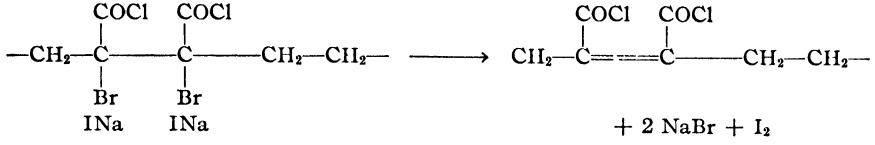
Acrylic acid, $CH_2{=}CHCOOH$, polymerizes in the light, on warming, or in the presence of catalysts. Polyacrylic acid is insoluble in the monomer and in hydrocarbons, but soluble in water and formamide. High polymeric products obtained at low temperature yield very viscous solutions in water. The viscosity of polyacrylic acid and of the alkali salts of polyacrylic acid have been investigated by Staudinger and Kohlschütter[100]; Kern[101] has studied their osmotic properties; Katchalsky has investigated the dissociation of polymethacrylic acid.[102] Aqueous solutions of poly-

[98] E. E. Blaise and M. Maire, *Bull. soc. chim.*, **3**, 265 (1908).
[99] C. S. Marvel and C. L. Levesque, *J. Am. Chem. Soc.*, **61**, 3234 (1939).
[100] H. Staudinger and H. W. Kohlschütter, *Ber.*, **64**, 2091 (1931).
[101] W. Kern, *Z. physik. Chem.*, **A181**, 249 (1938).
[102] A. Katchalsky and P. Spitnik, *J. Polymer Sci.*, **2**, 432 (1947).

acrylates have a limited commercial use, chiefly in the preparation of emulsions.

Acrylic esters polymerize in the light to form insoluble products, obviously of very high molecular weight (Kahlbaum[103]). By varying the conditions of reaction, products of various grades of polymerization may be obtained, ranging from oily liquids (molecular weight, according to Staudinger and Trommsdorff,[104] about 2000) to plastic masses and very viscous rubbery solids. The molecular weight of these cannot be determined, since they show limited swelling in benzene and all other solvents. Acrylonitrile also polymerizes, as do the chloride, the amide, and the anhydride.[105] Polyacrylonitrile may be converted into polyacrylic esters, so that various methods of preparing the polymerized esters are available. Highly polymerized polyacrylonitrile is insoluble, but the acid obtained on saponification is soluble in alkali (Kern[106]); this effect is due presumably to the high molecular cohesion of the nitrile groups, as displayed in the properties of succinonitrile.

The constitution of derivatives of polyacrylic acid differs from that of other vinyl polymers. Marvel[107] has shown that polyacrylic chloride (obtained from polyacrylic acid) can be brominated and the bromine removed by treatment with sodium iodide solution; this operation is only possible if the bromine atoms are attached to neighboring carbon atoms.



It is possible that the structure is irregular and that head-to-tail addition occurs as well as head-to-head.

Acrylic esters have been studied intensively by Otto Röhm, founder and director of the firm of Röhm and Haas, whose interest dates from the period during which he worked under Pechmann's direction, and has been maintained for many years.[108] Acrylic derivatives have been prepared system-

[103] G. W. A. Kahlbaum, *Ber.*, **13**, 2348 (1880); **18**, 2108 (1885).

[104] H. Staudinger and E. Trommsdorff, *Ann.*, **502**, 201 (1933).

[105] C. Moureu, *Bull. soc. chim.*, **9**, 386, 413, 424 (1893).

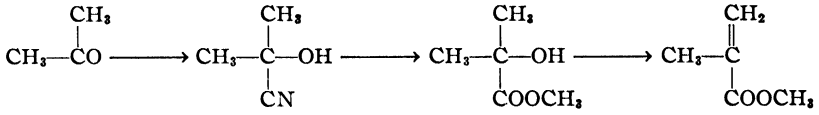
[106] W. Kern and H. Fernow, *J. prakt. Chem.*, **160**, 281 (1942).

[107] C. S. Marvel and J. C. Cowan, *J. Am. Chem. Soc.*, **61**, 3156 (1939). C. S. Marvel and C. L. Levesque, *ibid.*, **61**, 3244 (1939).

[108] O. Röhm, *Dissertation*, Tübingen, 1901. H. v. Pechmann and O. Röhm, *Ber.*, **34**, 427 (1901). O. Röhm, *ibid.*, **34**, 573 (1901).

atically in the laboratories of Röhm and Haas and their suitability for industrial purposes investigated; many have found applications in industry and are now manufactured on an ever-increasing scale.

The esters of α-methylacrylic acid (for which Röhm suggested the name methacrylic acid) are of even greater importance than the derivatives of unsubstituted acrylic acid. They are important starting materials for the preparation of high polymers. Methacrylic esters are obtained by the addition of hydrogen cyanide to acetone, saponification with alcoholic sulfuric acid to obtain the ester, and dehydration with thionyl chloride or some other suitable reagent:



Polymeric esters of acrylic and methacrylic acids with a great variety of alcohols have been described.[109,110] Polymerized esters of di- and multibasic alcohols, for example, polyglycol dimethacrylate, form hard infusible masses as a result of cross linkage. Degree of polymerization and viscosity of fractionated polymethyl methacrylate are related by the following equations:

$$DP = 2810 \times [\eta]^{1.32} \quad \text{(benzene)}^{111}$$

$$DP = 1810 \times [\eta]^{1.22} \quad \text{(chloroform)}^{112}$$

The density of pure vitreous polymethyl acrylate is 1.19, refractive index 1.50, dielectric constant 3.0–3.6.

All pure polyacrylic esters and methacrylic esters are vitreous, transparent, and colorless. They are fairly translucent to ultraviolet radiation and in this respect occupy a position intermediate between glass and quartz. For this reason polyacrylic esters have come into use in industry wherever complete transparency is required. First trade name Plexiglas.

By varying the initial materials and the degree of polymerization, as well as by copolymerization of various acrylates and methacrylates, it is possible to obtain substances differing not only in mechanical properties but also in solubility and in the viscosity of their solutions, so that the range of

[109] H. T. Neher, *Ind. Eng. Chem.*, **28**, 267 (1936).
[110] E. I. du Pont de Nemours & Co., *Ind. Eng. Chem.*, **28**, 1160 (1936).
[111] J. H. Baxendale, S. Bywater, and M. G. Evans, *J. Polymer Sci.*, **1**, 237 (1946).
[112] G. V. Schulz and A. Dinglinger, *J. Prakt. Chem.*, **158**, 136 (1940).

possible applications of acrylic derivatives is very wide. Polyacrylic esters are used in the varnish industry and as adhesives.[113] Emulsions of polyacrylic acid esters prepared by polymerization of the monomeric ester in the emulsified condition are used in the artificial leather industry for the preparation of surfaces to imitate the inner surface of the hide. Monomeric ester mixed to a paste with a hard inorganic filler can be polymerized by heating. In this way artificial teeth and plates are made.

The esters are also prepared as granular powders for injection molding. Trade Names: Plexigum, Lucite, Diakon, Perspex.

Polyacrylic acid ester has been used in the manufacture of safety glass, two plates of glass being cemented together by a layer of the viscous ester. The most interesting application employs methyl and ethyl methacrylates, which are prepared as vitreous sheets, under different trade names, for use as windows for airplanes and motor cars, protective goggles, and shields for scales on instruments. At a suitable temperature the material may be molded like celluloid. Of particular commercial importance on account of its useful rubberlike properties is a copolymer made from acrylonitrile and butadiene, known as Buna N or Perbunan (see Chap. C, sect. V 3*e*).

Polyacrylonitrile itself may be spun into fibers (Orlon) from a solution in dimethyl formamide.[113a] The fibers are said to have outstanding resistance to light and tropical weather conditions. Moreover, acrylonitrile can be obtained by a direct catalytic synthesis[113b] from acetylene and hydrocyanic acid; the economic prospects for this fiber appear to be very promising.

Esters of aconitic acid, $COOHCH_2C(COOH)=CHCOOH$, which can be obtained on a commercial scale from glucose by a special fermentation process, may be polymerized to form vitreous, translucent, and colorless masses similar to polymethacrylates.

8. Polystyrene (Polymerized Vinylbenzene)

Probably $-CH_2-CH(C_6H_5)-CH_2-CH(C_6H_5)-CH_2-$

Polystyrene (discovered about a hundred years ago by Simon)[114] has acquired considerable technical importance in recent years because of its excellent dielectric properties. The initial substance styrene (phenylethylene or vinylbenzene) is prepared commercially from benzene and ethylene, which unit to form ethylbenzene in presence of a catalyst, alumi-

[113] L. Klein and W. T. Pearce, *Ind. Eng. Chem.*, **28**, 635 (1936).

[113a] Inventor H. Rein, I. G. Farbenindustrie Wolfen.

[113b] O. Bayer and P. Kurtz, German Pat. 728,767 (1939).

[114] E. Simon, *Ann.*, **31**, 265 (1839).

num chloride. At a higher temperature ethylbenzene may be decomposed in the presence of suitable contact catalysts into styrene and hydrogen:

$$C_6H_6 + C_2H_2 \longrightarrow C_6H_5CH_2CH_3 \longrightarrow C_6H_5CH{=}CH_2 + H_2$$

Styrene, $C_6H_5CH{=}CH_2$, polymerizes slowly in the dark, more rapidly in the light[115] and at higher temperatures, to form the chain polymer, polystyrene. This seems to be invariably amorphous; even in the stretched condition it shows no sign of crystalline interferences. It is not yet known whether head-to-tail or head-to-head linkages are present. It has long been known that the solubility, melting point, and viscosity exhibited by solutions of the end products may be varied by changing the conditions of polymerization. Dilution by solvents and high temperature or the addition of chain interrupters such as quinone,[116] favor the formation of polymers of low viscosity; low temperature, high concentration, and, above all, a high degree of purity lead to the formation (although much more slowly) of highly viscous products. In emulsions results may be obtained similar to those in the liquid state. As Stobbe showed, the progress of polymerization is most easily followed by viscometric measurement. Staudinger and his collaborators[117] have made a special study of polystyrene and have obtained products of increasing molecular weight by various methods of polymerization. The lower polymers are liquid; those of moderately high molecular weight are solid, softening above 100°C. and readily soluble in ether and benzene; the high polymers are fibrous and swell slowly and eventually dissolve in these solvents.

The constitution of the polymer depends on the conditions of polymerization. According to Schulz,[118] polymers prepared at low temperatures are unbranched chains, while those prepared at higher temperatures are probably branched. If the intrinsic viscosities of products with the same mean molecular weight and similar homogeneity but prepared at different temperatures are compared, the viscosities are lower the higher the temperature at which the specimen was prepared. Since it is a general rule among isomers that the unbranched forms exhibit the highest viscosity, one may assume that in this instance also the specimens prepared at lower temperatures with the highest viscosity are also unbranched or branched to only a slight extent; studies of flow birefringence[119] are in agreement with this

[115] H. Stobbe and G. Posnjak, *Ann.*, **371**, 259 (1909).

[116] J. W. Breitenbach and H. L. Breitenbach, *Z. physik. Chem.*, **A190**, 361 (1942).

[117] H. Staudinger and W. Heuer, *Ber.*, **63**, 222 (1930). H. Staudinger and G. V. Schulz, *ibid.*, **68**, 2320 (1935).

[118] G. V. Schulz, *Z. physik. Chem.*, **B44**, 227 (1939).

[119] R. Signer, *Helv. Chim. Acta*, **19**, 897 (1936).

conclusion. The processes involved in branching are still obscure, but we may suppose that a secondary reaction occurs in that chains are ruptured only to reunite. In this way branched isomers are formed, whose free energy of formation is much lower than that of normal isomers, as shown in Table II of this chapter.

The mechanism of styrene polymerization has been studied by many workers and it is from their findings that the present view of the olefin polymerization process has been developed. If a trace of divinylbenzene is added to the styrene, polymerization products are obtained which show limited swelling (Staudinger and Heuer). It appears that the divinylbenzene residues form links between the chains so that a loose three-dimensional network arises.

High polymeric polystyrene (molecular weight 200,000–300,000) softens at temperatures from 100° to 180°C. and becomes elastic like rubber. It may then be worked into shape by molding or casting under pressure. The product is particularly useful for electrical purposes, owing to its great resistance toward chemical agents and its hydrophobic nature (due to the absence of hydrophilic groups). Furthermore, the valuable dielectric properties, resulting from the absence of polar groups, the isotropic electrical character, and the absence of inhomogeneities of texture render it superior to crystalline substances. It would be difficult to obtain a material superior to polystyrene in these respects. High polymeric styrene swells or dissolves in benzene but not in hexane. Signer has made a detailed study of styrene solutions; he has investigated birefringence of flow, viscosity, and behavior in the ultracentrifuge. The viscosity has been studied by several other authors.[120,121]

If heated above 150°C., polystyrene begins to decompose although Weith[122] found that, in the absence of oxygen, polystyrene resists decomposition below 250°C. At high temperatures the substance can be decomposed fairly completely into styrene. In injection molding, therefore, it is necessary to avoid elevated temperatures and the partial depolymerization which is likely to accompany it. When this happens, cracks appear in the molded articles, as monomer gradually escapes. High polymeric styrenes are broken down by ultrasonic waves to a certain molecular weight, after which further exposure is without effect. According to Schmid,[123]

[120] V. Z. Dancš, *Kolloid-Z.*, **68**, 110 (1934).

[121] A. I. Goldberg, W. P. Hohenstein, and H. Mark, *J. Polymer Sci.*, **2**, 503 (1947).

[122] A. J. Weith, *Ind. Eng. Chem.*, **32**, 1301 (1940).

[123] G. Schmid, E. Beuttenmüller, and A. Rief, *Kunststoff-Tech. u. Kunststoff-Anwend.*, **13**, 65 (1943).

the cause of breakdown is the mechanical vibration to which the molecules are subjected.

Polystyrene is resistant to alcohol, mineral oils, acids, and water, but is attacked by benzene, gasoline, and other organic solvents. (Density 1.05; refractive index 1.67.) The commercial preparation of styrene and polystyrenes was first undertaken by the I. G. Farbenindustrie at Ludwigshafen (trade name, Trolitul). Since then its manufacture has been taken up by many firms: Monsanto (Lustron), Dow (Styron), Bakelite (Bakelite), and Naugatuck (Victron). Commercially it is prepared both by bulk polymerization and by emulsion polymerization. Polystyrene is available in the form of a granular powder for pressure molding, as well as in the form of plates, rods, and tubes. It is used in the manufacture of radio cabinets, refrigerators, shields, flashlights, kitchen accessories, and kindred units. It is indispensable today in ultra-high-frequency technique (for television); it is used in the form of foils for cable insulation after these have been strengthened by transverse and longitudinal stretching (Styroflex foils). The wires of television cables are enveloped in Styroflex silk—threads prepared by extruding heated polystyrene through a duct and stretching it. The lower limit of the diameter of these fibers is about 10 μ.

Expanded polystyrene foam is marketed as Styrofoam. The cross-linked polymers obtained by polymerizing styrene together with a certain amount of divinylbenzene are used in the electrical industry in cases where heat-resistant rods are required. Copolymers with butadiene are used as synthetic rubber (Buna S, GR-S).

It is evident that in place of styrene substituted styrenes may be polymerized. Polymers from *o*-, *p*-, and *m*-methoxystyrene, from anethole ($CH_3OC_6H_4CH{=}CHCH_3$), and from *as*-diphenylethylene, have been described. Polymers of dichlorostyrenes prepared by the Mathieson Alkali Works are of technical interest. They are rather more difficult to work than polystyrene but are more heat resistant. With butadiene they copolymerize to form a heat-resistant rubber. Sulfonated polyanethole (Liquoid Roche) is said to have an effect like that of heparin in preventing clotting of the blood.

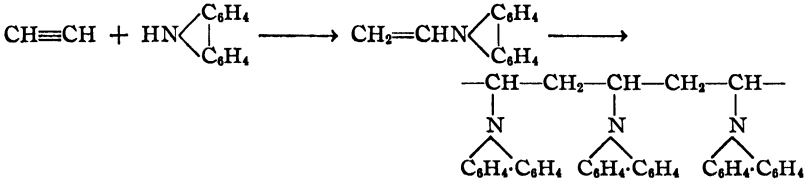
9. Polyvinyl Amines and Other Polyvinyl Derivatives

Polymerization takes place readily in substituted vinyl amines such as methylvinylaniline (prepared by von Braun[124]) and dimethylvinylamine, $CH_2{=}CHN(CH_3)_2$ described by Meyer and Hopff.[125]

[124] J. von Braun and G. Kirschbaum, *Ber.*, **52**, 2261 (1920).

[125] K. H. Meyer and H. Hopff, *ibid.*, **54**, 2274 (1921).

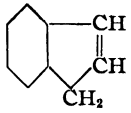
To the class of polymeric vinyl amines belongs polyvinylcarbazole, which is prepared by the addition of acetylene to carbazole and subsequent polymerization of the addition product:



Polyvinylcarbazole was prepared by the I. G. Farbenindustrie A.-G. under the name Luvican and was used as a synthetic in the manufacture of acid- and alkali-resistant moldings made either by press or injection molding (for example, as filter plates). Various brands were manufactured with heat resistance to 100–150°C.

Other olefins such as nitroethylene can also be polymerized.

Among other aromatic-substituted olefins mention must be made of indene:

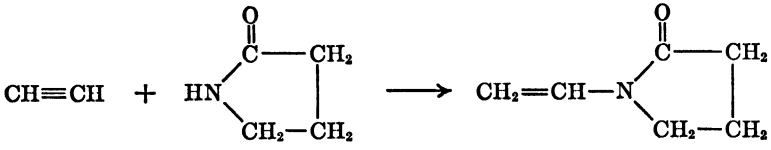


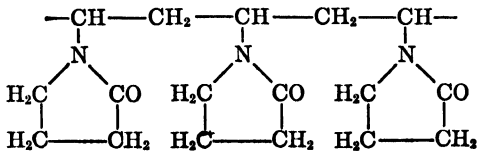
Indene

which may be caused to polymerize by light, heat, and metallic chlorides. The benzene nucleus in polymeric indene can be catalytically hydrogenated to give polyhexahydroindene.

1,2-Dihydronaphthalene can be polymerized with sodium naphthalene.

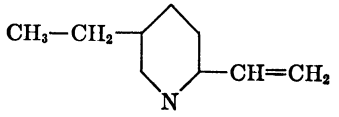
Poly-*N*-vinylpyrrolidone, obtained by polymerizing vinylpyrrolidone, is of some medical interest. Vinylpyrrolidone itself is obtained by the addition of acetylene to pyrrolidone:



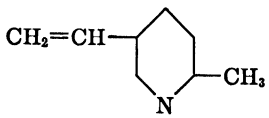


In water this forms highly viscous solutions which are used as a blood substitute when a plasma or serum transfusion is indicated (trade name, Periston).

Following Frank,[126] 2-vinylpyridine which can be prepared from picoline and formaldehyde can be copolymerized with butadiene to give elastomers of good technical quality. Similar experiments have been carried out with substituted vinylpyridines, especially 5-ethyl-2-vinylpyridine (*a*) and 2-methyl-5-vinylpyridine (*b*) which may both be prepared from 5-ethyl-2-methylpyridine:



(*a*)

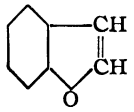


(*b*)

The latter is obtained through condensation of paraldehyde with ammonia.

Copolymers of vinylpyridine and styrene have been described by Fuoss.[127a] Unsaturated quaternary ammonium salts containing two olefinic groups, *e.g.*, triallyl butylammonium bromide polymerizes in the molten state if a suitable peroxide initiator is added.[127b] The reaction products are insoluble space network polymers which can serve as anion exchangers.

Mention must be made of cumarone:



Cumarone

which polymerizes in the presence of sulfuric acid or metallic chlorides, forming polymers of low or moderate degrees of polymerization which are commercially important as cumarone resins. The raw material is that fraction of the coal-tar distillate boiling at 160–200°C. to which, after the removal of acidic and basic constituents, about 1% of concentrated sulfuric acid is added. In this way indene and cumarone are caused to polymerize and are separated as resins. According to the initial material and the conditions of polymerization, soft, hard, or very hard grades with softening temperatures of 50–65°, 60–80°, 80–100°, or 110–140°C. may be obtained.

[126] R. L. Frank *et al.*, *Ind. Eng. Chem.*, **40**, 879 (1948).

[127a] R. M. Fuoss and U. P. Strauss, *J. Polymer Sci.*, **3**, 602 (1948).

[127b] G. B. Butler and R. L. Bunch, *J. Am. Chem. Soc.*, **71**, 3120 (1949).

They are used chiefly in making varnishes and insulating lacquers.

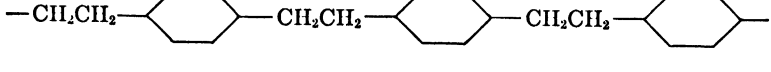
Readily polymerizing substances of this group also include the 1,3 dienes. The simplest representative of this class, butadiene and its homologues, will be considered later, since the polymerized products are closely related to rubber and gutta-percha, whose constitution is better known than is that of the synthetic products. It may be mentioned here that cyclopentadiene readily forms polymers which, on heating at a high temperature, can be cracked to form the starting material for another polymerization. Other diene derivatives, such as furan and pyrrole, are also easily polymerized, but seem not to give rise to products of very high molecular weight.

10. Crystallizability of Vinyl Polymers

Several of the polymers already considered crystallize spontaneously or upon mechanical deformation. These include polymethylene, polyisobutylene, polyvinyl chloride, polydichloroethylene, and polyvinyl alcohol and the series may be extended to include rubber and gutta-percha, to be considered later. On the other hand, polyvinyl acetate, polyacrylic derivatives, and polystyrene, as well as most synthetic rubbers, have not hitherto been crystallized; the same is true of all copolymers. As we have already seen with regard to copolymers of vinyl chloride and vinyl acetate, the crystallization of vinyl chloride can be prevented by the incorporation into the chains of a relatively small quantity of vinyl acetate. Such incorporation is known as internal plasticizing (internal softening).

Coarser irregularities in structure, such as occur in copolymers and in acrylic derivatives, completely prevent crystallization. But this does not explain why polystyrene, and in particular polyvinyl acetate, do not crystallize. The latter must have the same structure as polyvinyl alcohol, which crystallizes readily on stretching, as it is transformed into polyvinyl alcohol on saponification. Presumably we have here an example of that individual readiness to crystallize which is familiar to the organic chemist among organic compounds of low molecular weight; no quantitative interpretation of this phenomenon is as yet available. Since it is probable that the chains are not regularly built, but contain asymmetric *l* and *d* carbon atoms, which are statistically arranged, it is conceivable that the irregularity will be more conspicuous with the more voluminous groups, such as phenyl and acetyl, as substituents, than with chloride and hydroxyl.

11. Poly-*p*-xylylene

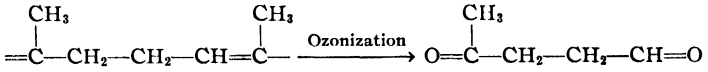


Under suitable conditions elastic-plastic condensation products are obtained by the action of ethylene chloride on benzene in the presence of aluminum chloride, their essential structure is based on the formula given above. They may be used as fillers for rubber or to a limited extent as bonding media for varnishes (AXF resins).[128] The same polymer is obtained as one of the reaction products of the pyrolysis of *para*-xylene.[128a]

IV. RUBBER AND GUTTA-PERCHA[129]

1. Constitutional Formula of Rubber

The chemical facts upon which the "formula" of rubber is based, may be summarized as follows: *Hevea rubber*, as well as that from *Ficus elastica*, when freed from resins and proteins, possesses the elementary formula $(C_5H_8)_n$. Each of these groups contains a double bond, the presence of which is shown by conversion into a dihalide; dry distillation yields isoprene. These experimental facts, which have been known for a long time, provide no information about the arrangement and the linkage of the C_5H_8 groups, and it was therefore a great advance when Harries[130] showed that levulinic aldehyde is obtained in considerable quantities by the action of ozone on rubber and subsequent treatment with water. Since, under the action of ozone, double bonds are fractured and give rise to CO groups, this demonstrated the presence of a structure which is convertible into levulinic aldehyde:



There was still, however, no evidence of the presence of the *cis* or *trans* configuration or of the size of the molecule held together by primary valences. Harries proposed as a formula that of a dimethyl cyclooctadiene, while Weber[131] suggested that the molecules consist of long carbon chains, to which he gave the name *polyprenes*. The same opinion was expressed

[128] S. D. S. Shinkle (to U. S. Rubber Co.). U. S. Pat. 2,016,026 (1935). See also K. Sisido and S. Katôo, *J. Soc. Chem. Ind. Japan*, **43**, 232B, 450B (1940); **44**, 25B (1941).

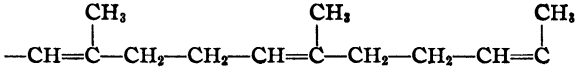
[128a] M. Szwarc, *J. Chem. Phys.*, **16**, 128 (1948).

[129] General monographs: K. Memmler, *Handbuch der Kautschukwissenschaft*, Hirzel, Leipzig, 1930. C. C. Davis and J. T. Blake, *Chemistry and Technology of Rubber*, Reinhold, New York, 1937.

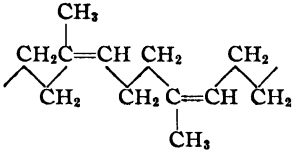
[130] C. Harries, *Ber.*, **37**, 2708 (1904); **38**, 3985 (1905).

[131] C. O. Weber, *The Chemistry of India Rubber*, Griffin, London, 1902; *Ber.*, **33**, 779 (1900); **35**, 1947 (1902); **36**, 3108 (1903).

very forcibly in 1906 by Pickles in a discussion at York. He wrote later[132]: "it is suggested that these unsaturated C_5H_8 nuclei unite to form long chains having the structure:



and that the number of C_5H_8 complexes may vary in different kinds of rubber, the difference being due, probably, to this variation in the number of complexes contained." A long time elapsed before this latter suggestion was generally accepted. The same interpretation was upheld by Staudinger[133] and supported by the preparation of paraffin-like products of very high molecular weight through the catalytic hydrogenation of rubber in the presence of nickel. Since that time, x-ray analysis has provided greater insight into the structure of organic molecules than was possible from chemical methods alone. As we shall see later, x-ray analysis has confirmed and made more precise our picture of the chain structure of rubber. According to Meyer and Mark,[134] the hydrocarbon of rubber possesses the *cis* configuration:



The *trans* configuration holds for the isomeric hydrocarbon, gutta-percha.[135] Rubber has an absorption band in the infrared at 12 μ which is not present in gutta-percha; for the rest, the absorption spectra of the two substances are very similar.[136]

2. Molecular Weight of Rubber

Natural rubber is incompletely soluble in hydrocarbons; almost invariably a highly swollen residue is left, which does not dissolve completely even after a long period of continuous stirring. Pummerer[137] observed this

[132] S. S. Pickles, *J. Chem. Soc.*, **97**, 1088 (1910).

[133] H. Staudinger and J. Fritschi, *Helv. Chim. Acta*, **5**, 785 (1922).

[134] K. H. Meyer and H. Mark, *Ber.*, **61**, 1939 (1928).

[135] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930, p. 205.

[136] S. B. Hendricks, S. G. Wildman, and E. J. Jones, *Arch. Biochem.*, **7**, 427 (1945).

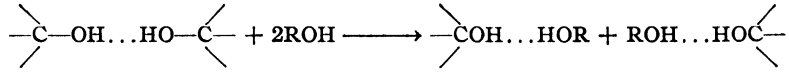
[137] R. Pummerer and H. Pahl, *Ber.*, **60**, 2152 (1927). R. Pummerer, A. Andriessen, and W. Gündel, *ibid.*, **61**, 1583 (1928).

phenomenon when dissolving rubber in ether, and suggested the name *sol rubber* for the soluble portion and *gel rubber* for the insoluble fraction.

The molecular weight of the fraction of Hevea rubber soluble in benzene or toluene has been estimated at between 300,000 and 500,000 by osmotic and ultracentrifuge methods. If the large drops of latex (0.6 to 1 μ in diameter) are separated from the small drops (0.1–1.6 μ) in natural Hevea latex, it is said that the former contain a rubber of higher molecular weight than that present in the latter (Hessels[138]). A comparative study of the different rubbers from different kinds of plants still has to be made.

After being broken down by mastication, rubber has a lower molecular weight. A value of 130,000 was obtained for the molecular weight of the product known as "feuille anglaise." Gel rubber owes its limited swelling to its structure: it consists of a loose, three-dimensional net in which the chains are linked together by bridges.

It has long been known to industrial chemists that gel rubber dissolves almost completely in benzene if a little butyl alcohol is added. According to Pummerer, Kroepelin, and Miedel[139] gel rubber may also be brought into solution in organic solvents by the addition of small quantities of bases, such as aliphatic amines. Links in the network which are broken by these reagents cannot be primary valence linkages but can only be hydrogen bonds such as associated C—OH, COOH or C=O groups. We conclude, therefore, that the chain molecules of rubber contain C—OH or C=O groups at certain points (perhaps as a result of changes subsequent to their extraction from the plant) and that these associate with corresponding groups of neighboring chains. This association is destroyed by the addition of substances containing dipoles which solvate the groups concerned:



Since the length of the chain may amount to 20,000 carbon atoms, an extremely small quantity of bound oxygen, namely 0.01% (corresponding to two atoms of oxygen per chain), suffices to bring about cross linking. It is possible that the groups which associate are formed by secondary reactions, particularly by oxidation in the air, since freshly tapped latex contains little gel rubber, and the gel rubber content increases progressively in the stored product. The gel rubber content also increases as a result of various coagulative or preservative treatments; "fine Para" from the

[138] J. H. E. Hessels, *Dissertation*, Delft, 1943.

[139] R. Pummerer, H. Kroepelin, and H. Miedel, quoted by K. Memmler, *Handbuch der Kautschukwissenschaft*. Hirzel, Leipzig, 1930, p. 203.

Brazilian forests, smoked over an open fire, is particularly rich in gel rubber.

Gel rubber which remains insoluble in all solvents contains a certain amount of oxygen, as Weber showed. It is possible that ether bridges have formed in samples of this kind, or it may have undergone some other change, which may, perhaps, be the same as that produced by the action of light or heat. It contains in all probability homopolar linkages formed with the loss of a double bond.

Branching may be regarded as a preliminary step toward the formation of a network of chains since a structure in which an oxygen bridge joins two chains at their middles has a branched form. It may be assumed that such branched molecules will arise as a result of secondary oxidations; there is no reason to suppose, however, that natural rubber has a structure other than of an unbranched hydrocarbon. The molecular weight of fractionated rubber is related to the viscosity in toluene by the following equation:[140]

$$\log M = 5.85 + 1.5 \log [\eta]$$

Rubber which has been broken down to a certain extent by treatment with oxygen, and the low molecular weight fraction of native rubber which is soluble in ether, may be obtained from very dilute solutions in ether in the form of crystalline aggregates (Smith, Saylor, and Wing[141]). (The melting point of these is 10–11°C.; refractive indices: $\epsilon = 1.535$, $\omega = 1.563$, at -5°C.)

3. X-Ray Analysis of Structure of Rubber

In the discussion of the results of x-ray analysis differences in solubility may be disregarded, since they are due to relatively inconsiderable changes in the structure of the chains and, as has been shown by Clark,[142] the x-ray diffraction patterns of the various soluble fractions are identical with one another.

(*a*) *X-Ray Diagram of Unstretched and Stretched Rubber*

The first x-ray diagrams of unstretched rubber were obtained by Scherrer.[143] They were typical amorphous diagrams and could not be evalu-

[140] W. C. Carter, R. L. Scott, and M. Magat, *J. Am. Chem. Soc.*, **68**, 1480 (1946).

[141] W. H. Smith and C. P. Saylor, *J. Research Natl. Bur. Standards*, **13**, 453 (1934). W. H. Smith, C. P. Saylor, and H. J. Wing, *ibid.*, **10**, 479 (1933).

[142] G. L. Clark, W. J. Warren, and W. H. Smith, *Science*, **79**, 433 (1934). G. L. Clark, E. Wolthuis, and W. H. Smith, *J. Research Natl. Bur. Standards*, **19**, 479 (1937).

[143] P. Scherrer, in R. Zsigmondy, *Lehrbuch der Kolloidchemie*. 3rd ed., Spamer, Leipzig, 1920.

ated. Katz[144] was able to obtain a fiber diagram from stretched rubber after Hock[145] had concluded that stretched rubber probably had an oriented

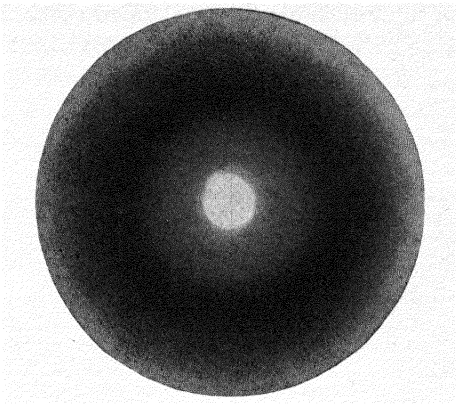


Fig. 4. X-ray diagram of unstretched, nonfrozen rubber (smoked sheet).

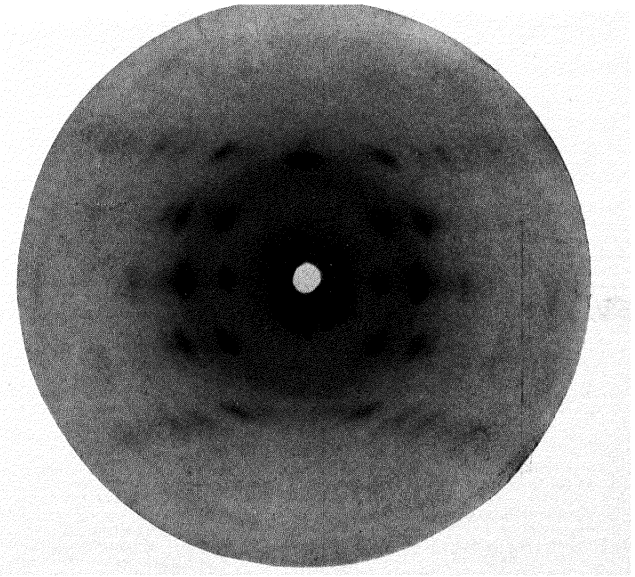
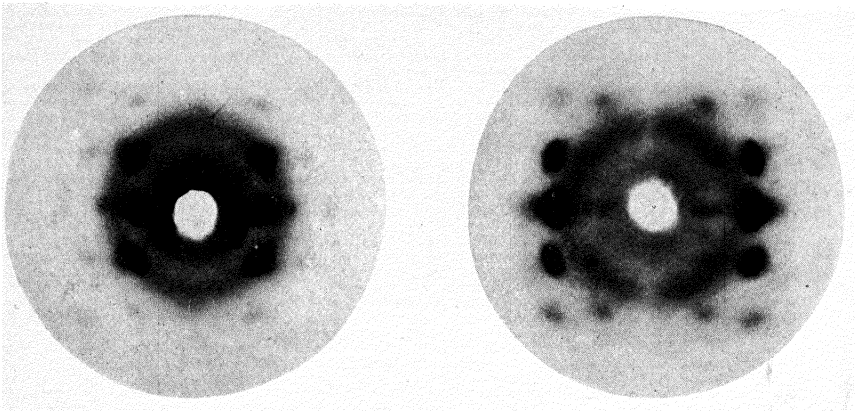


Fig. 5. Fiber diagram of stretched rubber. Preparation inclined at 8° to the normal. 002 is visible.

[144] J. R. Katz, *Naturwissenschaften*, **13**, 410 (1925).
[145] L. Hock, *Kolloid-Z.*, **35**, 40 (1924).

structure in view of the anisotropy of its properties. Figure 4 shows the x-ray diagram of unstretched rubber. The similarity between this diagram and that of a normal liquid is apparent. Figure 5 is the x-ray diffraction pattern of stretched rubber, showing well-defined reflections, the positions of which can easily be determined. Following Katz's discovery, the fiber



(*a*) (*b*)

Fig. 6. Rubber higher orientation: (*a*) incidence perpendicular to surface; (*b*) incidence parallel to surface.

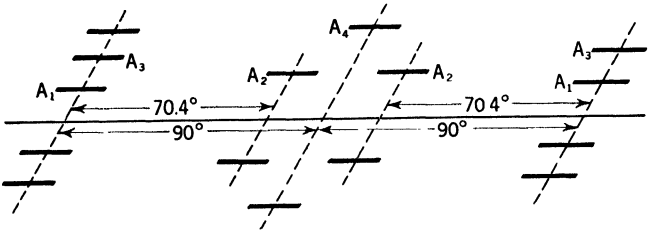


Fig. 7. Diagram showing doubling of the reflections in Weissenberg photographs.

diagram of stretched rubber was discussed in a number of publications with the purpose of establishing the unit cell, the space group, and a complete model of the crystal structure. Mark and von Susich[146] and Mark and Meyer[147] obtained more highly oriented preparations by stretching thin films. Such a film, irradiated at right angles to the plane of the film,

[146] H. Mark and G. von Susich, *Kolloid-Z.*, **46**, 11 (1928).

[147] H. Mark and K. H. Meyer, *Z. physik. Chem.*, **B38**, 395 (1937).

yields the diagram shown in figure 6*a*. When the preparation is irradiated by a beam parallel to the plane of the film, the diagram shown in figure 6*b* results; the reflections in figure 6*a* disappear for the most part and those appear which correspond to planes at right angles, or almost at right angles, to the planes responsible for the reflections in figure 6; that is, planes are now in a position to reflect which were not able to do so when figure 6 was taken.

In a paper by Morss,[148] no definite conclusion was reached, and indeed a definite answer to the problem cannot be expected from fiber diagrams such as those on which Morss's argument is based.

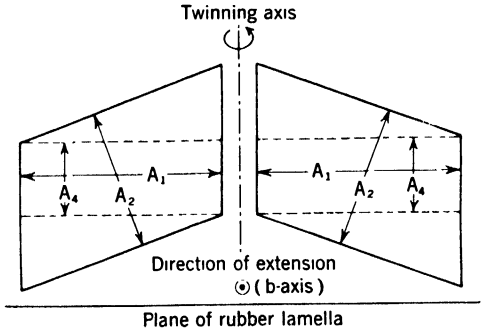


Fig. 8. Position of the crystallites in stretched rubber.

The question of the unit cell was re-examined by van der Wyk and Misch.[149] By means of Weissenberg and oscillating photographs, it was shown that certain reflections are doubled at the equator and on the first and second layer lines, as is shown schematically in figure 7. This doubling shows that in stretched rubber the crystallites tend to take up two preferred positions (the one derived from the other by rotation through 180°) during orientation (Fig. 8). A similar suggestion of double orientation or twinning was made by Morss.

The nature of the doubling and the appearance of sharp diatropic reflec tions in oblique fiber diagrams (see Fig. 5) show that the translation lattice may well be rhombic. The lattice constants obtained are:

$$a = 8.97 \text{ Å}.$$
$$b = 8.20 \text{ Å}.$$
$$c = 25.2 \text{ Å}.$$

[148] H. A. Morss, Jr., *J. Am. Chem. Soc.*, **60**, 237 (1938).

[149] A. J. A. van der Wyk and L. Misch, *J. Chem. Phys.*, **8**, 127 (1940).

The calculated density, assuming that 16 isoprene groups are present in the unit cell, is 0.97; this is in good agreement with the highest observed value of 0.965 in stretched and frozen rubber. Although this lattice accounts for all the observed reflections, the unusual dimensions of the unit cell and the abnormally high number of extinctions (93% of the possible equatorial reflections) are very striking. These facts support the suggestion that the rubber lattice may be represented by a smaller unit cell.

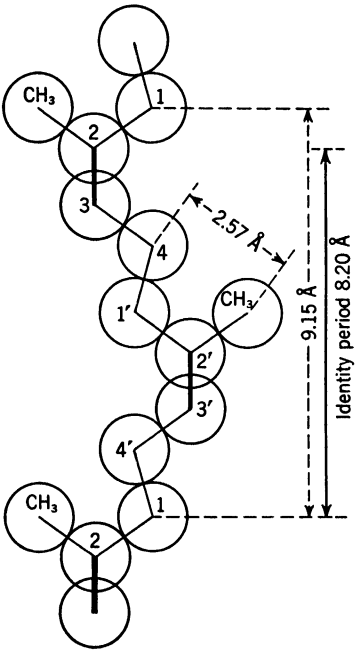


Fig. 9. Rubber chain stretched out flat; the identity period is greater than that observed.

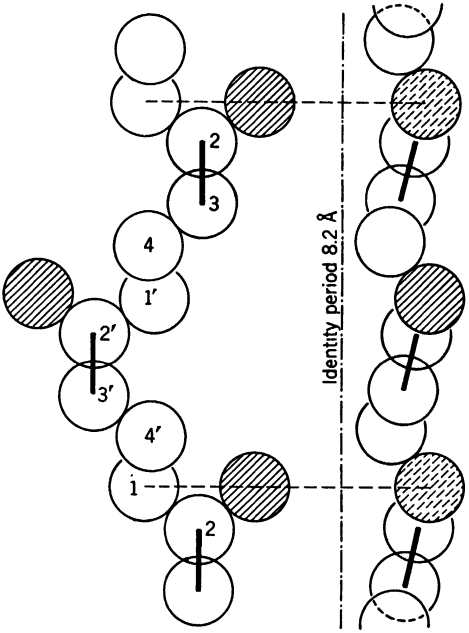


Fig. 10. Projections of the rubber chain: rotation of $\sim 50°$ in the same sense about all 1–2 linkages, and in the opposite sense about all 3–4 linkages.

Van der Wyk and Misch showed that this is possible only if one assumes that at least two unit cells are present in rubber, monoclinic and triclinic.

The question remains whether the two lattices occur independently, in which case rubber just like gutta can exist in two modifications, or whether the lattices must be regarded as parts of a larger lattice, formed by their mutual interpenetration. Up to the present this question has not been answered. The chains of the two modifications must be very similar in

shape, since the length of the two isoprene groups (that is, the identity period along the b axis) and the projection on the equatorial plane is the same in both.

A more recent suggestion is due to Bunn.[150] He suggests a monoclinic cell with the dimensions: $a = 12.46$ Å., $b = 8.10$ Å. (fiber axis), $c = 8.89$ Å., and $\gamma = 92°$. This solution is not entirely satisfactory since one inter-

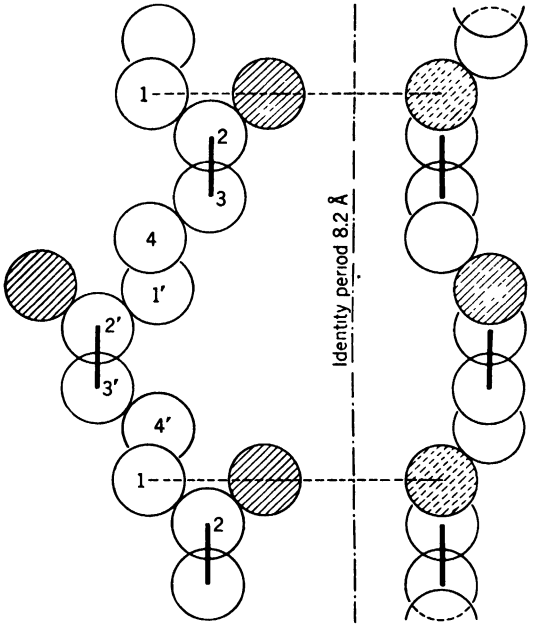


Fig. 11. Projections of the rubber chain; rotation of ~ 50° alternately about the 1–2 linkages, and in the opposite sense alternately about the 3–4 linkages.

ference on the second layer line is not adequately accounted for; moreover, the volume for the unit cell given by Bunn, 896 Å.^{3} is strikingly small and leads to a density of 1.01. Bunn's solution cannot therefore be regarded as final.

As Lotmar and Meyer[151] pointed out, the length of the b axis (8.20 Å.) does not permit the residues to be disposed in the fully stretched configuration, which would necessitate an identity period of at least 9.15 Å. (see Fig. 9). The small distance from carbon atom 4 to the nearest CH_3 group

[150] C. W. Bunn, *Proc. Roy. Soc. London*, **A180**, 40 (1942).
[151] W. Lotmar and K. H. Meyer, *Monatsh.*, **69**, 115 (1936).

(2.57 Å.), which results if one supposes that the chain is lying in the plane of the paper, as in figure 9, is also irreconcilable with common experience of intermolecular spacings. These anomalies disappear if a rotation of about 50° about the linkage of carbon atoms 3 and 4, and a second rotation of the same amount but in the opposite direction about the linkage of atom 1′ to 2′, are postulated. The identity period along the chain axis then becomes 8.20 Å. and the distance $CH_3 \ldots CH_2$ increases to 2.9 Å. As Misch and van der Wyk pointed out, this arrangement also makes possible the formation of two similar configurations of equivalent energy contents, according to whether the two rotations are in the same or alternately in opposite senses. The two rather flat configurations conforming to this behavior are shown in orthogonal projection in figures 10 and 11. These chain formulas satisfy all the requirements of the x-ray findings. Neither configuration possesses a center of symmetry or a plane of symmetry; in both cases optical antipodes are to be expected; four different forms of chains may thus be present in stretched rubber.

Bunn also discusses the shape of the chains and in connection with his unit cell suggests other forms for the chains which, like those considered above, are derived from each other by rotation about two linkages. In his models the isoprene residue is flatter, which is improbable theoretically.

(*b*) *Shape and Size of Crystallites*

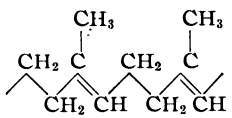
The size of the crystalline regions in stretched rubber was estimated by Hengstenberg[152] from the angular width of the reflections. The values obtained were: length (along the fiber axis) > 600 Å., width ~500 Å., and thickness ~150 Å. These values have little absolute accuracy but it is justifiable to assume that the crystallite has the shape of a long, flat box. This conclusion clarifies the circumstance that rubber crystallites can be brought to higher orientation with comparative ease.

The size of the crystallites varies with the previous history of the sample; thus, the spots become smaller when stretched rubber is stored for a long time in the cold, indicating that the crystallites have grown. According to Hengstenberg,[152] interference phenomena suggesting the existence of larger crystalline regions appear under certain conditions. It will be shown later that the rise in melting point of frozen rubber over a period of years indicates a very slow increase in the size of the crystallites.

[152] W. Hengstenberg, in K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*. Akadem. Verlagsgesellschaft, Leipzig, 1930, p. 196.

4. Constitutional Formula and Molecular Weight of Gutta-Percha and Balata

Chemical investigation has shown that the hydrocarbons present in balata and gutta-percha are composed of isoprene units and are closely related to rubber. The fact that neither of these products can be distilled and that their solutions are extremely viscous, suggests that very long chain molecules are present, although they may not be as long as in rubber. From measurements of osmotic pressure by Caspari,[154] a molecular weight of 32,000 may be calculated by extrapolating to zero concentration. More recent measurements by Wolff[155] on recrystallized Tjipetir gutta gave a value of 30,000, whereas Staudinger[156] obtained values around 100,000 for both gutta and balata if prepared from latex. Gutta-percha and balata are both completely soluble in benzene, chloroform, etc. It is is to be concluded, therefore, that no cross linkages are present. Both hydrocarbons, in contrast to rubber, are solid and only slightly extensible at room temperature; when heated to about 50°C., they become elastic and at the same time so plastic that the elastic properties are often masked. Hopff and von Susich[157] were able to demonstrate the identity of fiber diagrams from the two substances and, hence, their structural identity. The hydrocarbon present in both products will be described as gutta in the following account, and (as we shall see later) is to be given the *trans* formula.



Gutta may be precipitated from solutions in petroleum ether, since it is readily soluble on warming but relatively insoluble at 0°C.

Gutta melts at about 50°C.; that is, at this temperature all x-ray reflections disappear and the product becomes extensible, like rubber, but shows very rapid relaxation. Weakly vulcanized gutta crystallizes at room temperature; above 50°C. it is elastic like rubber. (Density of pure gutta, 0.95; refractive index at 20°, 1.52.)

[154] W. A. Caspari, *J. Chem. Soc.*, **105**, 2139 (1914).

[155] E. Wolff, *Helv. Chim. Acta*, **23**, 439 (1940).

[156] H. Staudinger and K. Fischer, *J. prakt. Chem.*, **157**, 19 (1940).

[157] H. Hopff and G. von Susich, *Kautschuk*, **6**, 234 (1930); *Rev. gén. caoutchouc*, **7**, No. 65, 23 (1930).

5. X-Ray Analysis of Structure of Gutta

Up to the present, a complete description of the crystal structure has not been given, owing to two main difficulties: (*1*) It is by no means easy to obtain fiber diagrams of gutta; more highly oriented preparations, the diagrams of which could be indexed with greater certainty, have not been prepared. (*2*) Gutta crystallizes in two different modifications (Hopff and von Susich[157]), the conditions of formation and mutual interconversion of which have been studied by Hauser and von Susich.[158] The slight vulcanization of gutta which has been used by Fuller[159] represents an important advance in technique. Gutta vulcanized in this way can be stretched at 60°C. without flowing and, after cooling in the stretched condition, gives a fiber diagram.

(*a*) *α-Gutta*

The modification which may be crystallized out from petroleum ether is known as the α-modification. The same modification is formed if gutta is heated to 70–75°C. (at which temperature all reflections disappear from the x-ray diagram and the product becomes plastic and elastic) and subsequently very slowly cooled. If rapidly cooled, the β form appears on solidification. From these observations Hauser and von Susich concluded that, below about 68°C., the α modification is stable, while the β form is stable above 68°C. At this temperature, which is above the melting point of both substances, as shown by the disappearance of the spots from the x-ray diagram, a conversion of one form into the other takes place. More recent observations by Fuller run counter to such a conclusion. It has been found that the β modification is converted into the α form at 80°C., if vulcanized gutta is stretched at this temperature. The following interpretation is, perhaps, the most satisfactory: Throughout the whole range of temperature the α form is the thermodynamically stable modification. The unstable, more symmetrical β form is the first to crystallize out from the melt. By rapid cooling, the mass may be "frozen" into this condition but it is converted into the α modification: (*1*) at higher temperatures, if the temperature range through which the crystalline phase persists is extended upward by stretching (see sect. VI 4*b*); (*2*) by prolonged tempering at 60°C.; and (*3*) by dissolving and crystallizing out from solution.

[158] E. A. Hauser and G. von Susich, *Kautschuk*, **7**, 120, 125, 145 (1931).
[159] C. S. Fuller, *Ind. Eng. Chem.*, **28**, 907 (1936).

Figure 12 shows a particularly clean fiber diagram of the α modification, obtained by Lotmar from vulcanized gutta. From this diagram, only the identity period along the fiber axis (8.70 Å.) can be determined accurately. Furthermore, the absence of the odd diatropic reflections lends support to the view that digonal screw axes parallel to the fiber axis are present. According to Misch, the reflections of the α-gutta diagram can be accounted for only by a monoclinic unit cell containing not less than 16

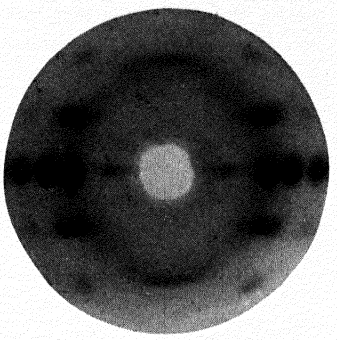


Fig. 12. Fiber diagram of stretched α-gutta (Lotmar).

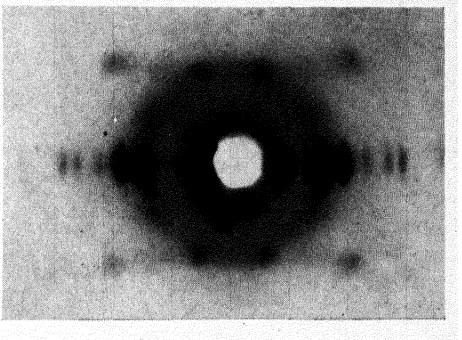


Fig. 13. Fiber diagram of β-gutta (Misch).

isoprene residues or 8 chains. Since it has not, as yet, been possible to obtain more highly oriented preparations, the indexing of the diagrams is somewhat uncertain.

(*b*) *β-Gutta*

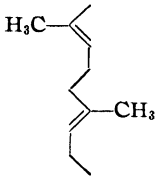
The β modification is formed by heating to 70°C., and cooling rapidly. If gutta is stretched at 30° to 40°C., fiber diagrams of the β modification are obtained (Fig. 13). The identity period along b is about half as great as that in the α modification; accurate measurements by von Susich and Hauser give 4.87 ± 0.07 Å., those of Fuller, 4.77 ± 0.03 Å. It may be assumed that the value is very close to 4.8 Å. Fuller considers also the possibility of an identity period of twice this value; we believe, however, that there is no evidence for this; no trace of a reflection on the corresponding layer line can be detected. From the remaining reflections, Fuller decides upon an orthorhombic unit cell with the dimensions:

a = 11.9 Å.
b = 4.8 Å. (identity period along the fiber axis)
c = 7.85 Å.

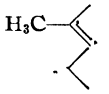
This unit cell contains four isoprene groups. If the chains are arranged parallel to the *b* axis, an identity period of 4.8 Å. is possible only if the chains have the *trans* and not the *cis* configuration. A glance at figure 10 shows that the *cis* form cannot be arranged in the same way. It is therefore certain that the *trans* form of the chains is present in gutta, while rubber consists of the *cis* modification (Meyer and Mark[160]).

(*c*) *Shape of Chains in α- and β-Gutta*

The two forms may be referred in principle to the structures represented in the following formulas:



α-Gutta
Identity period of two units



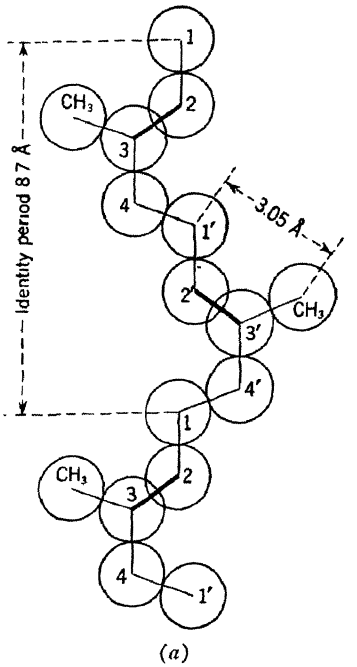
β-Gutta
Identity period of one unit

An arrangement of the isoprene units in one plane, as shown in figure 14, leads, on the assumption of the known values for primary valence spacings and valence angles, to an identity period of 8.7 Å., which corresponds exactly to the value observed in α-gutta. Moreover, as a result of the *trans* linkage, the smallest distance between the CH_3 group and the nearest CH_2 group is rather more than 3 Å., so that in this respect also (in contrast to the case of rubber) the plane arrangement appears extremely probable. The extinction of the odd diatropic reflections and the appearance of particularly intense equatorial reflections is in complete agreement with the shape of chain given here.

The situation is quite otherwise in the case of β-gutta. As figure 15 shows, an arrangement of the units in one plane leads to an identity period of 5.05 Å. instead of the observed value of 4.8 Å., and the distance of only 2.57 Å., between the CH_3 group and C atom 4, disagrees with experience. As in the case of rubber, these contradictions can be overcome by supposing a rotation of *ca.* 50° about the linkage 1–2 and one in the opposite sense about the linkage 3–4. The projections of the resulting chain of isoprene groups are represented in figure 16. The identity period agrees with the observed value (4.8 Å.) and the distance $CH_3 \ldots CH_2$ becomes *ca.* 2.9 Å.

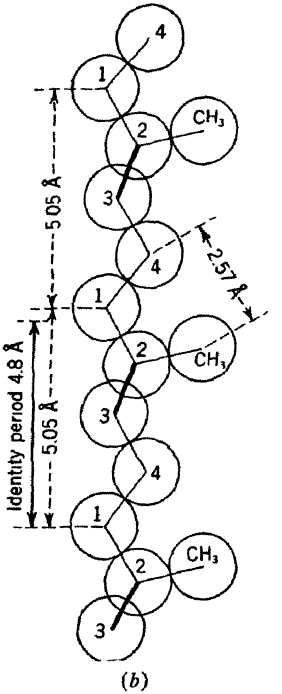
[160] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, p. 205.

The two configurations of the chains in α- and β-gutta differ much more than the two possible chain configurations of rubber. The chains of α-gutta possess a reflection plane (the plane of the paper in Fig. 14); optical antipodes are not, therefore, to be expected. On the other hand, the atoms



(a)

Fig. 14. Chain model of α-gutta. The fiber period of the flat, stretched form is equal to the observed identity period.



(b)

Fig. 15. β-gutta, stretched and flat. The fiber period is larger than the observed value.

of the β form, like the atoms in the two forms of rubber, are not arranged in one plane; no plane of symmetry or center of symmetry occurs. As in the case of rubber, optical antipodes are to be expected. We know that, in the crystal lattice of paraffins, the plane arrangement is preferred. This is possible only in the α-gutta, since the repulsion of the CH_3 groups in β-gutta (as in rubber) leads to a more open form of the chains.

Regarding the arrangement of the chains in the lattice, no satisfactory hypothesis can be advanced at the present time, either for the α or for the β modification. More recent suggestions of Bunn and Jeffrey for a three-

dimensional model of β-gutta are regarded by the authors as only tentative.[161]

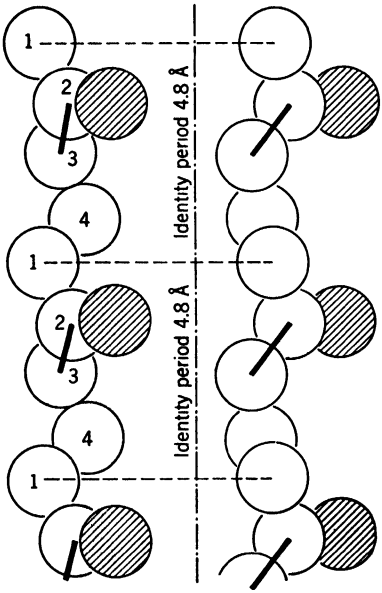


Fig. 16. Model of β-gutta (orthogonal projections).

6. Chemical Reactions of Rubber and Gutta-Percha

(a) *Vulcanization of Rubber*

By treating rubber with sulfur or sulfur chloride under suitable conditions, the mechanical properties and extensibility of rubber may be influenced, as is well known, to a very large extent. There is no longer any doubt that this process, known as vulcanization, depends essentially on a chemical reaction between sulfur and rubber, a conception first developed by Weber,[162] at a time when vulcanization was often looked upon as a simple process of adsorption. The nature of the reactions involved in heat vulcanization is uncertain, but during vulcanization in the cold it is known that both chloride and sulfur unite with the rubber (Henriques,[163] Weber, Hinrichsen and Kindscher[164]). In addition, however, one atom of

[161] C. W. Bunn, *Trans. Faraday Soc.*, **38**, 384 (1942). G. A. Jeffrey, *ibid.*, **40**, 517 (1944).

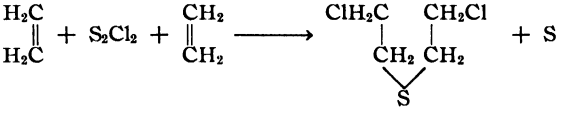
[162] C. O. Weber, *The Chemistry of India Rubber*. Griffin, London, 1906.

[163] R. Henriques, *Chem. Ztg.*, **17**, 634 (1893); **18**, 701, 1155 (1894).

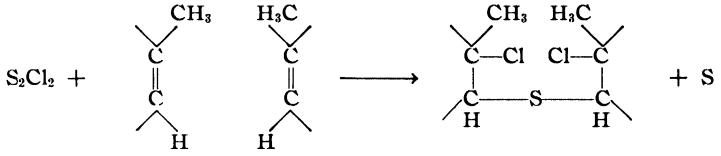
[164] F. W. Hinrichsen and E. Kindscher, *Kolloid-Z.*, **6**, 202 (1910).

sulfur per molecule of S_2Cl_2 is liberated and appears in the rubber as amorphous sulfur, soluble in sulfites but insoluble in acetone (Meyer and Hohenemser[165]); this was overlooked by the authors mentioned above.

The same reaction is known to occur with ethylene, and is used in the preparation of dichlordiethyl sulfide (mustard gas):

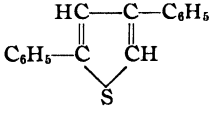


The process of cold vulcanization is therefore to be represented in the following way (Meyer and Mark[166]):



The bound sulfur is present as thioether sulfur, C—S—C; to this, CH_3I may be added to form trialkylsulfonium iodide. In this way the bound sulfur may be estimated.

In heat vulcanization the processes are more complex. The reaction of sulfur with olefins, which might be taken as a model, does not always proceed in the same way, nor are the end products homogeneous. In some cases disulfides are formed, together with mercaptans and other thiophenes. According to Meyer and Hohenemser, at the temperature of heat vulcanization the action of sulfur on cyclohexenes leads to the formation of cyclohexylmercaptan and dicyclohexyl sulfide, together with certain compounds of higher molecular weight. It is evident, therefore, that, in addition to the formation of sulfur bridges, hydrogenation occurs simultaneously, at the expense of other parts of the reacting substance. From styrene, on the other hand, diphenylthiophene:



is the only well-defined substance produced by the action of sulfur at the temperature of heat vulcanization; its formation involves dehydrogenation. Other possible reactions have been discussed by Farmer and Shipley.[167]

[165] K. H. Meyer and W. Hohenemser, *Helv. Chim. Acta*, **18**, 1061 (1935).

[166] K. H. Meyer and H. Mark, *Ber.*, **61**, 1948 (1928).

The addition of methyl iodide shows that sulfur is present in heat-vulcanized rubber in the form of the linkage C—S—C (Meyer and Hohenemser). In the action of sulfur on polyprene chains, it must be supposed that dialkyl sulfides are formed (where the term "alkyl" is equivalent to a polyprene chain residue) and that at the same time complicated hydrogenation and dehydrogenation reactions take place. Undesirable reactions such as the formation of rings within the chains may also occur if sulfur reacts with two double bonds of the same chain.[167]

Before vulcanization the sulfur is uniformly distributed and dissolves to some extent in the rubber during the process of mastication between hot rollers. At room temperature up to 1% of sulfur will dissolve in rubber; at 55°C., 3% of sulfur can be dissolved[168]; chemical reaction only occurs at higher temperatures. At 155°C. the velocity of vulcanization is about seven times greater than at 135°C. (Spence and Young[169]); the heat of activation calculated from these data is about 30,000 cal.; this value lies in the range of values observed for organic reactions.

Heat vulcanization is accelerated by many substances, as is well known. These accelerators include metallic oxides—in particular, finely divided zinc oxide—as well as many organic substances. The nature of the accelerating process and the question of whether or not the heat of activation is diminished cannot be decided from the results available. It may be mentioned that the velocity of reaction between sulfur and cyclohexene is also increased by vulcanization accelerators. It is possible that accelerants may have some effect in suppressing undesirable secondary reactions.

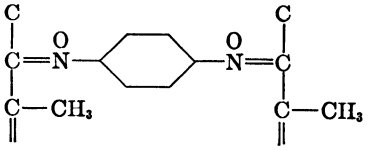
Sulfur which is liberated in the presence of rubber by reaction between hydrogen sulfide and sulfur dioxide leads to vulcanization at ordinary temperature (the Peachey process).

It is to be noted that, in the absence of accelerators, vulcanization begins at the temperature at which pure molten sulfur begins to transform into amorphous sulfur. In this process active nuclei—consisting, perhaps, of open S_8 chains with free valences—are formed and will certainly react rapidly with olefins. In the interaction of sulfur dioxide and hydrogen sulfide, amorphous sulfur is formed from the short open chains with free valences, which appear as intermediate products in the reaction. The high reactivity of such unstable intermediate products would explain the Peachey process.

Effects resembling those due to treatment with sulfur can be obtained

[167] E. H. Farmer and F. W. Shipley, *J. Polymer Sci.*, **1**, 293 (1946).
[168] A. R. Kemp *et al.*, *Ind. Eng. Chem.*, **32**, 1075 (1940).
[169] D. Spence and J. Young, *Kolloid-Z.*, **11**, 28 (1912).

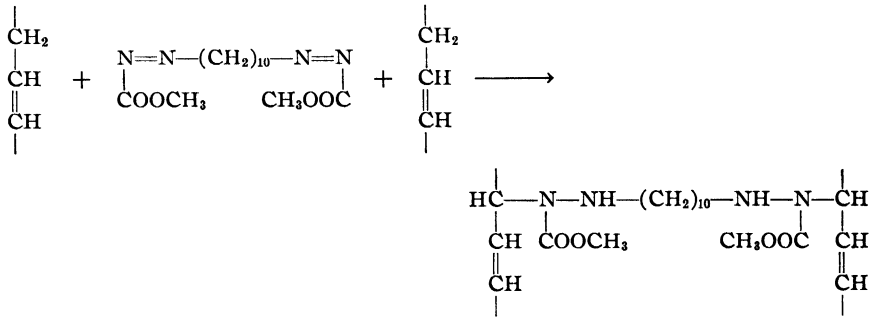
with 1,3,5-trinitrobenzene and *m*- and *p*-dinitrosobenzene. According to Rehner and Flory[170] the nitroso groups react with the rubber chains, without the double bonds disappearing to form cross linkages of the following kind:



Vulcanization is also possible with benzoyl peroxide. Benzoyl peroxide is a typical initiator of olefin polymerization and, in this case, vulcanization is probably due to a reaction between the double bonds of neighboring polyprene chains—a reaction which may, of course, be limited to a very small per cent of the olefin linkages.

The irreversible gelatinization of rubber exposed to ultraviolet light or to daylight in the presence of iodine is known as photopolymerization; it may be due to the same kind of reaction. Molecular oxygen also has a vulcanizing effect. The reaction may be of the same kind as that which occurs in the hardening of linseed oil in the presence of oxygen.[171]

Vulcanization may also be brought about by disazotetracarboxylic esters[171a] such as decamethylene dismethyl azodicarboxylate 10–1 according to:



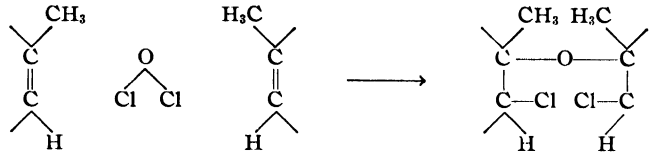
[170] J. Rehner, Jr., and P. J. Flory, *Ind. Eng. Chem.*, **38**, 500 (1946).

[171] K. H. Meyer and C. Ferri, *Helv. Chim. Acta*, **19**, 694 (1936). C. Ferri, *ibid.*, **20**, 88 (1937).

[171a] K. Alder, F. Pascher, and A. Schmitz, *Ber.*, **76**, 27 (1943); N. Rabjohn, *J. Am. Chem. Soc.*, **70**, 1181 (1948); P. J. Flory, N. Rabjohn, and M. C. Shaffer, *J. Polymer Sci*, **4**, 225 (1949).

A mixture of rubber and vulcanizing agent is dissolved in benzene and evaporated; the film thus obtained is heated for two hours at 60°C.

Hypochlorous acid or chlorine monoxide in aqueous solution also have a surface vulcanizing effect; it is suggested that the reaction responsible is as follows:



This behavior is exhibited by olefins of low molecular weight, although only as a side reaction.

(*b*) *Interpretation of Vulcanization in Terms of Molecular Morphology*

Changes in physical properties, such as lessening of plasticity, solubility, and degree of swelling, are immediately understandable if the chemical reaction which results in the linkage of two olefin compounds is interpreted in terms of molecular morphology.

Meyer and Mark[172] showed in 1928 that cross linkages progressively unite all rubber chains to form a three-dimensional net. The chains are then no longer able to slip over each other (a condition for plastic deformation) and can no longer pass into solution as free molecules. In its initial stages, cross linking leads only to an increase in the mean molecular weight as free chains are united in pairs; this has been shown by determinations of the molecular weight. Only when each chain is, on the average, united with two others is a three-dimensional network formed, after which the substance no longer dissolves in solvents but forms a swollen gel. Flory,[173] Stockmayer,[174] and others have calculated at what cross-linkage content material of any given mean molecular weight should become insoluble.

As cross links occur freely at any point along the molecule, the finished vulcanizate will always contain free chain ends which behave during deformation as if they were unvulcanized. The proportion of these "inactive" chain ends, according to Flory, is:

$$2\bar{M}^{x}/\bar{M}$$

[172] K. H. Meyer and H. Mark, *Ber.*, **61**, 1939 (1928).

[173] P. J. Flory, *J. Am. Chem. Soc.*, **63**, 3083, 3091, 3096 (1941); *Ind. Eng. Chem.*, **38**, 417 (1946).

[174] W. H. Stockmayer, *J. Chem. Phys.*, **11**, 45 (1943).

where $\bar{M}^x$ is the weight of the chain between two cross linkages and $\bar{M}$ is the molecular weight before vulcanization. The modulus of elasticity (stiffness) is depressed by the inactive chain ends.

Crystallization phenomena are also modified in vulcanized rubber; weakly vulcanized rubber shows very definite x-ray spectra on stretching; with moderately strong vulcanization, the diffraction pattern appears only at a very high degree of stretching and is then incomplete; highly vulcanized rubber shows no crystallization on stretching.

The more numerous the points of linkage, the smaller will be the meshes of the network until, finally, in hard rubber the structure of the network becomes like that of a material such as Bakelite. In a network as compact as this, the conditions for high extensibility are no longer fulfilled and, for this reason, hard rubber is a typical solid body. Finely divided fillers, such as amorphous carbon, zinc oxide or zinc sulfide, with surfaces possessing a considerable adsorption for hydrocarbon residues, tend to strengthen the texture and thus assist vulcanization. Moreover, following Stiehler and Wakelin,[175] zinc oxide forms bridges, —C—S—Zn—S—C—, with —C—S—H groups which appear in the course of vulcanization. We cannot, however, follow these authors in their theory that vulcanization consists mainly in the introduction of polar groups into the rubber chains and an increase of intermolecular attractive forces.

The properties of a vulcanized product depend essentially on the points of linkage. Such points of linkage may also arise as a result of the formation of small crystallites on slow cooling. If a number of neighboring segments become oriented to form a small crystalline particle (with reduction in the number of degrees of freedom of the chains and evolution of heat of fusion taking place), plastic flow is locally suppressed; a sample of rubber through which crystalline nuclei are scattered irregularly will behave like a vulcanized product ("vulcanization by cold" according to Le Blanc and Kröger[176]). The reverse of the process of vulcanization—regarded essentially as cross linking of the chain molecules—takes place during the regeneration of rubber scrap, in which process it is masticated between hot rollers, or heated in solvents under pressure. In this treatment the chains are ruptured, and the network broken up. The regenerated material must consist very largely, therefore, of irregular fragments of the network rather than unbranched chains. It should be possible to test the validity of this state ment by studying the behavior of solutions of regenerated rubber.

[175] R. D. Stiehler and J. H. Wakelin, *Ind. Eng. Chem.*, **39**, 1647 (1947).
[176] M. Le Blanc and M. Kröger, *Kolloid-Z.*, **37**, 205 (1925).

Gutta-percha may also be vulcanized—most readily with sulfur and accelerators. Weakly vulcanized gutta is crystalline at ordinary temperatures and solid like frozen rubber. Above 35°C. it shows rubberlike elasticity and at this temperature is scarcely to be distinguished in its mechanical properties from vulcanized rubber. On cooling, crystallization takes place rapidly. The most important applications of gutta and balata are as insulating material for cables and in the manufacture of golf balls.

(*c*) *Other Chemical Reactions*

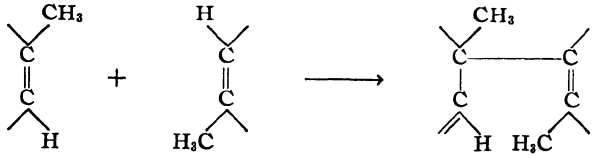
Rubber is extremely sensitive to decomposition by the action of oxygen. The reaction is accelerated in the presence of light and inhibited by antioxidants such as phenol, hydroquinone, or alkyl naphthylamines. At the same time, as we have seen above, oxygen also has the effect of decreasing the solubility; in measurements where it is desirable to keep the chain length constant, light and oxygen must therefore be excluded, and autooxidizable solvents (such as tetraline), which may contain peroxides, must be avoided.

For industrial purposes rubber is broken down by milling between hot rollers (mastication). The effect is reduced if oxygen is excluded, showing that oxygen also plays a part in this breakdown[177]; the process can be inhibited by adding antioxidants (for example, phenol, naphthylamine) or accelerated by adding other substances, such as phenylhydrazine. The effect of milling is minimal at 110°C., according to Busse.[178] At higher temperatures chemical decomposition is dominant, while at lower temperatures the breakdown which occurs is presumably purely mechanical, as it is not inhibited by antioxidants but is reduced by plasticizers such as oleic acid.

If milling is continued for long, degradation may be carried so far that the plastic properties begin to mask the elastic properties (over-masticated rubber). Diminution in size of the chains and, finally, splitting up into monomers (that is, isoprene) may also be caused by heating dry rubber. If heating takes place under pressure, a secondary reaction occurs, namely, the linkage of neighboring chains with the loss of a double bond, according to:

[177] B. D. Poritt, *Trans. Faraday Soc.*, **16**, 81 (1921). Review of more recent papers: D. F. Twiss, *Nature*, **156**, 9 (1945).

[178] W. F. Busse and E. N. Cunningham, *Proceedings of the Rubber Technology Conference, 1938, London.* Heffer, Cambridge, 1938, p. 288.



The reaction bears some resemblance to the effect of metallic halides. Metallic halides, phosphorus trichloride, etc., convert rubber (as well as gutta-percha) to a material which at ordinary temperatures is a tough solid, but which becomes plastic on warming (thermoprenes). This material has acquired commercial importance. One such product obtained from rubber by the action of chlorostannic acid (H_2SnCl_6) is marketed under the name Plioform (manufactured by the Goodyear Tire and Rubber Co.[179]); it is used in the manufacture of lacquer and as a thermoplastic mass for molding. Mixed with rubber, it is used instead of gutta-percha in the manufacture of golf balls and as insulating material for cables. A survey of such thermoprenes is to be found in Bruson[180] and in Thies and Clifford. At high temperatures such masses can be stretched and (as is shown by their fibrous structure and tendency to longitudinal splitting if pulverized at low temperatures) orientation of the chains takes place, without, however, the formation of a crystal lattice (Ferri[181]). The regularity of the chains present in rubber has, therefore, disappeared in the reaction product. One may assume, with Ferri, that a partial isomerization of *cis* to *trans* linkages has taken place and that such linkages are distributed in the reaction product in purely statistical manner. The hydrocarbon present in thermoprenes should be regarded, then, as an isomer of gutta as well as of rubber. In addition to this isomerization, there may also be isomerism of unbranched and branched chains, linkage (as in ethylene polymerization), and shortening of the chains. Staudinger[182] formerly supposed that 6-membered rings are formed under the action of metallic halides and he invented the term "cyclo-rubber" to designate them. It seems unnecessary to use the term "cyclo-rubber" for the product, or "cyclization" for a reaction which is complex and has nothing to do with ordinary types of cyclization.

Inasmuch as it is an olefin, rubber also undergoes various addition reactions. Hydrorubber is obtained by the catalytic hydrogenation of rubber

[179] H. R. Thies and A. M. Clifford, *Ind. Eng. Chem.*, **26**, 123 (1934).

[180] H. A. Bruson, L. B. Sebrell, and W. C. Calvert, *Ind. Eng. Chem.*, **19**, 1033 (1927); U. S. Pat. 1,797,188 (1931).

[181] C. Ferri, *Helv. Chim. Acta*, **20**, 1393 (1937).

[182] H. Staudinger and E. Geiger, *ibid.*, **9**, 549 (1926).

or gutta-percha (Pummerer and Burkard,[183] Staudinger[184]). If hydrogenation takes place at a low temperature, the products obtained are tough and reversibly extensible like rubber (Pummerer, Burkard, and Koch[183]); at a higher temperature the hydrogenated products are viscous pastes and, at a still higher temperature, oily liquids. Hydrogenation is therefore accompanied by breakdown, just as is the case with paraffins:[185]

$$C_2H_6 + H_2 \longrightarrow 2CH_4$$

Hydrorubber does not crystallize on stretching. This is very remarkable, since the compound must be regularly built and should readily form a regular lattice structure; no explanation is forthcoming.

Rubber forms addition products with halogens. The products are solid at ordinary temperature and become plastic on heating. When they are stretched, the x-ray diffraction pattern shows no crystal interferences; this is understandable in view of the possible formation of diastereoisomers by addition. It is not surprising that the halogen derivatives are solid at ordinary temperature (that is, are more viscous than rubber), since the viscosity of halogen substitution products of hydrocarbons is in general higher than that of the unsubstituted hydrocarbons. Highly chlorinated products of molecular weight 150,000–400,000 are five times less viscous than rubber of the same degree of polymerization when dissolved in toluene[186]; from this it seems probable that the rubber has been converted to branched isomers during the somewhat brutal chlorination reaction.

Staudinger[186] on the other hand assumes that chlorinated rubber is a "polycyclic highly chlorinated terpene." This view cannot be reconciled with the analytical results published by Staudinger himself, according to which the constitution is represented by the open formula: $C_nH_{2n-x}Cl_x$. One such product, obtained by chlorinating rubber dissolved in carbon tetrachloride, is Tornesite, manufactured by the Hercules Powder Co. It is used as a thermoplastic mass for injection molding and as a base for lacquer. It is resistant to acids, chlorine, and aliphatic hydrocarbons, but is attacked by fatty oils and aromatic hydrocarbons, and by esters and ketones.

Rubber hydrochloride: $—CH_2—CH_2—CClCH_3—CH_2—CH_2—CH_2—CClCH_3—$, is formed by the reaction of gaseous hydrochloric acid on dissolved rubber. At room temperature it is hard like gutta and only becomes rubberlike at about 115°C. Stretched specimens give a fiber diagram with

[183] R. Pummerer and P. A. Burkard, *Ber.*, **55**, 3458 (1928). R. Pummerer and A. Koch, *Ann.*, **438**, 294 (1924).

[184] H. Staudinger and W. Feisst, *Helv. Chim. Acta*, **13**, 1361 (1920).

[185] H. Staudinger, *Ber.*, **57**, 1203 (1924).

[186] H. Staudinger and H. J. Staudinger, *J. prakt. Chem.*, **162**, 148 (1943).

a fiber period of 8.95 Å. Following Bunn,[187] the unit cell is rhombic with $a = 5.83$ Å., b (fiber axis) $= 8.95$ Å., and $c = 10.38$ Å., and there are four C_5H_9Cl units in the cell. The product has not acquired commercial importance.

Rubber exhibits practically all the reactions characteristic of olefin hydrocarbons, and reacts with nitrous gases, ozone, etc. Within the limits of this account it is not necessary to consider these reactions in detail.

7. Some Points in Manufacture of Rubber

The rubber hydrocarbon occurs in the milky sap (latex) of certain plants (in particular, *Hevea brasiliensis*, belonging to the Euphorbiaceae) emulsified in the form of fine droplets. The diameter of the droplets may amount to 3 μ; the proportion of particles less than 0.5 μ is probably small. The composition of fresh latex is as follows:

Water.....	about 60%	Stearin, fats, soaps.....	about 1 %
Rubber hydrocarbon...	about 35%	Inositol-*l*-methyl ether..	about 1 %
Proteins.....	about 2%	Inorganic constituents..	about 0.4%

On the addition of acids (generally of formic acid) the hydrocarbon is coagulated. The coagulum is washed between rolls and exported as crêpe. "Smoked sheet" is the term applied to the crude coagulum after smoking for export.

This crude rubber is worked up in the factory in the following three stages (*1*) The rubber is kneaded (masticated) between warm rolls; at the same time, materials necessary for the manufacture of the particular product required are mixed into the rubber. The nature and significance of the substances added will be considered later. In the kneading process the rubber gradually loses its reversible extensibility and becomes plastically deformable as a result of the breakdown of the chains. Masticated rubber is almost completely soluble in benzene or carbon disulfide. (*2*) The mixture of masticated rubber and added substances is transferred to molds; and (*3*) it is subjected to heat vulcanization or vulcanized in the cold by immersing the molded object in a dilute solution of S_2Cl_2 in carbon disulfide or benzene.

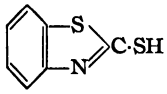
The following ingredients must be considered:

(*1*) *Regenerated Rubber.*

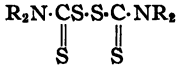
(*2*) *Sulfur*, for heat vulcanization (in present-day practice 1.4–4% for soft rubber).

[187] C. W. Bunn and E. V. Garner, *J. Chem. Soc. London*, **1942**, 654.

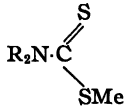
(*3*) *Accelerators.* These are organic substances, containing nitrogen or sulfur or both, which accelerate the process of vulcanization and lower the temperature at which vulcanization takes place. In order of effectiveness, accelerators are grouped as normal, semiultra-, and ultraaccelerators. The first include derivatives of guanidine and aldehyde imines. A favorite semiultra-accelerator is mercaptobenzothiazole:



Among the ultraaccelerators may be mentioned tetramethylthiuram monosulfide and disulfide, as well as zinc salts of dithio acids, such as zinc dimethyldithiocarbamate:



Thiuram disulfide



Dialkyldithiocarbamate

Thiuram sulfides also vulcanize in the absence of sulfur. The proportion of accelerators used ranges from 0.25 to 1.5%.

(*4*) *Acceleration Activators.*—The action of the accelerators attains a maximum in the presence of 1 to 5% of finely divided zinc oxide or lead oxide and from 1 to 2% of fatty acids. There are no satisfactory theories of the action of accelerators and activators.

(*5*) *Protectors against Aging,* that is, against the gradual decline in mechanical properties which occurs in the course of time. These substances inhibit the reaction of rubber with atmospheric oxygen and are, therefore, known as antioxidants. The compounds used are aromatic amines, chiefly those of the naphthalene series, in proportions varying from 0.25 to 1.5%.

(*6*) *Softeners.*—These facilitate mastication and have a favorable influence on the viscosity of the vulcanized product (determined from the recoil elasticity). For this purpose, mineral oils, vegetable oils, waxes, resins, pitch, and tar are used.

(*7*) *Reinforcing Agents.*—In order to increase the modulus of elasticity and to improve the resistance of the product to abrasion, a number of solid, finely divided fillers may be added in considerable quantities. The most important strengthener is gas black, which is essential to the manu-

facture of goods of high tensile strength and wear resistance. Other reinforcing fillers include zinc oxide, magnesium carbonate, barium sulfate, and certain kinds of clay.

(*8*) *Fillers.*—In cases where the mechanical properties of the goods warrant it, diatomaceous earth, clay, and other finely divided, cheap materials may be added to lower the cost of the product. Organic fillers, such as asphalt or factice (rubber substitute made by vulcanizing vegetable oils), may also be used.

(*9*) *White and Colored Inorganic and Organic Pigments.*—After mixing in a rubber mill, the batch is either molded by itself (in the case of belting or tubing this may take place continuously), or in conjunction with other materials, such as fabric in the manufacture of automobile tires. The composition, temperature, and duration of vulcanization may be varied according to the physical properties desired. The optimum conditions for vulcanization of each mixture are unique. If the optimum temperature and length of vulcanization are exceeded, tensile strength and resistance to aging are diminished.

To obtain the high extensibility required for stationer's bands, balloons, rubber tubing, etc., compounds are used which are low in sulfur and free from gas black; sulfur and carbon reduce the extensibility and increase the modulus of elasticity, the tensile strength, and the resistance to abrasion. Table V indicates the composition of typical compounds.

TABLE V

Composition of Typical Rubber Compounds

Highly elastic articles		Automobile tires	
Pale crêpe	100	Smoked sheet	100
Stearic acid	1	Pine tar	3
Antioxidant	0.5	Stearic acid	4
Zinc oxide	5	Antioxidant	1
Sulfur	2.5	Zinc oxide	5
Mercaptobenzothiazole	1	Gas black	50
Vulcanized for 20–30 minutes at 140°C.		Sulfur	3
Tensile strength, kg./cm.^{2}	200–240	Mercaptobenzothiazole	1
Modulus of elasticity, kg./cm.^{2}, at 500% elongation	20–28	Vulcanized for 60 minutes at 134°C.	
Breaking extension, %	825–850	Tensile strength, kg./cm.	273
		Breaking extension, %	535
		Hardness (Shore scale)	70

Latex stabilized by the addition of ammonia may be exported as such. It reacts readily with sulfur, vulcanization accelerators, and other sub-

stances; smears of such mixtures coagulate as soon as the water evaporates and may then be vulcanized by warming. By this process it is possible to avoid the breakdown of the chains through mastication—a breakdown which is compensated to some extent by the effects of vulcanization. For this reason latex is rapidly replacing solutions of rubber in organic solvents: (*1*) in the manufacture of various articles by the dipping process; (*2*) for the impregnation of fabrics; and (*3*) for making rubber threads by extrusion into a coagulating bath, etc.

TABLE VI

Some Physical Constants of Pure Rubber, Soft Vulcanized Rubber, and Hard Vulcanized Rubber[189]

	Pure rubber	Commercial product	Soft rubber (2% S)	Hard rubber (32% S)
Specific gravity...	0.906[189]	0.911[190]	0.923[191]	1.173[191]
Refractive index..	1.519[192]	1.519[192]	1.5264[192]	1.6[193]
Specific heat (cal./°C.).....	0.449[194]	—	0.510[195]	0.34[195]
Heat of combustion (cal./g.)...	10.82[196]	—	10.63[196]	7.92[196]
Cubic coefficient of expansion.....	670×10^{-6} [189]	670×10^{-6} [190]	661×10^{-6} [191]	194×10^{-6} [194]
Compressibility (cm.²/dyne)....	53.7×10^{-12} [191]	—	51.0×10^{-12} [191]	24.3×10^{-12} [191]
Poisson's ratio at elongations < 300%.....	—	—	0.500[197]	—
Dielectric constant	2.37[189]	2.45[190]	2.86[198]	2.82[198]
Velocity of sound (m./sec.).....	—	—	37[199]	1560[200]

[189] A. T. McPherson, *J. Research Natl. Bur. Standards*, **8**, 751 (1932).

[190] H. L. Curtis and A. T. McPherson, *Sci. Papers U. S. Bur. Standards*, **560**, 385 (1927). A. T. McPherson and A. H. Scott, *ibid.*, **560**, 398 (1927).

[191] A. H. Scott, *J. Research Natl. Bur. Standards*, **14**, 99 (1935).

[192] A. T. McPherson and A. D. Cummings, *ibid.*, **14**, 553 (1935).

[193] W. E. Ayrton and J. Perry, *Phil. Mag.*, **12**, 196 (1881).

[194] N. Bekkedahl and H. Matheson, *J. Research Natl. Bur. Standards*, **15**, 503 (1935).

[195] A. T. McPherson and N. Bekkedahl, *ibid.*, **14**, 601 (1935).

[196] R. S. Jessup and A. D. Cummings, *ibid.*, **13**, 357 (1934).

[197] W. L. Holt and A. T. McPherson, *ibid.*, **17**, 657 (1936).

[198] A. H. Scott, A. T. McPherson, and H. L. Curtis, *ibid.*, **11**, 173 (1933).

[199] F. Exner, *Sitzber. Akad. Wiss. Wien, Math. naturw. Klasse*, **69** (II), 102 (1874).

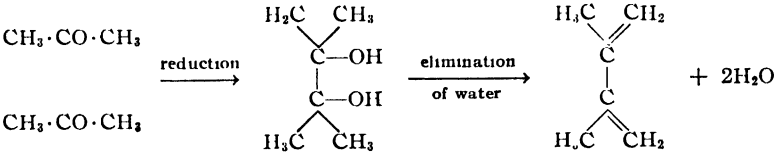
[200] E. J. Irons, *J. Sci. Instruments*, **7**, 323 (1930).

V. SYNTHETIC OR "CHEMICAL" RUBBERS

1. Problem of Artificial Rubber

The impressive advance of organic chemistry, which led to the synthesis, among other things, of the most important natural dyestuffs, suggested that the problem of synthesizing as valuable a natural product as rubber was not without hope of solution. Since it has long been known that isoprene may be derived from rubber, numerous attempts were made to convert isoprene into rubber and, as early as 1891, Tilden[200a] observed that isoprene yielded a rubberlike mass on standing or as a result of the action of hydrochloric acid. Working independently, Hofmann and Coutelle[201] and Harries[202] converted isoprene into "rubber" in 1909, the first two authors by heating, the last by treatment with acetic acid. In England, Matthews and Strange[203] discovered that diolefins polymerize in the presence of sodium. This was discovered independently by Harries[204] a few days after the announcement of the British patent. It is now known that branched chains are formed during this polymerization; it is not known whether *cis* or *trans* linkages occur. In any case, the products obtained are no more closely related to rubber than is a branched hydrocarbon to a straight-chain hydrocarbon. The synthesis of rubber, that is, of a mixture of homologous, polymeric *cis*-polyprenes, such as is present in rubber, has not as yet been carried out, and at the present time there is still no suggestion as to how this synthesis may be accomplished.

Inasmuch as rubber appeared to be a unique substance in view of its elastic character, and in the absence of a theory of rubber elasticity providing suggestions for the synthesis of substances with rubberlike properties, investigators were reluctant to depart widely from isoprene and turned their attention first to 2,3-dimethylbutadiene, which could be prepared more easily than isoprene, by reducing acetone to pinacone and eliminating water:



[200a] W. Tilden, *Chem. News*, **1891**, 265.
[201] F. Hofmann and C. Coutelle, German Pat. 250,690 (Bayer and Co.).
[202] C. Harries, *Ann.*, **383**, 157 (1911); **385**, 116 (1911–12).
[203] F. E. Matthews and E. H. Strange, Brit. Pat. 24,790 (1910).
[204] C. Harries, U. S. Pat. 1,058,056 (1910).

2,3-Dimethylbutadiene polymerizes to form what is known as methyl rubber. This particular approach was used by F. Hofmann but was abandoned in 1918 and was never taken up again.

With the development of large-scale continuous catalytic methods for the commercial preparation of intermediate products, the likelihood of manufacturing butadiene at a reasonable price increased. Accordingly in the years following 1925, the preparation of butadiene and its polymerization were undertaken by the I. G. Farbenindustrie A.-G. Somewhat later, work on 2-chlorobutadiene began in America and led to the synthesis of neoprene. Several hundred tons of sodium butadiene rubber had been produced in a pilot factory at Ludwigshafen up to the year 1930, under the energetic direction of C. Schumann. The enormous development of the synthetic rubber industry since that time owes much to that pioneering work in Ludwigshafen.

Parallel with this activity, the commercial development of olefin polymerization took place and rubberlike properties were discovered in saturated hydrocarbon derivatives. At the same time, the constitution of rubber and synthetic rubbers was investigated by x-ray analysis, the theory of rubber elasticity was propounded, and copolymerization was developed, so that the problem could now be formulated in an entirely different way. The object in view was no longer the synthesis of rubber, but the discovery of chain polymers of any constitution with rubberlike properties, and the selection and development of the most suitable products for various industrial applications. The original objective was lost sight of as a result of the cheapening of plantation rubber and overproduction. It is hardly possible now to prepare a product equivalent to and at the same price as natural rubber, but products like butadiene-styrene mixed polymers, which can completely or almost completely replace natural rubber in certain fields, for example, tires, have already reached astronomically large production figures. Moreover, synthetic rubbers whose oil resistance is superior to that of natural rubber can be marketed at even higher prices.

From the point of view of practical application the work of I. G. Farbenindustrie was undoubtedly the most successful. This work led to butadiene-styrene copolymers[204a] (buna S, known as GR-S in America) and butadiene-acrylonitrile copolymers (perbunan) widely used at the present time. As a result of an understanding which provided for a complete exchange of patents and experience reached with the I. G. Farbenindustrie in 1929, the Standard Oil Company of New Jersey had the process

[204a] E. Tschunkur and W. Bock (I. G. Farbenindustrie), German Pat. 570,980 (1929).

at its disposal and during the war issued licenses to numerous other works supported by the U. S. Government. Investigations were carried out to improve the process and to adapt it to large-scale manufacturing, so that today an enormous quantity of these synthetic rubbers is produced in the United States; moreover the research work carried out under the sponsorship of the Office of the Rubber Reserve has led to products highly superior in quality to the original German Buna S.

Of less significance are polymerized 2-chlorobutadiene (neoprene) and polyethylene polysulfide (thiokol). Butyl rubber, a copolymer of isobutylene and butadiene, appears more promising.

2. Butadiene and Its Polymerization

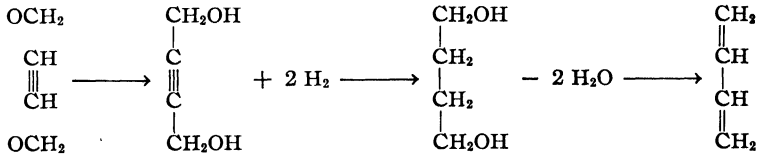
The derivatives of ethylene which polymerize readily include the diolefins with conjugated double bonds; after suitably controlled polymerization, they yield high polymeric products with rubberlike properties.

Butadiene is prepared from acetylene in the manner indicated by the following scheme:

$$\underset{\text{acetylene}}{CH{\equiv}CH} + H_2O \longrightarrow \underset{\text{acetaldehyde}}{CH_3CHO} \longrightarrow \underset{\text{aldol}}{CH_3CHOHCH_2CHO} + H_2$$

$$\longrightarrow \underset{\text{1,3-butylene glycol}}{CH_3CHOHCH_2CH_2OH} \longrightarrow \underset{\substack{\text{butadiene}\\ \text{(elimination of water in the}\\ \text{presence of a catalyst)}}}{CH_2{=}CH{-}CH{=}CH_2}$$

Another method, due to Reppe,[205] starts from formaldehyde and acetylene, which are caused to combine under pressure in the presence of copper or silver acetylide to form butindiole:



Vinylacetylene, prepared from acetylene, can also be hydrogenated to form butadiene. In countries producing petroleum, the catalytic dehydrogenation of butane, through butylene, to butadiene is a practicable procedure, while in agricultural countries the direct catalytic conversion of ethyl alcohol or better an equimolecular mixture of alcohol and acetaldehyde will doubtless be followed.

Butadiene and its substitution products polymerize in the liquid state, in solution, or when emulsified.

[205] W. Reppe, U. S. Pat. 2,232,867.

(*a*) *Sodium Polymers*

Sodium polymerization is brought about by the addition of metallic sodium, in the form of fine wire, to liquid butadiene. The polymerized product is formed in the neighborhood of the metallic sodium and from that region polymerization spreads out into the liquid. During this process, there develops in the neighborhood of the sodium an intense orange-brown coloration, which is probably due to the formation at the double bond of an addition product with the metal. The reaction mechanism has been made clear by the work of Ziegler[206] and his collaborators. They showed, first of all, that alkyl compounds of alkali metals form addition products with butadiene and that these substances react with other butadiene molecules by a kind of organo-metallic synthesis in which products are formed which themselves react with other butadiene molecules:

$$H_2C{=}CH{-}CH{=}CH_2 + LiR \longrightarrow RH_2C{-}CH{=}CH{-}CH_2Li + C_4H_6 \longrightarrow$$
$$RH_2C{-}CH{=}CH{-}CH_2{-}CH_2{-}CH{=}CH{-}CH_2Li, \text{ etc.}$$

In subsequent additions, new butadiene molecules may be bound in the 1,2 linkage, so that branching takes place. The same author showed later that the metal reacts first with diene and diene is subsequently added to the organo-metallic compound thus formed:

$$C_4H_6 + 2Na \longrightarrow Na_2C_4H_6 + C_4H_6 \longrightarrow Na_2C_8H_{12}, \text{ etc.}$$

The kinetics of sodium polymerization have been studied by Bolland[207] in the case of isoprene. After an induction period, probably due to the presence of traces of impurities, the velocity increases. Free radicals are formed at the sodium surface and act as nuclei for chains. In the presence of toluene the chains are interrupted while still quite short. In the presence of toluene only 1,4 addition occurs; in the absence of toluene 1,2 addition also occurs.

The higher sodium polymers are insoluble but they swell in solvents. They are not as extensible as rubber, and do not crystallize when stretched. After vulcanization, they become more rubberlike, but it is impossible to prepare products from them which are as extensible as moderately vulcanized rubber. It is possible, however, by vulcanization with sulfur and by the addition of gas black, to obtain products which resemble natural rubber of a similar degree of vulcanization in impact resistance, resistance

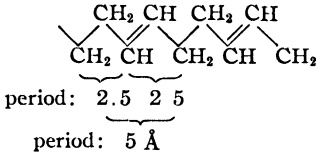
[206] K. Ziegler and K. Bähr, *Ber.*, **61**, 253 (1928). K. Ziegler and H. Kleiner, *Ann.*, **473**, 57 (1929). K. Ziegler, F. Dersch, and H. Wollthan, *ibid.*, **511**, 13 (1934). K. Ziegler and L. Jakob, *ibid.*, **511**, 45 (1934). K. Ziegler, L. Jakob, H. Wollthan, and A. Wenz, *ibid.* **511**, 64 (1934).

[207] J. L. Bolland, *Proc. Roy. Soc. London*, **A178**, 24 (1941).

to abrasion, and recoil elasticity. These observations indicate that branched chains are present in the polymer and that these are united through a fairly high percentage of sulfur to form a three-dimensional network of moderate mesh, such as is obtained in rubber by mild vulcanization.

(*b*) *Emulsion Polymers*

In the presence of protective colloids and catalysts the dienes also polymerize in aqueous emulsion. Starting with pure butadiene, extensible products may be obtained which show a fiber diagram in the stretched condition.[207a] This indicates that the chains are entirely or almost entirely unbranched. The identity period along the fiber axis is given as 2.5 Å. It may be assumed that the true identity period amounts to 5 Å. and that this is subdivided into two very similar subperiods, according to the scheme:



Butadiene rubber therefore exhibits the *trans* configuration; hence, it corresponds to gutta and not to rubber. Strictly speaking, the compound should be called norgutta.

A considerable number of *cis* linkages has been detected by means of infrared absorption in other specimens of emulsion polybutadiene; it seems that high polymerization temperature favors these irregularities, whereas at lower temperature only *trans* linkages are formed.[207b]

Recent investigations[207c] have shown that 20–24% of laterally attached vinyl groups due to 1,2 addition are found in polybutadiene. Whereas, according to Meyer,[207c] the temperature of polymerization is of little influence on the degree of branching, Johnson and Wolfangel[207d] report that polybutadiene, if polymerized at 50°, is highly branched, while the low temperature polymer is not.

3. Copolymers

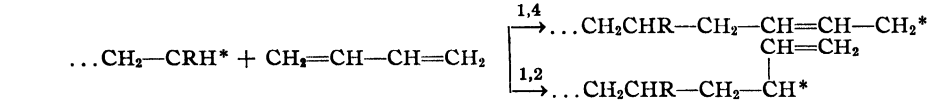
The copolymers of butadiene and styrene (buna-S or GR-S) and of butadiene and acrylonitrile (perbunan) are commercially superior to pure butadiene rubber.

[207a] X-ray diagrams obtained in 1930 by I. G. Farbenindustrie, Ludwigshafen.
[207b] E. J. Hart and A. W. Meyer, *J. Am. Chem. Soc.*, **71**, 1980 (1949).
[207c] A. W. Meyer, *Ind. Eng. Chem.*, **41**, 1570 (1949).
[207d] B. L. Johnson and R. D. Wolfangel, *Ind. Eng. Chem.*, **41**, 1580 (1949).

(a) *Preparation of GR-S (Government Rubber with Styrene; Buna S)*

Butadiene and styrene are copolymerized in emulsion. The original GR-S recipe (1941) consisted of (expressed in parts by weight): butadiene 75, styrene 25, $K_2S_2O_8$ 0.30, fatty acid soap (90% sol) 4.6, dodecylmercaptan 0.60, H_2O 180. The polymerization is carried out with stirring at 50°C. and is interrupted after 15–17 hours at 77% conversion. The polymerized product contains butadiene and styrene residues in the ratio 79:21. This recipe has been subsequently slightly modified. As has been pointed out on page 156, butadiene is more readily added to the growing chain than is styrene. The mixture of free monomers thus becomes gradually richer in styrene until finally pure styrene is left which gives undesirable polystyrene if the polymerization is continued. The modifier, dodecylmercaptan, is incorporated in the chain; commercial GR-S contains, therefore, one atom of sulfur per chain.[208] In Germany[209] Nekal (sodium salt of diisobutylnaphthalene sulfonic acid) and linseed oil soap were used as emulsifiers, the soap also acting as a modifier. Diisopropylxanthogen disulfide was also used as a modifier.

Polymerization consists for the most part in the 1,4 addition of butadiene; in 1,2 addition a fair proportion of vinyl groups may be formed simultaneously which lead to undesirable branching:



Recent experiments in Ludwigshafen showed that, in the absence of modifier, the chain grows without branching until 30% conversion has taken place. At this point a modifier must be added if the reaction is to continue[209] in the same way.

The products first obtained were difficult to work and had to be made plastic between hot rollers before the addition of sulfur, which led to oxidative breakdown; presumably they were of too high a molecular weight or were branched. This difficulty has been avoided by the addition of modifiers which bring about chain transfer and chain stop. The more recent brands, GR-S and also the German brand buna S_3, can be worked without preplasticizing; they contain *ca.* 25% of styrene units.

Lately in America[210] and until 1945 in Germany, intensive study has

[208] F. T. Wall, F. W. Banes, and G. D. Sands, *J. Am. Chem. Soc.*, **68**, 1429 (1946).
[209] E. R. Weidlein, Jr., *Chem. Eng. News*, **24**, 771 (1946).
[210] A. E. Boss and R. W. KixMiller, *Chem. Eng. News*, **24**, 336 (1946).

been made of butadiene-vinyl copolymers. GR-S, the copolymer of butadiene and styrene, has been greatly improved, especially with regard to abrasion. Several new types have been introduced; GR-S 10, using resin soap as an emulsifier, has better tack than standard GR-S. Other types have been developed for insulating purposes: X 116 GR-S is made from isoprene alone; X 141 GR-S is a copolymer of isoprene and styrene.

Polymerization at low temperatures (+5°C. or even −10°) with redox systems such as cumen hydroperoxide + ferrous sulfate and rosin soap as emulsifier has given very promising results.[211] Vulcanized so-called "cold rubber" is reported to have better tensile strength and better resistance to flexion ("flex life") than has the original GR-S.

In Germany [212] all possible vinyl derivatives have been systematically investigated with respect to their suitability for copolymerization with butadiene: for example, isopropenyl methyl ketone, methyl acrylate, methyl methacrylate, *N*-diisobutylacrylamide, β-vinylnaphthalene, 2-chlorobutadiene, and vinylidene chloride. No copolymer proved better than buna S_3. Vinyl chloride, methyl vinyl ether, and vinyl carbazole could not be copolymerized with butadiene.

GR-S and perbunan may both be vulcanized with sulfur. The quality of the vulcanizate obtained depends very much upon the quantity and quality of the added gas black, which has much more effect on buna than it has on rubber. With rubber, the optimum vulcanization time must not be exceeded, otherwise the quality falls off, but buna S and perbunan are much less sensitive in this respect.

Vulcanizates of buna S are valuable on account of their resistance to abrasion, which exceeds that of vulcanized rubber, and they are, therefore, particularly suitable for the manufacture of automobile and bicycle tires. Their great resistance to the action of hot air and steam makes them suitable for other special purposes, such as cable insulation. The emulsion can also be used in the same way as natural latex, for applications such as impregnating textiles. Finally, it may be mentioned that a very good quality of hard rubber can be made both from GR-S and from perbunan.

Molecular weight and intrinsic viscosity of GR-S in toluene follow the relation:[212a]

$$[\eta] = 5.4 \times M^{0.66}$$

[211] W. H. Shearon, J. P. McKenzie, and M. E. Samuels, *Ind. Eng. Chem.*, **40**, 769 (1948).

[212] E. R. Weidlein, Jr., *Chem. Eng. News*, **24**, 771 (1946).

[212a] J. A. Yanko, *J. Polymer Sci.*, **3**, 576 (1948).

(*b*) *Determination of Constitution of Synthetic Butadiene Copolymers*

The relative proportions of butadiene and styrene residues can be determined spectrophotometrically from the intensity of the absorption band of the styrene residue at 262 mμ.[213] Moreover, 90% of the double bonds present can be determined rapidly by titration with chlorine iodide in nitrobenzene.[214] Oxidation with ozone and subsequent treatment with permanganate, as well as direct oxidation with permanganate, are useful in determining the distribution of styrene residues in the chain. When styrene units are adjacent to butadiene units, β-phenyladipic acid is formed; while, if styrene units are grouped together, polyphenyl dibasic acids are formed.

To obtain some indication of the extent of branching the ratios of the osmotic molecular weight to the intrinsic viscosity may be compared. For this purpose fractionated material must be used, since this ratio is also influenced by the degree of polydispersity.

(*c*) *Copolymers of High Styrene Content*

Copolymers of butadiene and styrene in the ratio of 15:85 form thermoplastics resistant to oxygen and chemicals (for example, Pliolite S3 made by the Goodyear Tire and Rubber Co.).[215] They can be mixed with natural and synthetic rubber and indeed improve the electrical properties of the product.

(*d*) *Copolymers with Dichlorostyrene*

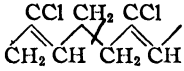
Copolymers of butadiene and dichlorostyrene (with 30–40% dichlorostyrene) are manufactured by the Mathieson Alkali Works as heat- and oil-resistant rubber.

(*e*) *Copolymers of Butadiene and Acrylonitrile* (*Perbunan, Perbunan Extra*)

Copolymerization is carried out in emulsion. A special feature of perbunan is its resistance toward the action of oil and benzene, due to the presence of incorporated polar groups, C—CN. Perbunan extra is a polymer containing more acrylonitrile than perbunan; it is more resistant toward hydrocarbons but less resistant toward acetone than is perbunan.

[213] E. J. Meehan, *J. Polymer Sci.*, **1**, 175 (1946).
[214] E. R. Weidlein, Jr., *Chem. Eng. News*, **24**, 771 (1946).
[215] A. M. Borders, R. D. Juve, and L. D. Hess, *Ind. Eng. Chem.*, **38**, 955 (1946).

4. Polymeric 2-Chlorobutadiene, Polychloroprene, Neoprene



Neoprene was developed by Carothers[216] in the laboratories of du Pont de Nemours and Co. at Wilmington; the impulse which led to its development came from the work of Nieuwland[217] on vinylacetylene.

(*a*) *Preparation and Properties of Neoprene*

The starting point in manufacture of neoprene is the catalytic conversion of acetylene into vinylacetylene:

$$2\ CH{\equiv}CH \longrightarrow CH_2{=}CH{-}C{\equiv}CH$$

A mixture of copper, cuprous chloride, ammonium chloride, and water is used as a catalyst. One molecule of hydrochloric acid is added to vinylacetylene, forming 2-chlorodivinyl ($CH_2{=}CCl{-}CH{=}CH_2$), also known as chloroprene. This reaction is accelerated by copper ions. The polymerization of 2-chlorobutadiene is accomplished in emulsion. Williams and Walker[218] have studied the question of suitable emulsifiers and they find that triethanolamine stearate, together with sodium stearate, forms a suitable combination. According to the conditions of polymerization, namely, temperature, proportion of accelerators, emulsifiers, and antioxidants, viscous products of a moderate degree of polymerization or tough, elastic substances of higher molecular weight may be obtained (Bridgwater and Krisman[219]).

The product of polymerization displays elastic properties like those of rubber. Since the organically bound chlorine atom occupies about the same space as a methyl group, one might expect a certain structural similarity with rubber. In actual fact the product belongs not to the *cis* series but to the *trans* series (gutta-percha), as appears from its x-ray diagram when stretched, which resembles the diagram of β-gutta-percha (identity period along the fiber axis 4.9 Å. (Carothers, Williams, Collins, and Kirby[220]). It is therefore related to gutta rather than to rubber and may be

[216] W. H. Carothers, *Ind. Eng. Chem.*, **26**, 30 (1934). This paper and other publications of the late W. H. Carothers are reprinted in this Series, Vol. I.

[217] J. A. Nieuwland, W. S. Calcott, F. B. Downing, and A. S. Carter, *J. Am. Chem. Soc.*, **53**, 4197 (1931).

[218] J. Williams and H. W. Walker, *Ind. Eng. Chem.*, **25**, 199 (1933).

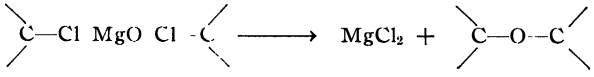
[219] E. R. Bridgwater and E. H. Krisman, *ibid.*, **25**, 280 (1933).

[220] W. H. Carothers, J. Williams, A. M. Collins, and J. E. Kirby, *ibid.*, **53**, 4203 (1931); reprinted in this Series, Vol. I, p. 281.

regarded as chloronorgutta. Certain commercial neoprenes are soluble in rubber solvents; the solutions are about as viscous as solutions of masticated rubber of the same concentration. From this observation it may be assumed that the molecular weight is 100,000–300,000. Neoprene swells but does not dissolve in benzene and petroleum. Although elastic like rubber, the modulus of elasticity is higher, even in the unvulcanized condition; between 70° and 90°C. it becomes soft and plastic like masticated rubber. Neoprene is more stable in the presence of oils than is rubber; in particular it is resistant to lubricating oil and in this respect is superior to rubber. It is said to be less permeable to gases. The density is 1.27.

(*b*) *Vulcanization of Neoprene*

Neoprene can be vulcanized with metallic oxides (most readily with magnesium oxide). This observation indicates clearly the nature of vulcanization: the chemistry of the particular vulcanization process is relatively unimportant, the essential feature is that the chains are cross linked as a result of the reaction in question. In the present case, magnesia effects the linkage of the chains by the formation of ether bridges:



Sulfur accelerates the reaction but is unsuitable for vulcanization. It has been found advisable to add a certain amount of pine resin as well as an antioxidant (such as phenyl-α-naphthylamine) before vulcanization. Like rubber, neoprene may be loaded with fillers, such as amorphous carbon (gas black), whereby the resistance to oils may be increased.

Neoprene is used for oil-resistant tubes, washers, plates, and other objects in which a certain degree of elasticity is desired (such as rollers). It is also used as a heat-resistant insulating material (for example, for ignition cables).

5. High Polymers of Other Butadiene Derivatives

Isoprene, piperylene, and 2,3-dimethylbutadiene polymerize to form rubberlike products. 2-Methoxy, ethoxy, and butoxy dienes have been prepared by Dykstra.[221] They polymerize more slowly than isoprene and

[221] H. B. Dykstra, *J. Am. Chem. Soc.*, **57**, 2255 (1935).

chloroprene but yield rubberlike substances, which are not commercially important or likely to become so.

Resinous materials have been prepared from 1-phenylbutadiene and 2,3-diphenylbutadiene; 1,3-diphenylbutadiene forms a dimeric product which does not polymerize further.[222]

Rubberlike polymers of isobutylene have been described in sect. III 1*d*. They can only be vulcanized if copolymerized with a few per cent of butadiene, and are more resistant toward hydrochloric and sulfuric acids, halogens, and oxygen than is natural rubber because they contain fewer double bonds (Butyl rubber, made by Standard Oil.[223]) The mechanical properties of vulcanized Butyl rubber and their dependence on the molecular weight of the unvulcanized hydrocarbon and its double bond content have been investigated in detail by Flory.[224]

A copolymer of 95% ethyl acrylate and 5% chloroethyl vinyl ether (Lactoprene E.V.) seems to have good oil resistance similar to that of perbunan, and is said also to be heat resistant. It can be vulcanized with sulfur and accelerators.[225]

VI. STATES OF AGGREGATION OF CHAIN POLYMERS

Chain polymers may occur in the following states: the amorphous vitreous, the rubberlike, and the plastic or liquid state of aggregation. Many polymers crystallize under certain conditions; in this case, in addition to the amorphous state, there is the crystalline state.

1. Solid Amorphous State

All noncrystallizing polymers become solid and vitreous at low temperatures. Elastomers capable of crystallization like rubber may form an amorphous vitreous mass if cooled very rapidly. Firmness in this condition depends on pretreatment: objects obtained by injection molding or by calendering are stiffer than those made from the same material and at the same temperature by press molding. Obviously, a parallel arrangement of parts of the chains is obtained by these treatments and the cohesion is thus increased.

[222] G. S. Whitby and W. Gallay, *Can. J. Research*, **6**, 280 (1932).

[223] R. M. Thomas, I. E. Lightbown, W. J. Sparks, P. K. Frolich, and E. V. Murphree, *Ind. Eng. Chem.*, **32**, 1283 (1940).

[224] P. J. Flory, *Ind. Eng. Chem.*, **38**, 417 (1946).

[225] T. J. Dietz, W. C. Mast, R. L. Dean, and C. H. Fisher, *Ind. Eng. Chem.*, **38**, 960 (1946).

(a) *Second-Order Transition*[226]

If a solid, amorphous high polymer is heated, a sudden change in several properties is observed at a certain temperature: the thermal expansion coefficient increases and the specific heat exhibits a maximum. If volume and heat content are plotted against temperature a sudden change in the slope of the curve is found. The transition thus manifested is known as second-order transition. At the same temperature the stiff substance gradually becomes soft; if the temperature is raised further the substance becomes rubbery and, finally, at still higher temperatures, plastic. This means that the viscosity of 10^{12} c.g.s. is gradually decreased by several powers of ten.

Table VII shows the transition temperature for various materials. Samples of the same substance with different pretreatment may, however, give slightly different results. In a polymeric series the transition temperature at first rises with the degree of polymerization and becomes constant after a molecular weight of about 20,000.

TABLE VII
Transition Temperatures in °C.

Selenium (amorphous)[226a]	30.7	Polyvinyl acetate[231]	28
Polyisobutylene[227]	−74	Polyvinyl chloride[230]	75
Rubber[228]	−73	Polymethyl methacrylate[232]	60
Buna S (GR-S)[229]	−61	Polystyrene[233]	81
Cellulose acetate[230]	70		

As Ueberreiter has shown, it is often possible to draw conclusions about internal arrangement and previous history of the specimen from a study of volume–temperature curves. Cold-stretched polystyrene, for example, is often somewhat expanded. This is shown by the change in volume of the

[226] See R. F. Boyer and R. S. Spencer, in *Advances in Colloid Science*, Vol. II. Interscience, New York, 1946, p. 1.

[226a] G. Tammann and A. Kohlhaas, *Z. anorg. allgem. Chem.*, **182**, 67 (1929).

[227] J. D. Ferry and G. S. Parks, *J. Chem. Phys.*, **4**, 70 (1936).

[228] N. Bekkedahl, *J. Research Natl. Bur. Standards*, **13**, 411 (1934). N. Bekkedahl and H. Matheson, *ibid.*, **15**, 503 (1935).

[229] R. D. Rands, Jr., W. J. Ferguson, and J. L. Prather, *J. Research Natl. Bur. Standards*, **33**, 63 (1944).

[230] R. F. Clash, Jr., and L. M. Rynkiewicz, *Ind. Eng. Chem.*, **36**, 279 (1944).

[231] E. Jenckel, *Kolloid-Z.*, **100**, 163 (1942).

[232] F. E. Wiley, *Ind. Eng. Chem.*, **34**, 1052 (1942).

[233] W. Patnode and W. J. Scheiber, *J. Am. Chem. Soc.*, **61**, 3449 (1939).

sample after heating and recooling. The transition point is generally determined at rising temperature; but the same point may be found if a rubberlike substance is slowly cooled; in this case, however, hysteresis is not excluded.

(*b*) *Brittle Point*

As the temperature is lowered elastomers gradually lose their rubbery properties; they harden and become brittle. At a temperature slightly above the transition point the sample shatters upon the sudden application

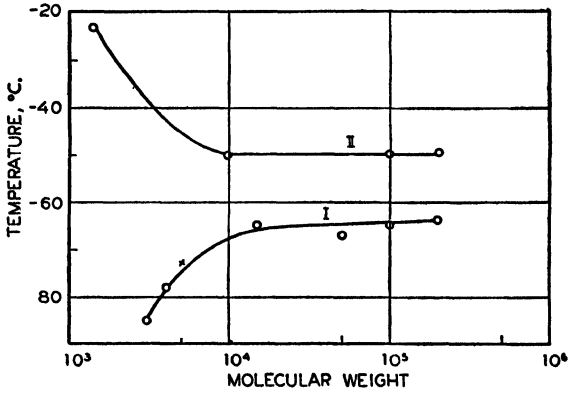


Fig. 17. Effect of molecular weight on transition point (curve I, data of Jenckel and Ueberreiter) and brittle point (curve II, data of Selker *et al.*) of polyisobutylene.[226]

of force. This temperature is known as the brittle point; it is of great practical importance for it determines the lower temperature limit of serviceability.

The best method of determining the brittle point is apparently the method of Selker, Winspear, and Kemp[234]: Strips of the sample are bent rapidly—the temperature at which breaking occurs is determined. It is, however, less reproducible than the transition point and depends, for example, on the dimensions of the sample. As figure 17 shows the brittle point is found at a somewhat higher temperature than the transition point. The difference between these points is quite large for the lower polymers; these are plastic at a higher temperature and become brittle at a lower temperature without passing through a distinctly rubbery state.

[234] M. L. Selker, G. G. Winspear, and A. R. Kemp, *Ind. Eng. Chem.*, **34**, 157 (1942).

(c) *Theory of Second-Order Transition*

As has been pointed out by Meyer, Susich, and Valkó,[235] a considerable part of the heat content of elastomers consists in rotational movements of smaller or larger segments of the chains. By rotations about the C—C bond the form of the molecule may change, as is shown in figure 18. We

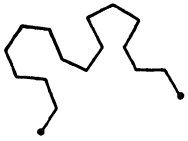


Fig. 18. Configurations of a chain molecule. Only one stretched configuration is possible, but many twisted configurations are possible (Meyer).



shall see later that these intramolecular rotational movements account for the elastic force in the rubberlike state. These movements are frozen in the stiff amorphous state. The phenomena observed at the transition point are attributed to the beginning of rotation about the C—C bonds. The many possible modes of movement which may occur once this rotation sets in would explain the gradual and marked increase in heat capacity once the transition temperature is exceeded. Transition temperature as well as brittle point is lowered by addition of softeners (suitable swelling agents).

2. Rubberlike State

(a) *Molecular Arrangement*

The state of aggregation of chain polymers above the transition or melting point is characterized by a reversible, long-range extensibility and a low modulus of elasticity. Ordinary glasses are cross linked in three dimensions either by primary valences as in glass or by secondary valences as in supercooled alcohol. Melting, therefore, consists in a loosening of all cross links and the melt behaves like any ordinary liquid. In frozen elastomers the repeating groups (segments) are bound by primary valences with two neighbors, and by much weaker secondary valences with all other neighbors. Melting, therefore, affects only the latter valences; thereby a state of aggregation is created which is a definite intermediate between the solid and the liquid states. As this state was first observed for rubber it is

[235] K. H. Meyer, G. von Susich and E. Valkó, *Kolloid-Z.*, **59**, 208 (1932).

known as the rubberlike or rubbery state. All synthetic, high molecular chain polymers display rubbery properties at least within certain limits of temperature; the same is true for sulfur, selenium, and polyphosphonitrilic chloride. Rubberlike elasticity is also exhibited by biological systems such as muscle fibers, elastic bands, and membranes of animal cells.

X-ray analysis has shown that all substances investigated are amorphous in the rubbery state; only in the amorphous condition, therefore, is the property of high reversible extensibility present; even unstretched rubber which has crystallized in storage (as is often the case with commercial crêpe) is rigid and does not become elastic until it is warmed. The amorphous x-ray diagram indicates that no parallel oriented stretched chains are present. On stretching the chains are aligned in parallel and finally arrange themselves into a crystalline lattice. The tensile strength of rubber in amorphous condition (measured on material cooled at very low temperatures) is only a fraction of that of stretched and crystallized rubber.[236]

Rubber	Tensile strength, kg /mm.
Native rubber, unstretched	5.1
stretched 9.6 times	35.1
Soft vulcanized rubber, unstretched	5.3
stretched 9 times	44.4

On pulverization in liquid air, small, irregular fragments are obtained (Hock[237]), whereas fibers are obtained on pulverization of stretched frozen rubber. These observations indicate an entirely random arrangement of the chains and segments in the amorphous state. The chains are coiled and are elongated and aligned in parallel on stretching.

(*b*) *Kinetic Theory of Rubberlike Elasticity*[238]

The theory of rubberlike elasticity generally accepted at the present time is based on the observation, originally made by Gough,[239] that rubber becomes warm on stretching (Gough-Joule effect). At the suggestion of Thomson[240] (Lord Kelvin) Joule made a careful study of the thermoelastic behavior of various solids. Joule classified elastic substances according to their thermoelastic behavior into four groups: (*1*) The equivalent of the work done on the substance is evolved as heat; in this case the elastic force is due to thermal agitation. (*2*) The work done on the substance is

[236] M. Polanyi and A. Schob, *Mitt. deut. Materialprüfungsanstalt.*, **42**, 22 (1924).

[237] L. Hock, *Kolloid-Z.*, **35**, 40 (1924); *Kautschuk*, **3**, 125 (1927).

[238] Review: K. H. Meyer, *J. Soc. Chem. Ind.*, **57**, 439 (1938). *Advances in Colloid Science*, Vol. II, Interscience, New York, 1946, p. 253.

[239] R. Gough, *Memoirs Lit. Phil. Soc. Manchester*, **1**, 288 (1805).

[240] R. Joule, *Phil. Trans. Roy. Soc. London*, **149**, 91 (1859); *Phil. Mag.*, 227 (1857).

stored as potential energy, not evolved as heat. (*3*) The work done on the substance is partly given off as heat, partly stored as potential energy. (*4*) More heat is evolved than the equivalent of the work done on the system. Metals and other ordinary solids behave according to rule for the second class or substances, while rubber occurs in classes (*1*) or (*4*). There can thus be no doubt that Joule had already recognized at that time (1859) that the contraction of stretched rubber is due to thermal agitation.

In accordance with the state of knowledge of his time, Joule made no attempt to analyze still further the kinetics of the contraction process; Wöhlisch,[241] on the other hand, sought to explain this in terms of the thermal movements of crystals, micelles, or rod-shaped molecules oriented by stretching. The energy stored in a piece of stretched rubber is of a different order, however, from the energy of thermal agitation of such particles. For this reason, Wöhlisch's assumption must be rejected.

As has been shown by Meyer, Susich, and Valkó,[242] the elastic force of deformed rubber is due to thermal movements of the segments of primary valence chains stretched by deformation. Whereas the thermal movements of a methylene group are connected with the movements of the neighboring groups of the chain, they will be independent of the behavior of groups further away; parts of the chains, which will be termed segments, may therefore perform thermal movements independently of one another. Since there is only one stretched form, but many twisted forms, the chains tend to return to one or another of the statistically more probable twisted forms, such as they assume in unstretched rubber. The elastic force of rubber and other elastomers is thus based on a phenomenon similar to the pressure of a gas; according to Meyer, Susich, and Valkó, the elastic force is indeed (at least within a large range of elongation) proportional to the absolute temperature.

(*c*) *Behavior of Segments*

As mentioned above, coiled chain molecules are stretched and aligned in parallel if a rubbery body is stretched; after the deformation any individual segment will have other neighbors (with the exception of the two neighbors of its own chain) than it had before. The segments must therefore be able to slip over each other like the molecules of a liquid when flowing. Just as in the latter case internal friction will slow down the rate of the

[241] E. Wöhlisch, *Verh. phys.-med. Ges. Würzburg*, **N. F. 51,** 53 (1926).

[242] K. H. Meyer, G. von Susich, and E. Valkó, *Kolloid-Z.*, **59,** 208 (1932). See also W. T. Busse, *J. Phys. Chem.*, **34,** 2870 (1932), and E. Karrer, *Phys. Rev.*, **39,** 857 (1932). W. Kuhn, who is often called the author of this theory, did not publish his first work until several years later.

deformation and heat of friction will be evolved. In rubber this friction is quite high; it amounts at room temperature to 10^6 poises, which is one hundred million times as large as the friction observed in water. In contrast to an ordinary liquid the segments of an elastomer form long flexible chains; if these chains are sufficiently long they form a sort of a felt so that the elastomer returns to the original form when the deforming stress vanishes. The felt texture is best maintained by a small number of cross links which unite the chains into one giant spongy molecule.

If the internal friction between portions of the chains exceeds a value of 10^{12} poises, the body is an amorphous solid. Examples of amorphous solid high polymers are provided by polyvinyl acetate and polystyrene, the viscosity of which only falls at higher temperatures to the limiting value at which the rubberlike state appears. Other polymers are crystalline at ordinary temperatures and become reversibly extensible on melting; examples are polyvinyl chloride and gutta-percha.

A further possible means of lowering viscosity and so obtaining rubberlike bodies from amorphous solids is provided by swelling agents; polyvinyl acetate and nitrocellulose become rubbery in the presence of certain swelling agents. In this case all of the segments are bound firmly in one direction but dissolved in the other two directions, so that the swelling may be regarded as two-dimensional solution. Many animal tissues exhibit this variety of the rubberlike state.

(*d*) *Anisotropy of Elastomers*

We have already seen that a deforming force aligns the chain molecules in rubber. Under certain conditions the orientation may persist after the applied force has ceased to act, leaving the specimen with a certain degree of anisotropy; this is very noticeable during calendering. As a result of milling the chains are aligned in the direction of rolling, and the elastic modulus and refractive index are higher in that direction than in directions at right angles.

Such anisotropy must always be considered when studying the mechanical properties of high polymers; almost invariably traces of anisotropy persist as a result of the method of preparation or molding the material. A film of rubber prepared by allowing a solution to evaporate contracts—an indication that the arrangement of the chains is different in different directions. Even a single short extension of a rubberlike substance changes its physical properties: the "virgin" stress–strain curve has a somewhat different form from those obtained later under otherwise identical conditions.

Anisotropy can be removed by annealing for a sufficiently long period;

if internal friction is very great, however, the balancing out of anisotropies does not occur in practice. In materials which are only rubberlike at high temperatures, anisotropies are frozen in as soon as they are cooled. Thus, threads spun under high tension or stretched after spinning have a high tensile strength; flexible, tough foils of polystyrene are prepared by stretching both in length and in breadth during calendering.

(e) *Effect of Cross Linking*

A certain amount of cross linking does not abolish rubberlike elasticity. A rubberlike substance does not become a solid until three-dimensional cross linking has so stiffened the internal structure that the slipping of the links over their neighbors becomes impossible. This occurs when rubber is vulcanized with large quantities of sulfur (*ca.* 40%); the product obtained in this way is known as ebonite or hard rubber.

A reasonably high proportion of cross links is reconcilable with rubberlike properties. A sample of rubber containing 7% of sulfur, half of which was in the form of thio ether, so that on an average each chain fragment of molecular weight 100 includes a bridge linkage, can still be extended to five times its original length. Even greater vulcanization, up to one cross link per seven isoprene residues, does not abolish rubberlike properties. It is not easy to estimate the dimensions of the meshes in such a three-dimensional network.

If a piece of rubber which has become anisotropic through milling is carefully vulcanized, the anisotropy is fixed by cross linking: the modulus of elasticity and the stiffness are then permanently higher in one direction than in any other.

In nature, systems which are rubberlike but display marked anisotropy play an important part, such as elastic fibers and muscle fibrils. It may be assumed that their anisotropy is to be referred to the same cause as that of oriented vulcanized rubber: a network which prevents the chain molecules from assuming all possible positions and configurations. When we come to consider elastic fibers we shall return to this point, particularly in our discussion of their thermoelastic properties.

(f) *Limit of Elastic Deformation*

If unvulcanized rubber is stretched quickly, the elastic modulus begins to increase rapidly after an elongation of 400–600%, and if it is extended further, the rubber breaks. This may be explained by the segments being

completely aligned in parallel. If crystallization does not occur, however, as with polystyrene, or is suppressed as a result of heating, or by a solvent, it is possible to continue stretching long after the chains are aligned parallel to one another. In this way extensions over 1000% may be reached; they are not reversible, however, so that plastic deformation must have occurred during stretching.

It is a remarkable fact that in many other rubberlike substances this orientation of the chains is complete at about the same degree of elongation; this is true, for example, of elastic sulfur, polyphosphonitrilic chloride, and many organic chain polymers. Conversely, oriented, crystalline fibers contract to about one seventh of their original length when the crystallites are fused by heating.

In amorphous, unstretched rubber, the chains must be tightly intertwined, since the density of amorphous rubber differs little from that of crystallized rubber; the molecular texture must approximate that of threads in a felt or in compressed cotton wool. If this material is stretched, parts of the threads are stretched parallel to the direction of stretching, others are pulled into folds which can only be removed by applying a greater tension—which quickly leads to rupture. There is no reason to suppose that the processes occurring in the deformation of rubber are essentially different; in fact, the behavior of substances in the rubberlike state fits in completely with this picture.

If stretched to the breaking point, a cube of an elastomer is converted to a parallelipiped whose length is about $7\sqrt{7} = 20$ times as long as its breadth. Conversely, an extended sample of these dimensions is converted to a cube when it contracts. In molecular terms this means that a straight chain fragment of 25 isoprene units (100 Å. long, *ca.* 5 Å. wide) will be converted to an isodiametric particle when it contracts. In amorphous rubber this particle will not be compact, but to some extent distended by the presence of portions of neighboring chains.

Many polymers can be extended to their breaking extension without showing any tendency to return to their initial length; stretching of this kind is usually referred to as cold drawing. Examples of such behavior are provided by polyamide fibers (to be discussed later), the contents of the silk glands—which are converted into the crystalline silk thread on stretching, and swollen hydrated cellulose. Here again we may suppose that the unstretched, amorphous material has a molecular structure similar to that of an unstretched elastomer. It very often happens in such materials that oriented crystallites appear during stretching as in silk and hydrated cellulose.

(g) *Effect of Swelling on Form of Chains*

If unstretched rubber is allowed to swell in a suitable solvent the molecular felt is expanded; when the solvent evaporates, the specimen regains its original form. This means that points of linkage in the felt remain unchanged and it is only the flexible, much contorted portions of the chains that are straightened during swelling. In the swollen condition, the chain molecules must be less contorted than in pure rubber (Müller[243]). The meshes can be fixed in the extended form if a thin layer of rubber solution is allowed to evaporate on a solid surface. In this way a film is formed which adheres closely to the surface, and which when lifted off immediately contracts as if it had been stretched in all directions (see Fig. 19). On the other hand, a film prepared by allowing a solution of latex to evaporate shows no such internal tension.

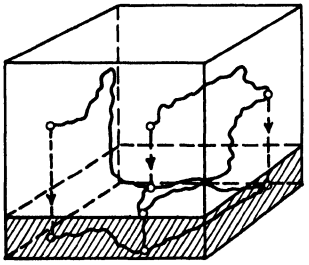


Fig. 19. Behavior of chain molecules on evaporation of a solution. (F. H. Müller).

3. Plastic State

Chain polymers become plastic if the temperature is raised far above their transition point: they begin to flow. Plastics in this state, however, still exhibit rubbery properties; their viscoelastic behavior is thus much more complicated than that of glycerol, melted glucose, or melted glass; it will be treated in detail in Chapter I.

In a polymeric homologous series the softening temperature on heating rises with molecular weight; lower polymers pass from the solid amorphous state directly into the plastic state; polymers of molecular weight 20,000 to 500,000 display, over a considerable interval of temperature, rubber-like properties, while still higher polymers become plastic only at temperatures at which chemical degradation sets in.

4. Crystallization and Fusion in Rubber and Other High Polymers

(a) *Peculiarity of Crystallization*

After Katz[244] had discovered the x-ray diffraction pattern of stretched rubber, the question arose as to whether true crystallization or some en-

[243] F. H. Müller, *Kolloid-Z.*, **103**, 144 (1943).

[244] J. R. Katz, *Naturwissenschaften*, **13**, 410 (1925).

tirely different phenomenon was involved. At the present time we are fairly well informed about the phenomena of crystallization in high polymers and it is possible to give a general account of this subject. In so doing, it will be necessary to consider also observations on homologous series of substances of lower molecular weight.

We have already seen that as molecular weight increases in normal paraffins, the melting points tend toward the limiting value of 145°C. This behavior is quite general, as Fairweather[245] showed in the case of dicarboxylic acids, in which the limiting value for even members is 123°C. This has also been shown by Garner and King.[246] Mixtures of higher homologous polymers form mixed crystals, the melting point of which does not differ from that of the pure substances. Two higher members of a homologous polymeric series have, therefore, the same melting point. Since at the melting point the heat of fusion, ΔH_f, becomes equal to the product of the temperature of fusion, T_f, and the entropy of fusion, ΔS_f, the following expression is valid for all higher members of the series:

$$\frac{1}{T_f} = \frac{\Delta S_f}{\Delta H_f}$$

The molar heat of fusion increases by a constant amount, Δh, for each CH_2 group added to the chain. From this it follows that the entropy of fusion also increases by a constant amount, Δs, for each CH_2 group added (King and Garner). For a paraffin of z monomeric units, we have therefore:

$$\frac{1}{T_f} = \frac{z\Delta s}{z\Delta h}$$

The increase in entropy with each CH_2 group corresponds to an increase in possibilities of arrangement due to the mobility of the added groups. The entropy of melting of long-chain substances depends predominantly therefore on the mobility of the individual links at fusion. The mobility of the molecule as a whole and the difference in entropy associated with this mobility are overshadowed by the mobility of the links. In the case of members of a series of moderate or low molecular weight, however, the mobility of the molecule as a whole cannot be neglected. The equation given above may be completed by adding a term B, the increment in the entropy which is practically the same for all members of the homologous series, corresponding to the mobility of the molecule as a whole. We obtain then:

[245] D. A. Fairweather, *Phil. Mag.*, [7], 1, 944 (1926).

[246] A. M. King and W. E. Garner, *J. Chem. Soc.*, **1936**, 1368, 1372.

$$\frac{1}{T_f} = \frac{z\Delta s + B}{z\Delta h}$$

Writing $\Delta s/\Delta h = a$ and $B/\Delta h = b$, we have:

$$\frac{1}{T_f} = a + \frac{b}{z}$$

This is the empiric formula for the melting point in a series of homologous paraffins developed by van der Wyk.

Certain consequences follow: (*1*) the melting point of chain polymers (such as rubber) is independent of the chain length, provided that the chains are not too short. As far as observations are available, they are in agreement with this conclusion. The melting point of rubber of the highest molecular weight never exceeds 42°C. when measured in the absence of tension. The melting point of gutta-percha is fairly definite and for the α form is 47–48°C. (*2*) This conclusion concerns the process of melting in very long chain polymers. From what has been said, it is clear that the entropy of fusion—*e. g.* of rubber with a molecular weight of 300,000—will consist practically entirely of the intramolecular fraction. Fusion is therefore a process in which parts of the molecules, that is, the segments of the chains, become more mobile.

Because of the great length of the molecules, it follows that one part of a chain may participate in a crystal lattice while another part is amorphous. The links at the beginning, in the middle, and at the end of a chain are so far removed from each other that they are able to crystallize or fuse independently of each other. Very small crystalline regions may thus be formed and complete crystallization will be prevented by entanglement of the molecular threads uniting the crystallites.

A certain percentage of amorphous material will thus be present in any crystallized chain polymer. The fact that crystallization is not complete explains why the melting point in a homologous series first increases with molecular weight, then falls, and finally reaches a constant value. The limiting value of the melting point of the normal paraffin series should be 145°; there has been observed, however, a melting point of about 130° in the case of a medium degree of polymerization, whereas high polymeric polythene becomes entirely amorphous at 115°.

Melting does not occur at a sharp and defined temperature. The specific heat increases well below the real melting point; this has been found for polythene by Raine, Richards and Ryder.[247] If the heat of melting is esti-

[247] H. C. Raine, R. B. Richards, and H. Ryder, *Trans. Faraday Soc.*, **41**, 56 (1945).

mated from the heat content–temperature curve the following values are found: 43 cal. at 20°, 40 cal. at 60°, and 22 cal. at 100°. The thermal movements of segments which characterize the rubbery state therefore set in over a large range of temperature.

The process of crystallization occurs in two different ways in many high polymers. A number of these (including gutta-percha, degraded acetyl and methyl cellulose, and, according to Smith, Saylor, and Wing,[248] degraded rubber) may be obtained from suitable solvents in microcrystalline form. It is also possible, by evaporating solutions, to obtain opalescent films which break up readily into powder, thereby indicating that they consist of aggregates of discrete particles. This type of crystallization does not occur among high polymers of very great chain length.

The second type of crystallization occurs during the solidification of concentrated solutions or melts. If a film of gutta, obtained in the manner mentioned above, is melted and cooled, it becomes rigid (although it was elastic in the molten state) and now shows birefringent areas, with optically isotropic regions lying between them. The film is slightly extensible and the tensile strength so great that we must assume the crystalline regions to be still interconnected by primary valence chains.

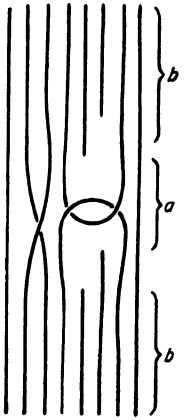


Fig. 20. Arrangement of chain molecules in stretched elastomers: (*a*) amorphous; (*b*) crystalline regions.

Hence, the limits of the crystallites are not also the limits of the chain molecules. The crystallites are to be regarded, rather, as ordered regions from which the chains project and are continued into nonoriented regions and into other crystallites. Such crystallites are often called fringed micelles, following Gerngross, Herrmann, and Abitz,[249] who first clearly recognized this peculiar type of crystallite formation. All chain polymers of very high molecular weight form fringed micelles only, for example, polymethylenes, other polyvinyl derivatives, elastic sulfur, cellulose, and silk. In branched polymers like amylopectin the formation of individual crystals is prevented by irregularities due to the branching points. These polymers therefore crystallize in fringe micelles only.

[248] W. H. Smith and C. P. Saylor, *J. Research Natl. Bur. Standards*, **13**, 453 (1934). W. H. Smith, C. P. Saylor, and H. J. Wing, *ibid.*, **10**, 479 (1933).

[249] O. Gerngross, K. Herrmann, and W. Abitz, *Biochem. Z.*, **228**, 409 (1930). L. Hock, *Kautschuk*, **3**, 207 (1927).

A third kind of crystallization occurs on stretching. This is understandable when it is remembered that the chains are aligned parallel by stretching. Figure 20 shows diagrammatically the arrangement of the chains in stretched fibers or films. This crystallization, however, is also incomplete as is shown in figure 20.

(*b*) *Dependence of Melting Point on Tension and Deformation*

In rubber crystal interferences appear at room temperature on stretching to 80%. The intensity of the interference spots increases approximately in proportion to the degree of stretching, while their position is not perceptibly changed. Hock[250] made the important observation that the heat developed on stretching was proportional to the increase in intensity of the spectra. This suggested very strongly that the heat evolved was to be interpreted as the sum of the relatively small work of extension and the heat of crystallization. Hock and Bostroem[250] showed that the heat of extension amounted to 0.84 cal./g. at 186%, 2.86 cal./g. at 428%, and 6.8 cal./g. at 821% elongation. In the case of rubber in which, by suitable vulcanization, plastic deformability has been suppressed, equilibrium between crystalline and amorphous fractions is determined by the temperature and the degree of deformation. Raising the temperature displaces the point of equilibrium in favor of the amorphous fraction, so that a melting point curve is obtained (according to von Susich[251]) if the temperatures at which the diffraction pattern disappears are plotted against the deformation. At 600% elongation, this temperature is about 80–90°C.

At the melting point the relation:

$$H_{am} - H_{cryst} = T(S_{am} - S_{cryst})$$

holds (where H is the heat content in the amorphous or in the crystalline condition). It can be shown that the entropy of a chain in the stretched condition is less than that of an unstretched chain.

Since S_{am} is smaller in the stretched condition than in the unstretched condition ($S_{am} - S_{cryst}$) will also be smaller; and since the heat of fusion ($H_{am} - H_{cryst}$) may be assumed to be independent of temperature, T (the temperature of fusion) must rise in the stretched condition. The dependence of the melting point on the tension applied is shown with particular clearness by gutta-percha, when slightly vulcanized and stretched. If a sample of gutta-percha is warmed in a water bath at various loads, the

[250] L. Hock and S. Bostroem, in K. Memmler, *Handbuch der Kautschukwiss.* Hirzel, Leipzig, 1930, p. 489. L. Hock, *Kautschuk*, **3**, 207 (1927).

[251] G. von Susich, *Naturwissenschaften*, **18**, 915 (1930).

melting point can be determined quite sharply by the sudden contraction; figure 21 represents the melting point curve of gutta.[252] By means of the Clausius-Clapeyron equation, it is possible to calculate the heat of fusion, λ_f, from the relation between the temperature of fusion and the tension applied.[253]

Since rubber (and gutta-percha) has a higher density in the crystalline than in the amorphous condition, the melting point must be raised by increase of pressure. It is estimated that the rise in melting point amounts to 0.035°C. per atmosphere.[254]

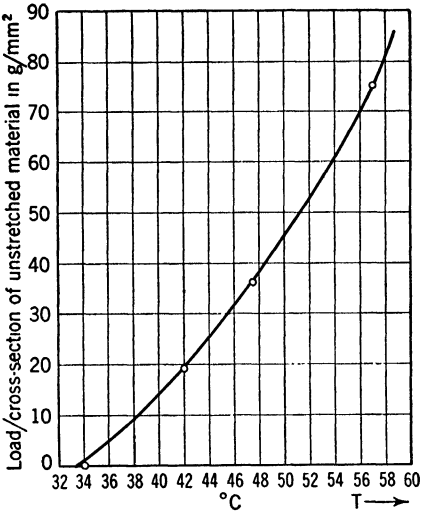


Fig. 21. Melting point of gutta plotted as a function of tension.

At constant pressure (as also under constant tension) the melting point remains reasonably sharp; only the temperature at which fusion occurs changes with these variables. On the other hand, at constant volume or, correspondingly, under constant deformation, the melting extends over a range of temperature; for here the tension increases, whereby the melting point of the parts which have not yet melted is raised.

[252] K. H. Meyer, *ibid.*, **26**, 199 (1938).

[253] E. Wöhlisch and R. du Mesnil de Rochemont, *Z. Biol.*, **85**, 406 (1927).

[254] W. Kirsch, *Z. angew. Chem.*, **50**, 964 (1937). L. A. Wood, N. Bekkedahl, and R. E. Gibson, *J. Research Natl. Bur. Standards*, **35**, 375 (1945).

(c) *Velocity of Crystallization and Size of Crystallites*

The velocity of crystallization of high polymers may vary within wide limits. Gutta-percha, which crystallizes rapidly, may be contrasted with rubber, which will remain for months in the supercooled condition. Even when crystallization has begun, the process continues very slowly for years, as was shown by the experiments of van Rossem and Lotichius.[255] Sheet rubber which had been frozen for four years melted at 31° to 33°C. and showed a reduction in density of 2.9%. Twelve years later the same rubber melted at 35° to 37°C., and the diminution in density amounted to 3.1%. The writer found the melting point of a specimen of sixteen year old crêpe to be 40° to 42°C. If rubber is rapidly crystallized by stretching, fusion takes place below 20°C. It may be that the differences in melting point are due to differences in the size of the crystallites, as Dostal[256] has suggested.

Irregularities in molecular structure arising from vulcanization have the effect of lowering the melting point of the specimen, in the same way as a dissolved substance. Vulcanized rubber, for example, does not freeze at about 0°C. In weakly vulcanized rubber, however, slow crystallization occurs over a period of months or years, as is shown by dilatometer measurements at 0°C.; the final proportion of crystallized material depends on the quantity of sulfur or accelerators added. Another example of very slow crystallization in a high polymer is provided by selenium, which can be kept indefinitely in the supercooled vitreous condition at room temperature.

The velocity of crystallization is increased by parallel orientation of the primary valence chains; thus, amorphous selenium becomes crystalline if kept in the stretched condition for a short time. Molten selenium becomes covered with a crystalline skin, presumably because of the orienting influence of surface forces. Rubber, which in the unoriented condition can be kept supercooled for a long time, crystallizes extremely rapidly when stretched; and the amorphous rubberlike contents of the silk glands of the silkworm and arachnids rapidly become crystalline on stretching.

[255] A. van Rossem and J. Lotichius, *Kautschuk*, **5**, 2 (1929).
[256] H. Dostal, *Oesterr. Chem.-Ztg.*, **41**, 20 (1938).

D. POLYMERIC ETHERS, ESTERS, SULFIDES, ETC.

The high polymeric hydrocarbons are related to the polymeric ethers, esters, and the like, in which many short carbon chains are united by other atoms. The reactions leading to the formation of such compounds are so diverse that no general scheme can be given for the polymerization or condensation reactions. Most of the compounds described below, however, are formed by condensation with the elimination of water, so that this type of condensation or polycondensation (as it leads to the formation of high polymers) will be discussed in some detail.

I. CONDENSATION REACTION

1. Polycondensation

Polycondensation does not proceed as a chain but as a step reaction: one molecule after another is added on, for example, with the elimination of water, and the probability that a new link will be established is practically the same for all groups capable of reacting. For this reason it is easier to get a general picture of the course of the reaction and, in the case of the formation of chain polymers, of the distribution of molecular sizes prevailing at any particular stage in the reaction, than in polymerization. According to Flory:[1]

$$\phi_i = iB^{i-1}(1 - B)^2 = i\alpha^2(1 - \alpha)^{i-1}$$

where ϕ_i is the weight fraction of chains of the degree of polymerization i, and $B = (1 - \alpha)$ is the degree of conversion, that is, the number of linkages established divided by the number which can be established. In this way one can calculate the proportion of polymers of different grades of polymerization which are present; distribution curves such as those shown in figure 1 are obtained. From these one can estimate both the mean molecular weight (arithmetic mean) and the weight average (or average molecular weight). The same distribution is obtained when a chain polymer is regularly degraded; $\alpha = (1 - B)$ is the degree of degradation, that is the number of linkages which have been ruptured divided by the number originally present.

[1] P. J. Flory, *J. Am. Chem. Soc.*, **58**, 1877 (1936).

Supposing that the reaction is 98% complete ($B = 0.98$), that is, 98% of the linkages have been established for a monomer of molecular weight 118 which occurs as a residue of weight 100 in the polymer, then the mean molecular weight (arithmetic mean) is 5000 (degree of polymerization = 50), and molecules of this size are also the most numerous. Only 26% by weight of the polymer has a molecular weight less than 5000; 74% on the other hand has a molecular weight of 5000 or more and 40% has a molecular weight exceeding 10,000. The arithmetic mean is about half as large as the weight average, which is 9900.

If condensation takes place in the presence of aldehydes, particularly formaldehyde, it continues virtually to an end; equilibrium lies entirely

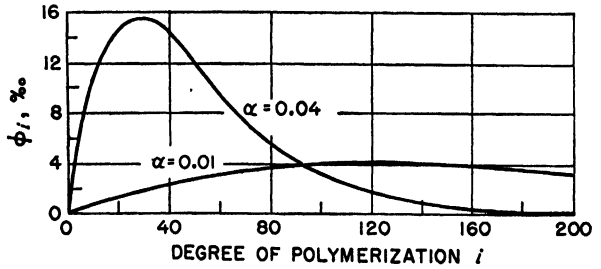


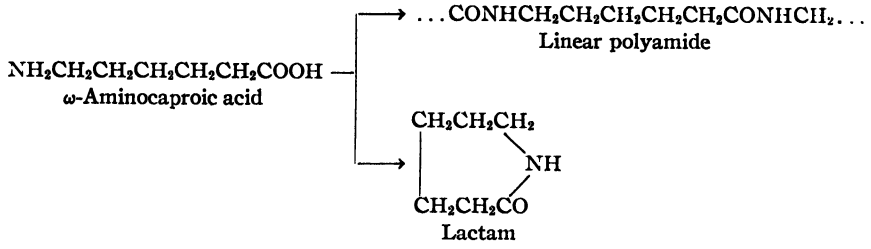
Fig. 1. Weight fractions, ϕ_i, for different degrees of polymerization, i, at degree of conversion $1 - \alpha = 0.96$ and 0.99.

on the side of the polymer + water. If carboxyl groups are condensed with alcoholic hydroxyls, for example, to form esters, or with amines to form amides, equilibrium is soon reached unless the water is removed. In order to obtain polymers of molecular weight 10,000—referred to by Carothers[2] as "superpolymers"—such as are required for synthetic fibers, the water must be removed during the reaction, preferably by working at very low pressures. Carothers, who was particularly successful in his work on the condensation of bifunctional monomers to form high polymers, made use of the *molecular distillation apparatus* in which water molecules emerging from the surface of the mass in a high vacuum are immobilized by freezing. The polymerizing material must be heated in very thin layers because, as a result of the increasing viscosity of the mass, water diffuses very

[2] E. W. Spanagel and W. H. Carothers, *J. Am. Chem. Soc.*, **58**, 654 (1936). W. H. Carothers, *Chem. Revs.*, **8**, 353 (1931). H. Mark and G. S. Whitby, eds., *Collected Papers of Wallace Hume Carothers on High Polymer Substances* (*High Polymers*, Vol. I), Interscience, New York, 1940.

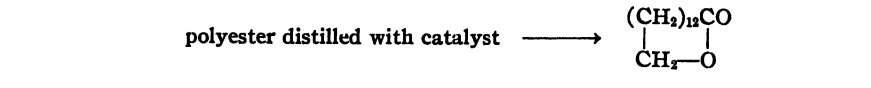
slowly and it is desirable to make the path to the surface as short as possible.

The condensation of bifunctional monomers may lead, not only to the formation of linear polymers, but also to rings of the same analytical com position as the high polymer, but differing in their physical behavior, for example:



These cyclic compounds may arise from one or several monomers. If six-membered rings can be formed, the polymerization reaction is negligible. For this reason commercially available bifunctional substances, such as lactic acid, are unfortunately unsuitable for the preparation of chain polymers.

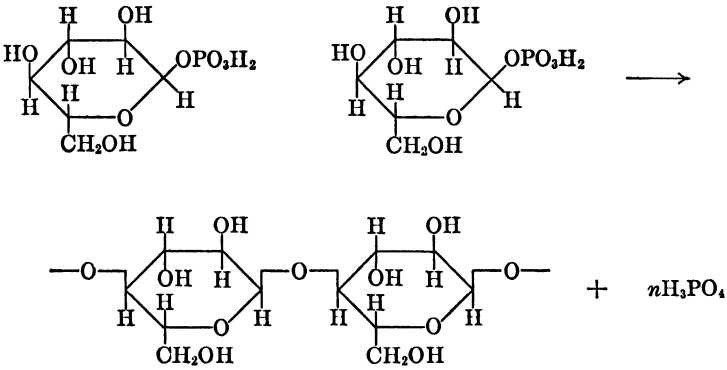
According to Ruggli[3] and Ziegler,[4] ring formation is favored by dilution with inert solvents; solvents are therefore to be avoided in the preparation of polymers. Solvents may also have a specific influence on the velocity of reaction: in the condensation of ω-hydroxyundecanoic acid, *e.g.*, lactone formation is favored by solvents in which the carboxyl groups are not associated; on the other hand, polyesters are formed if the solvent causes the carboxyls to associate.[5] If a cyclic product is formed, it can usually be removed from the reaction mixture by warming, as it is more volatile. If catalysts are added to accelerate the establishment of equilibrium polymer $\rightleftharpoons$ cyclic product (according to Carothers, $SnCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 2H_2O$, and similar metallic chlorides are suitable for this purpose), the polymer can be almost completely depolymerized to the cyclic product, even if this is only present in minute quantities at equilibrium. For example:



[3] P. Ruggli, *Ann.*, **392**, 92 (1912).
[4] K. Ziegler, H. Eberle, and H. Ohlinger, *Ann.*, **504**, 94 (1933).
[5] M. M. Davies, *Trans. Faraday Soc.*, **37**, 804 (1941).

Conversely, many cyclic compounds can be isomerized in part or entirely to polymers[6]; obviously in this case the reaction is not carried out at reduced pressure. Whether equilibrium in the melt lies on the side of the ring form or on that of the polymer depends on the constitution of the compound. If five- or six-membered rings can be formed the ring form predominates. On the other hand, a seven-membered ring, such as the lactam of ϵ-aminocaproic acid, is converted almost quantitatively to the chain polymer. Nothing is known of the effect of temperature on the equilibrium; but by analogy with the equilibria: cyclic sulfur $S_8 \rightleftharpoons$ chain polymeric sulfur S_n, and triphosphonitrilic chloride $\rightleftharpoons$ polyphosphonitrilic chloride, one may assume that high temperature favors the polymeric form in the melt.

In cases where the ring form predominates at equilibrium, ring formation can often be prevented by choice of the initial material. While glycine or glycine ester always yields predominantly diketopiperazine, the cyclic carbaminate of glycine polymerizes with elimination of carbon dioxide to form a chain polymer (Chap. G, III1). Even a polysaccharide, amylose, can be synthesized by an indirect method of this type. It is formed from glucose 1-phosphoric acid in aqueous solution in the presence of an enzyme, phosphorylase (Chap. F, VII9*e*). In this case not water but phosphoric acid is split off:



It is possible that the formation of proteins from amino acids in living organisms does not occur directly with the elimination of water, but indirectly from some derivative of the amino acids which is still unknown.

[6] E. W. Spanagel and W. H. Carothers, *J. Am. Chem. Soc.*, **58**, 654 (1936). W. H. Carothers, *Chem. Revs.*, **8**, 353 (1931).

2. Cocondensation

Copolymers can be prepared by condensing mixtures of different components; bifunctional monomers yield chain polymers. By copolymerization the crystallization of the polymer can be reduced or suppressed, which is an advantage in glass-clear synthetics. Solubility and softening temperature can be varied, and this may be important in the preparation of artificial fibers or of materials for injection molding.

Cocondensation is applied particularly to thermosetting resins to obtain alcohol- or oil-soluble intermediate products which can be condensed by heating *in situ* to form insoluble protective coatings. Thus, butyl alcohol can be built into a urea formaldehyde condensate to give an intermediate product soluble in oil.

A combination of condensation and polymerization is also possible. Maleic acid can be condensed in the absence of a catalyst to form a soluble linear polyester with a dihydric alcohol. If a little vinyl chloride is introduced, bridges are established between the double bonds of the maleic ester residues, so that an insoluble net polymer is formed.[7]

3. Polyaddition

Alcohols form semiacetals with aldehydes and hydroxy ethers with epoxides such as ethylene oxide. These intermediates in turn add a second molecule of aldehyde or epoxide, respectively; this addition continues and leads to linear high polymers. The reaction proceeds like polycondensation as a step reaction; it is therefore suggested that this type of reaction be called polyaddition, and that olefinic polyaddition, which is governed by a radical chain mechanism, be termed polymerization. Polymerization of cyclic imines and addition of diisocyanates to glycols are further examples of polyaddition.

II. POLYMERIC ETHERS

—R—O—R—O—R—

1. Polyoxymethylene

The structure of this compound, formerly known as paraformaldehyde, was cleared up by Staudinger's[8] observation that, on decomposition in the

[7] J. B. Rust, *Ind. Eng. Chem.*, **32**, 64 (1940).

[8] H. Staudinger and M. Lüthi, *Helv. Chim. Acta*, **8**, 41, 65, 67 (1925). H. Staudinger and collaborators, *Ann.*, **474**, 145 (1929).

presence of acetic anhydride, a mixture of polyoxymethylene diacetates was formed in which from 2 to 30 formaldehyde groups were united to form a single molecule:

$$CH_3CO \cdot O \cdot CH_2 \cdot O \ldots . C \cdot CH_2 \cdot O \cdot COCH_3$$

By fractional distillation, the compounds of lowest molecular weight (containing up to 20 formaldehyde groups) could be isolated and, from their properties, shown to be homogeneous substances. The possibility of determining the molecular weight by chemical methods, that is, by estimation of the acetyl content, was particularly important. Comparison of the compounds with one another showed that the vapor pressure and the solubility diminish with increasing molecular weight. In addition to the polyoxymethylene diacetates which could be isolated, insoluble substances were formed, the low acetyl content of which indicated the presence of longer chains containing at least 30 formaldehyde groups. These apparently crystalline substances are certainly not homogeneous. The value obtained for the molecular weight is, therefore, only a mean value.

Staudinger and his collaborators were also able to explain the differences between various polyoxymethylenes described in the literature. Not only the average length of the primary valence chains, but also the nature of the end groups may vary. α-Polyoxymethylene is a hydrate, while β-polyoxymethylene, obtained by the action of concentrated sulfuric acid on formaldehyde, is presumably a sulfuric acid ester of polyoxymethylene; its special reactivity is certainly due to the sulfuric acid content. γ-Polyoxymethylene, which is formed in the presence of methyl alcohol, is a polyoxymethylene dimethylether; it is chemically very inert. In contrast to other polyoxymethylenes, it does not dissolve in solutions of sodium sulfite.

All polyoxymethylenes are crystalline and yield well-defined powder diagrams. Some of them have been obtained in the form of fibers and give fiber diagrams. The evaluation of these has been undertaken by Hengstenberg,[9] who interprets the lattice of paraformaldehyde as a primary valence chain lattice with 18 CH_2O groups in the unit cell. His results have been questioned by Sauter[10] who reaches other conclusions.

From the results of work on polyoxymethylenes, Staudinger has drawn a number of general conclusions, some of which have proved to be wrong, others to be correct. He concluded, and rightly so, that natural high polymers, such as cellulose, are mixtures of homologous polymers, just as is paraformaldehyde. On the other hand, he elaborated the theory that the physical properties of such polymers are determined by the length of

[9] J. Hengstenberg, *Ann. Physik*, **84**, 245 (1927).
[10] E. Sauter, *Z. physik. Chem.*, **B21**, 161, 186 (1933).

the chain, while the end groups decide the chemical properties. This conception is entirely erroneous; both chemical and physical properties result rather from the interaction of all the groups.

Mention may be made here of thiometaformaldehyde (Wohl; Baumann and Fromm[11]) to which, in view of its properties, a chain structure similar to that of paraformaldehyde must be ascribed, and the recently discovered polyacetaldehyde.[11a] The latter is, however, not yet described with sufficient accuracy.

2. Polymeric Glycol Formals

$$O—CH_2—O—(CH_2)_n—O—CH_2—O—(CH_2)_n—$$

As Hill and Carothers[12] showed, higher glycols (from tetramethylene glycol onward) can be converted into high polymeric polyacetals by heating with dibutyl formal at 150°C., during which reaction butyl alcohol is liberated and distilled off. The α-polyacetals have a mean molecular weight of 2200 and on heating in the molecular distillation apparatus at 230–260°C. are converted in part to high polymers of molecular weight 10,000–20,000, which can be spun to silklike threads of considerable tensile strength, and in part to cyclic monomers or dimers which are liquid and can be distilled off. On heating, the latter can be in part reconverted to polymers. The x-ray diagram of the fibers does reveal an orientation of the molecules. Polyformals have been prepared from 1,4-butanediol and 1,10-decanediol.

3. Polyethylene Oxide

$$HO—CH_2—CH_2—O—CH_2—CH_2—O—CH_2—CH_2—$$

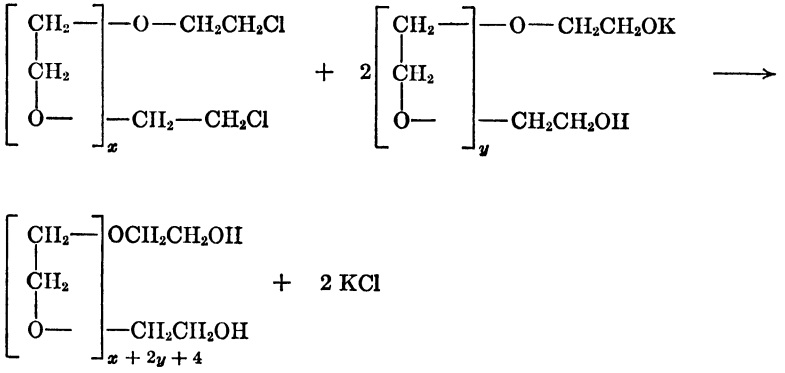
Following Lourenço,[13] who prepared and determined the formula of the first homologous polymeric series, that of the polyethylene glycols, Wurtz,[14] Roithner,[15] and Staudinger[16] have all worked on the polymerization of ethylene oxide. Staudinger and his collaborators prepared polymers of

[11] A. Wohl, *Ber.*, **19**, 2344 (1886). E. Baumann and E. Fromm, *ibid.*, **24**, 1457 (1891).
[11a] M. Letvet, *Compt. rend.*, **224**, 50 (1947).
[12] J. W. Hill and W. H. Carothers, *J. Am. Chem. Soc.*, **57**, 925 (1935).
[13] A. V. Lourenço, *Ann. chim. phys.*, **67**, 275 (1863).
[14] A. Wurtz, *Ann. chim. phys.*, **69**, 330, 369 (1863).
[15] E. Roithner, *Monatsh.*, **15**, 679 (1894).
[16] H. Staudinger and O. Schweitzer, *Ber.*, **62**, 2395 (1929). H. Staudinger and H. Lohmann, *Ann.*, **505**, 41 (1933).

various degrees of polymerization. The lower homologues are viscous liquids, the higher are crystalline.

The process of polymerization differs fundamentally from that of vinyl derivatives—as is clear, for example, from the fact that light and peroxides have no effect on the reaction. According to Hibbert and Perry,[17] the first step is the formation of glycol or alkali glycolate on the addition of water or alkali. This then adds on ethylene oxide to form in succession the diglycol, the triglycol, and so on.

Polyethylene oxides of moderate molecular weight and known chain length have been synthesized by Hibbert[18] according to the following scheme:



In this way polymers of grades 6, 18, 42, 90, and 186 have been prepared. From measurements of the viscosity in carbon tetrachloride it was found that the ratio of the intrinsic viscosity to the molecular weight is not a constant but that the following empiric equation holds:

$$[\eta] = K_m M + \beta$$

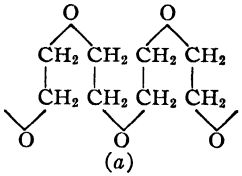
where K_m and β are constants.

Polyethylene oxides are water-soluble; the lower members are also soluble in ether. The solubilities in various media correspond approximately with those of polyvinyl alcohol. Admirable photomicrographs of fibrous

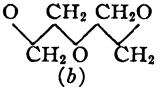
[17] H. Hibbert and S. Z. Perry, *Can. J. Research*, **8**, 102 (1933).

[18] R. Fordyce, E. L. Lovell, and H. Hibbert, *J. Am. Chem. Soc.*, **61**, 1905 (1939). R. Fordyce and H. Hibbert, *ibid.*, **61**, 1910, 1912 (1939). E. L. Lovell and H. Hibbert, *ibid.*, **62**, 2140 (1940).

polyethylene oxide have been published by H. and M. Staudinger.[19] From the fiber diagram of a high polymeric ethylene oxide, Sauter[20] determined the identity period along the fiber axis: $b = 19.5$ Å.; the other data which he gives ($a = 9.5$ Å.; $c = 12.0$ Å.; $\beta = 101°$; 36 C_2H_4O residues in the unit cell) require confirmation by photographs made with preparations of higher orientation before they can be considered to be correct. Sauter assumes that nine CH_2—CH_2—O groups of a chain occur within the period 19.5 Å., and that the unit cell is traversed by four chains. Accordingly, the chains are not stretched but are folded (meandrine) according to scheme *a*:



(*a*)



(*b*)

Sauter's experimental results, however, are equally in agreement with the assumption that six chains are present and that there are six CH_2—CH_2—O groups of each chain in the unit cell, so that $19.5 \div 6 = 3.3$ Å. represents the length of each residue. This assumption is in good agreement with existing data for valence angles and interatomic distances, and leads to the chain formula (*b*) which, by analogy with other compounds, appears more probable. It will be possible to decide between the two formulas only after detailed investigation.

Polyethylene oxide, under the trade name Postonal Bayer, is recommended for the preparation of suppositories.

Propylene oxide and other homologues of ethylene oxide also polymerize.

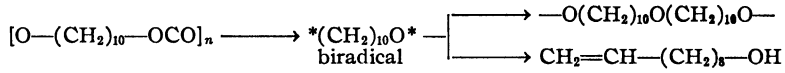
4. Polytetramethylene Oxide and Polydecamethylene Oxide

Polytetramethylene oxide is formed, according to German Patent 741,478, from tetrahydrofuran by catalytic conversion using $SbCl_5$.

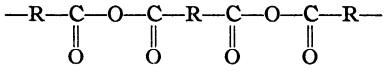
Polydecamethylene oxide was obtained as a waxlike mass (F.P. 58–60° C.) of molecular weight *ca.* 1200 by heating polydecamethylene carbonate *in vacuo*. 1-Decen-9-ol is also formed. It is to be supposed that carbonic acid is split off from the polyester, and that the free decamethylene oxide radical reacts in two ways:

[19] H. Staudinger and M. Staudinger, *Z. physik. Chem.*, **B37**, 403 (1937).

[20] H. Staudinger, *Die hochmolekularen organischen Verbindungen*, Springer, Berlin, 1932. E. Sauter, *Z. physik. Chem.*, **B21**, 161, 186 (1933).



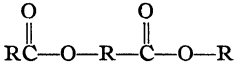
III. POLYMERIC ACID ANHYDRIDES



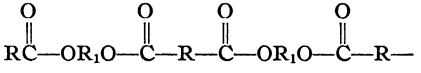
Dibasic acids, from adipic acid ($C_6H_{10}O_4$) upward, may be converted into polymers by heating with acetic anhydride or acetyl chloride; these have been studied by Hill and Carothers.[21] On being heated strongly, the polymeric anhydrides are mostly depolymerized to the monomeric cyclic anhydrides. Polymers from unbranched acids are solid and crystalline, which is scarcely surprising in view of their regular structure. The lower polymers (α polymers) are converted on melting into higher polymers (ω polymers); at the same time, the breakdown mentioned above occurs to some extent. High polymeric sebacic anhydride $(C_{10}H_{16}O_3)_n$ softens at 130°C. and may be drawn out into threads which are highly reflecting and solid. The anhydrides are gradually decomposed by moisture.

IV. POLYMERIC ESTERS

1. Linear Esters



(R = oxy-acid residue)
(*a*)



(R = dicarboxylic-acid residue)
(R_1 = dialcohol residue)
(*b*)

Chain polymeric esters may be obtained by dehydration of oxy acids (*a*), and by the action of dibasic acids on dibasic alcohols (*b*). The polymeric products of lactic acid have been known for a long time. Lactide is a solid material obtained by heating lactic acid to 200°C. (Gay-Lussac,[22] Krafft and Dyes[23]). It is sparingly soluble in alcohol and ether. Glycolic acid also yields a polymer. These polymers have adhesive properties similar to those of the resins obtained from phthalic acid and glycerol (alkyd resins). They will be discussed later.

Of the higher hydroxy acids, ricinoleic acid:

$$CH_3(CH_2)_5CH(OH)CH_2CH{:}CH(CH_2)_7COOH$$

[21] J. W. Hill and W. H. Carothers, *J. Am. Chem. Soc.*, **54**, 1569, 1579 (1932).
[22] J. Gay-Lussac and J. Pelouze, *Ann.*, **7**, 40 (1833).
[23] F. Krafft and W. A. Dyes, *Ber.*, **28**, 2589 (1895).

according to Meyer,[24] is converted at room temperature into a mixture of polyesters of high molecular weight, from which the original acid is obtained on saponification. Carothers and coworkers[25] converted ω-hydroxydodecanic acid and ω-hydroxypentadecanoic acid into polymers of molecular weight 3000–5000 which they then transformed into superpolymers of molecular weight > 10,000 in the molecular distillation apparatus. The substances obtained could be spun into sparkling fibers of high tensile strength.

The second method (*b*) offers more possibilities of variation. The solubility of the products obtained may be greatly influenced by the choice of acids or of alcohols: the presence of many CH_2 groups favors swelling in organic solvents. Medium and higher polymers of succinic acid and glycol have been described; the medium polymers of molecular weight *ca.* 3000 crystallize out from solution in a manner similar to that observed in the case of degraded acetyl celluloses and methyl celluloses. The appearance of such microscopically visible crystals is, therefore, no indication of chemical homogeneity or low molecular weight; it is rather to be assumed that the molecules, although of different length, are so similar in structure that they can associate to form crystal-like aggregates, just as happens in the higher paraffins.

Carothers and Hill[26] obtained from hexadecamethylene dicarbonic acid and trimethylenediol, first a brittle, waxlike polymer of mean molecular weight 3000, and from this a superpolymer (M.W. > 12,000) which could be drawn out into threads and spun from a solution in chloroform. If spun under considerable tension the strength and flexibility of the fibers is increased. The lower polymer melts at 75°; the higher polymer becomes transparent and rubberlike at the same temperature.

Synthetic esters have been subjected to x-ray analysis by Fuller and Erickson.[27] They obtained fiber diagrams from a number of esters; their results are shown in Table I. Column 4 shows the calculated lengths of the individual molecules, if these are supposed to have a zigzag structure like that of the paraffins.

It is evident that esters with an odd number of carbon atoms in the structural unit have a unit cell containing two molecules in the direction of the fiber axis; the others have a unit cell containing a single molecule. Certain

[24] H. H. Meyer, *Arch. Pharm.*, **235**, 186 (1897).

[25] W. H. Carothers and G. L. Dorough, *J. Am. Chem. Soc.*, **52**, 711 (1930).

[26] W. H. Carothers and J. W. Hill, *J. Am. Chem. Soc.*, **54**, 1559, 1579 (1932); reprinted in this Series, Vol. I, pp. 156, 179.

[27] C. S. Fuller and C. L. Erickson, *J. Am. Chem. Soc.*, **59**, 344 (1937).

TABLE I

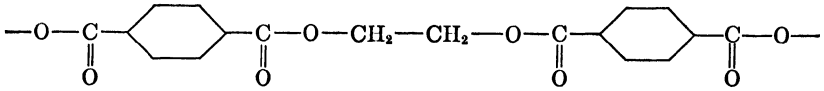
X-Ray Data on Synthetic Esters[27]

Polyester	Structural unit	Observed fiber period, Å.	Calculated for units, Å.	Difference, Å.
Polyethylene succinate.....	$[CO\cdot(CH_2)_2CO\cdot O\cdot(CH_2)_2O]$	8.3	9.70	1.4
Polyethylene adipate.....	$[CO\cdot(CH_2)_4CO\cdot O\cdot(CH_2)_2O]$	11.7	12.2	0.5
Polyethylene azelate.....	$[CO\cdot(CH_2)_7CO\cdot O\cdot(CH_2)_2O]$	31.2	16.0	0.4
Polyethylene sebacate.....	$[CO\cdot(CH_2)_8CO\cdot O\cdot(CH_2)_2O]$	16.7	17.2	0.5
Trimethylene sebacate.....	$[CO\cdot(CH_2)_8CO\cdot O\cdot(CH_2)_3O]$	33.5	18.5	1.7
Diethylene oxide sebacate.....	$[CO\cdot(CH_2)_8CO\cdot O(CH_2)_2O(CH_2)_2O]$	38.0	20.8	1.8

esters appear to be somewhat shortened, for example, polyethylene succinate, trimethylene sebacate, and diethylene oxide sebacate. They do not appear to have the stretched, zigzag configuration. Fuller[28] has made x-ray analyses of mixed esters and mixtures of esters. Some mixed esters show rubberlike elasticity.

According to Staudinger and Nuss,[29] high polymeric esters of hexanediol and sebacinic acid must be branched, because their intrinsic viscosity is not proportional to their molecular weight. Staudinger's conclusion must be rejected since most chain polymers behave in the same manner.

Fibers of polyethylene terephthalate, prepared by esterifying terephthalic acid and glycol, have valuable commercial properties.



The polymers can be drawn into threads which are remarkably water-resistant. They are used (like nylon, which will be discussed later) in the manufacture of bristles and artificial textiles, and have received the trade name of Terylene from the firms responsible for their manufacture—Calico Printers' Association and Imperial Chemical Industries, Ltd. The fibers cannot be dyed. According to Astbury and Brown[30] the fibers give a good

[28] C. S. Fuller, *Ind. Eng. Chem.*, **30**, 472 (1938).

[29] H. Staudinger and M. Nuss, *J. prakt. Chem.*, **157**, 283 (1941).

[30] W. T. Astbury and C. J. Brown *Nature*, **158**, 871 (1946).

fiber diagram revealing the presence of a triclinic unit cell with $a = 5.5$ Å., b (fiber axis) $= 10.9$ Å., $c = 4.1$ Å.; $\alpha = 107°$, $\beta = 92°$, and $\gamma = 112°$. The chains lie flat in the (101) plane.

Liquid or soluble polyesters of maleic acid may be polymerized at ordinary temperature and pressure into cross-linked insoluble and heat-resistant materials by addition of suitable peroxydic initiators. These resins are known as low pressure or contact resins.

The kinetics of ester polymerization have been studied by Flory.[31] According to this work, the reaction is of the third order: the elimination of a molecule of water and the formation of an ester linkage require the interaction of two carboxyl groups and an alcoholic hydroxyl group. It appears that one carboxyl group acts as a catalyst.

Natural shellac is also to be regarded as a polymeric ester which yields several dibasic, hydroxyl-containing acids[32] on hydrolysis. Since it is not a *high* polymeric product in the strict sense, however, we shall merely refer to its existence.

2. Artificial Resins and Compositions from Glycerol and Polybasic Acids (Alkyd Resins)

From tri- and polybasic alcohols and dibasic acids, net polymers may be obtained; of these, the polyesters of glycerol with various acids (in particular with phthalic acid, which is easy to prepare on a commercial scale) have been studied intensively from the point of view of their practical application, and are manufactured under various names; alkyd resins, glyptals, etc. If glycerol is heated with phthalic anhydride or maleic anhydride, the mass, which is at first fluid, becomes more viscous and finally solid; at the same time, the solubility is reduced. The reaction is simply a progressive esterification in which, ultimately, nets are formed, as glycerol is trifunctional and so cross links easily. When warm, the products have very striking adhesive properties, particularly toward metals. Products which are still fusible may be hardened subsequently and are then markedly heat-resistant; in the presence of water, however, they are gradually saponified. This sensitivity to water, which is also manifested in damp air, has considerably reduced the practical application of these cheap products, otherwise of value owing to their resistance to high temperature and to oils.

On the other hand, the mixed esters of glycerol, phthalic acid or maleic

[31] P. J. Flory, *J. Am. Chem. Soc.*, **59**, 466 (1937).

[32] For a discussion of shellac see C. E. Barnes, *Ind. Eng. Chem.*, **30**, 449 (1938). B. B. Schaeffer, H. Weinberger, and W. H. Gardner, *ibid.*, **30**, 333, 451, 454 (1938).

acid, and varying quantities of fatty acids, in particular linoleic acid, have acquired considerable importance. By the addition of fatty acids, the properties of the polymers may be influenced, particularly if drying fatty acids are built into the molecule. If esterification is continued until products of a moderate degree of polymerization are formed, substances are obtained which are still soluble but which, exposed to the air, become insoluble, as does linseed oil ("drying"). The drying components are responsible for the linking of the molecules to form a coherent cross-linked film. Varnishes consisting of solutions of these products occupy a position intermediate between varnishes with a basis of linseed oil and those prepared from natural resins and solvents. They offer advantages over both types, since the quality of the products may be adapted to suit every requirement by varying the choice and proportion of components and the duration of polymerization.

3. High Polymers from Fatty Oils

If several unsaturated fatty acids occur in a triglyceride, bridges to neighboring molecules may be formed at the double bonds by suitable reactions. When three points of linkage are present in the molecules, three-dimensional net polymers are formed as insoluble coherent masses. Such reactions have been known for a long time. They occur in the drying of linseed oil (in which process, under the influence of oxygen, insoluble linoxyn is formed) and also in the preparation of factice.

It is well known that drying oils through which air is bubbled become more viscous and absorb oxygen. The rise in viscosity indicates an increase in molecular weight. After prolonged exposure to the air, linseed oil becomes insoluble. If, as happens with oil paint, it is spread out in thin layers, an insoluble, durable film is formed. By the oxidation of linseed oil in the presence of sawdust and other fillers, linoleum is manufactured. The reaction is accelerated by traces of metallic oxide and by light, in particular by ultraviolet light. Advantage is taken of this in the manufacture of patent leather; leather painted with linseed oil is exposed to sunlight or to ultraviolet radiation.

The reactants are highly unsaturated fatty acids or their esters, such as linolic acid and linolenic acid of linseed oil:

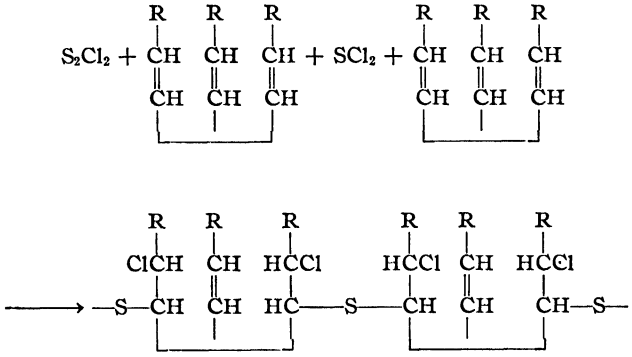
Linolic acid: $CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$

Linolenic acid: $CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$

and the α- and β-eleostearic acids of Chinese tung oil. When linseed oil is heated migration of the double bonds occurs so that a conjugated system

is formed, as revealed by the ultraviolet spectrum. As a result of this transposition the oil becomes more reactive. Presumably two phenomena are superimposed during drying: (*1*) cross linkage, which results from the formation of oxygen bridges, each double bond first forming a peroxide which then reacts with a neighboring molecule to form a bridge; and (*2*) polymerization which is catalyzed by light, and which also takes place at the double bonds, as in the normal polymerization of styrene, butadiene, or other substituted olefins. Similar reactions are known in the case of rubber; it is "vulcanized" by the action of ultraviolet light and may be rendered insoluble by the action of oxygen.

The term factice (often called rubber substitute) is used for elastic materials which may be mixed with rubber in the manufacture of rubber goods, partly to lower the price of the product, partly in order to confer on it particular properties. Erasers, for example, are always prepared with the addition of factice. White factice is obtained by the action of S_2Cl_2 on oils, chiefly colza or castor oil. It forms a solid, pulverizable, insoluble mass. The reaction is analogous to cold vulcanization and may be represented in the following way:



Brown factice is obtained from dried or half-dried oils by the action of 15–20% of sulfur at 140–160°C. The reaction is comparable to the heat-vulcanization of rubber. Since in the commercial preparation of factice it is customary to subject the oils to a blast of air or water vapor before treatment with sulfur, it is probable that oxidation and bridge building by oxygen, as well as saponification, reconstitution, and perhaps breakdown by oxidation or cracking also play a part. It is therefore difficult to describe the course of the reaction with accuracy.

4. Basic Polyesters

Esters of Phthalic Acid and Triethanolamine

By the esterification of triethanolamine with phthalic acid a high polymeric substance with basic properties is obtained (Meyer and Sievers[33]). On the addition of methyl iodide, a high polymeric quaternary ammonium iodide is formed.

5. Polyphosphoric Acid Esters

(*a*) *Synthetic Esters*

It may be assumed that tribasic phosphoric acid, which forms fairly easily saponifiable esters with monohydric alcohols but relatively stable esters with phenols, is able to react with polyhydric alcohols to form three-dimensional net polymers. The data available about such compounds are very scarce.

On heating glycerol and phosphoric acid in equimolecular proportions in a high vacuum, a solid spongy mass with the composition $(C_3H_5O_3PO)_n$ is formed with the elimination of water (Carré[34]); this is certainly a highly cross-linked high polymer. The high polymeric phosphates, in particular those of the polyhydroxyphenols, are worthy of greater attention than that which they have received up to the present.

(*b*) *Nucleic Acids*[35]

The name nucleic acids denotes a group of high polymeric substances which occur, either free or, in combination with protein, as nucleoproteids in many organisms, and are of great interest as constituents of the cell nucleus and, in particular, of the chromosomes. The best known nucleic acids are thymonucleic acid, obtained from the thymus, and yeast nucleic acid. Nucleic acids have also been isolated from cancer tissues, from chick embryos, from nitrogen-fixing bacteria (*Azotobacter*), from wheat germ, and

[33] K. H. Meyer and J. F. Sievers, *Helv. Chim. Acta*, **19**, 665 (1936).

[34] P. Carré, *Ann. Chim.*, **5**, 345 (1905); *Compt. rend.*, **137**, 1070 (1903); *ibid.*, **138**, 47 (1904). A Contardi, *Gazz. chim. ital.*, **42, II**, 270 (1912).

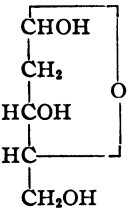
[35] General accounts: S. J. Thannhauser, *Lehrbuch des Stoffwechsels und der Stoffwechselkrankheiten*, Springer, Berlin, 1929; P. A. Levene and L. W. Bass, *Nucleic Acids*, Reinhold, New York, 1931; P. A. Levene and L. W. Bass, "Nucleic Acids, etc.," in Abderhalden, *Biochem. Handlexikon*, Vol. 14 (Ergänzungsband 7), Urban und Schwarzenberg, Berlin, 1933. F. Schlenk, in F. F. Nord, ed., *Advances in Enzymology*, Vol. IX, Interscience, New York, 1949, page 455.

from tubercle bacilli (tuberculinic acid). Diphtheria toxin contains a nucleic acid as does also the virus of tobacco mosaic disease.

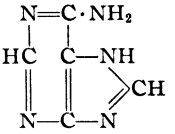
Nucleic acids may be hydrolyzed and yield phosphoric acid, purine or pyrimidine bases, and sugars—pentoses, hexoses, or desoxy sugars. The structural plan is the same in all nucleic acids, but the nature of the individual carbohydrates and bases varies. Nothing is known with certainty about the character of the linkage between nucleic acid and protein in nucleoproteins. This may involve the formation of a salt by the acid and the protein (which is pronouncedly basic in character). On the other hand, we have a fairly clear idea of the linkage of the structural units in nucleic acid itself as a result of the work of Hammarsten,[36] Levene and Jacobs,[37] Feulgen,[38] Thannhauser,[35] Fischer,[39] and others. In the preparation of nucleic acids, the protein of the organ in question is generally eliminated by peptic digestion, and the nucleic acid, which is soluble in alkali and precipitated by acids, is thrown down as a precipitate. A method of preparation for thymonucleic acid is given by Hammarsten.[36]

Nucleic acids are colloids with the properties of high polymeric polybasic acids. Insight into the linkage of the residues may be obtained by progressive hydrolysis. By hydrolysis with pancreatic enzymes (ribonuclease, desoxyribonuclease), the mononucleotide, consisting of one molecule of each of the products of hydrolysis, is obtained. The mononucleotide may be hydrolyzed by dilute hydrochloric acid into purine or pyrimidine and a compound of sugar and phosphoric acid, or, by the nucleotidase secreted by the intestinal epithelium, into phosphoric acid and a compound of purine or pyrimidine and sugar known as nucleoside. In the nucleotide, therefore, purine and phosphoric acid must be attached to the sugar, which forms the link between the other two compounds; the nucleotide residues themselves are united by the phosphoric acid molecules, which link the nucleotides to the sugar residues by ester linkages. The phosphoric acid is united with the hydroxyl groups of the carbohydrate, while the base is attached as a *N*-glucoside to the aldehydic carbon atom. As an example, thymonucleic acid will be considered in rather more detail. The sugar is 2-desoxyribose, and adenine, cytosine, thymine, and guanine have been isolated as bases:

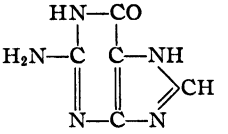
[36] E. Hammarsten, *Biochem. Z.*, **144**, 383 (1924).
[37] P. A. Levene and W. A. Jacobs, *Ber.*, **44**, 746 (1911). P. A. Levene, see footnote 35.
[38] R. Feulgen, *Chemie und Physiologie der Nucleinstoffe*. Bornträger, Berlin, 1923.
[39] F. G. Fischer, *Naturwissenschaften*, **30**, 377 (1942).



Desoxyribose as furanose

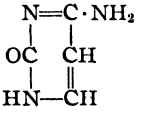


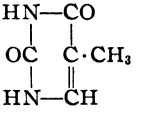
Adenine



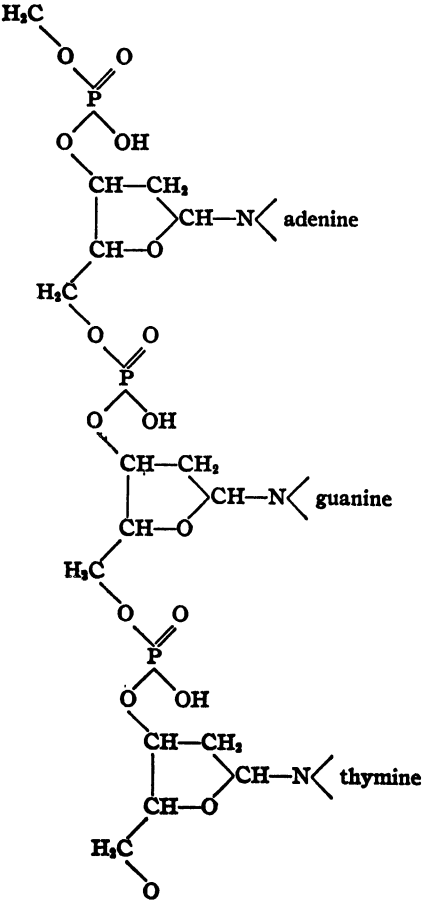
Guanine

Cytosine





Thymine



In yeast nucleic acid, on the other hand, adenine, cytosine, uracil (2,4-dioxypyrimidine), and guanine have been identified. The sugar is D-ribose, but small quantities of desoxyribose are also found.[39a] Both types of acids may be distinguished by the reaction with fuchsin sulfurous acid (Feulgen reaction), which gives a red color only with desoxyribonucleic acid.

Bredereck's finding, that nucleic acids are broken down to tetranucleotides by chemical hydrolysis, was not confirmed by Fischer, who obtained, both on acid and alkaline hydrolysis, a mixture of oligonucleotides of different molecular weights. This is in agreement with what one would expect from the chain structure of the molecule.

Important work by Signer, Caspersson, and Hammarsten,[40] in which the birefringence of flow was measured and compared with the viscosity, gave some indication of the shape and molecular weight of the particles in solution. The authors conclude that the molecules of the sodium salt of thymonucleic acid are in the form of thin rodlets, the length of which is about 300 times their diameter. The molecular weight of the preparation which they examined lay between 500,000 and 1,000,000. This value is in agreement with experiments by Caspersson[41] on the filtration of this substance. Preliminary and unpublished measurements by Svedberg also indicate a very high molecular weight. The molecules show very strong uniaxial negative birefringence; their greatest polarizability is therefore in the direction at right angles to the longitudinal axis, with the ring systems of adenine, thymine, etc., in the plane of the rings, at right angles to the chain axis.

The arrangement of nucleic acids in the chromosomes has been studied by Caspersson,[42] using the ultraviolet microscope. This work was greatly facilitated by Hammarsten's[43] observation that nucleic acid is hardened and converted *in situ* into insoluble salts by treatment with lanthanum salts, so that it can be freed from proteins by enzyme action. Nucleic acids display a characteristic adsorption in the ultraviolet at 261 mμ (Dhéré[44]).

Nucleic acids form insoluble compounds with basic dyes; they are responsible for the marked basophilic character of the cell nucleus, which is evident from their staining with methylene blue and other basic dyes.

An important biological property of nucleic acids is the possibility of their causing mutation of certain bacterial types. Several authors have an-

[39a] E. Chargaff and S. Zamenhof, *J. Biol. Chem.*, **173**, 327 (1948).
[40] R. Signer, T. Caspersson, and E. Hammarsten, *Nature*, **141**, 122 (1938).
[41] T. Caspersson, *Biochem. Z.*, **270**, 161 (1934).
[42] T. Caspersson, *Skand. Arch. Physiol.*, **73**, Suppl. 8 (1936).
[43] T. Caspersson and E. Hammarsten, *Trans. Faraday Soc.*, **31**, 367 (1935).
[44] C. Dhéré, *Compt. rend. soc. biol.*, **58**, 34 (1906); *ibid.*, **101**, 1124 (1929).

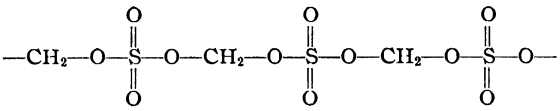
nounced the preparation of desoxypentose nucleic acid fractions capable of inducing these transformations.[44a]

6. Polymeric Borates, Silicates, and Sulfates

High polymeric substances are formed from glycerol and boric anhydride (vitreous masses, according to Schiff and Bechi[45]) and from glycerol and orthosilicic esters (amorphous, infusible masses, according to Knorr and Weyland[46]). Various soluble resins from silicic acid and glycerin derivatives with at least one free OH group have been described by du Pont.[47]

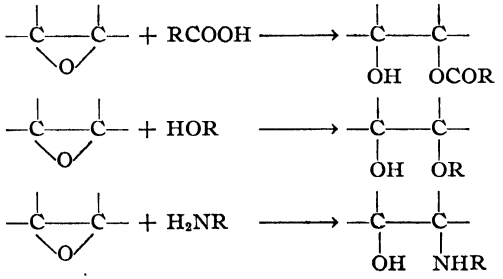
It is generally known that boric acid readily forms complexes with polyhydroxylic compounds. Its ability to convert polyvinyl acetates and cellulose esters which retain some free hydroxyl groups into insoluble and infusible masses probably depends on this property (cross linking with the formation of a complex). Water breaks up the complex.

In conclusion, mention may be made of the methylene sulfate obtained by Délépine[48] on treating paraformaldehyde with fuming sulfuric acid containing 50% SO_3. It has the empirical composition $CH_2O_2SO_2$. It may be regarded without hesitation as a polymer, since it is insoluble in all media and decomposes on heating to 153°C. The formula is probably:



7. Epoxy Polymers

Substituted epoxides can add carboxylic acids, alcohols, and amines:



[44a] O. T. Avery, C. M. MacLeod, and M. McCarty, *J. Exptl. Med.*, **79**, 137 (1944); **83**, 97 (1946). A. Boivin, *Cold Spring Harbor Symposia Quant. Biol.*, **12**, 7 (1947); *Bull. soc. chim. biol.*, **29**, 136 (1947).

[45] H. Schiff and E. Bechi, *Z. Chem.*, **9**, 147 (1866).

[46] L. Knorr and H. Weyland, German Pat. 285,285 (1914).

[47] U. S. Pat. 2,048,799 (1931); 2,053,474 (1932).

[48] M. Délépine, *Compt. rend.*, **129**, 831 (1899).

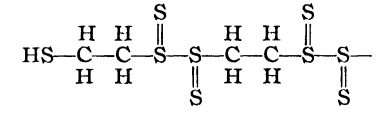
Compounds containing two epoxy groups are obtained by etherification of dihydroxy compounds or diphenols with epichlorhydrin; they form chain or space network polymers by polyaddition with polyvalent alcohols, acids, or amines.[48a] The products are used as lacquers and as adhesives for metals, glass, and porcelain. Trade name: Araldite Ciba.

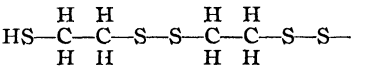
V. POLYMERIC SULFIDES, SULFONES, AND IMINES

1. Polymeric Sulfides and Polysulfides

In 1926 Baer[49] made the observation that high polymeric, rubberlike substances are formed during the reaction between ethylene dichloride (and other dihalides) and alkali sulfides. Working independently, Patrick and Mnookin[50] showed in 1927 that rubberlike masses are produced by the action of polysulfides on dihalides. These authors were the first to realize the importance of polysulfide sulfur in the reaction. Patrick has systematically studied this group of compounds and founded a new industry in the manufacture of Thiokol.[51]

The chemical composition of ethylene polysulfide is $(C_2H_4S_4)_n$ (Patrick).[52] In the presence of alkali two sulfur atoms per residue may be removed, leaving a solid colorless substance of composition $(C_2H_4S_2)_n$, softening at 130°C.—that is, of fairly high molecular weight. Patrick suggests the formulas:





On treating the latter substance with sulfur (passing between hot rollers), the original rubberlike material is obtained. For this reconversion, it is not necessary to reach the original sulfur content; half a sulfur atom per $(C_2H_4S_2)$ group is sufficient. It seems not impossible that the sulfur re-

[48a] French Pat. 881,981, 884,271; German Pat. 731,030 (I.G. Farbenindustrie); Swiss Pat. 211,116, 236,594 (de Trey A.G.); 251,647 (Ciba A.G.).

[49] J. Baer, French Pat. 640,967 (1927).

[50] J. C. Patrick and N. M. Mnookin, Brit. Pat. 302,270 (1928).

[51] J. W. Crosby, *India Rubber World*, **106**, 133 (1942).

[52] J. C. Patrick, *Trans. Faraday Soc.*, **32**, 347 (1936). S. M. Martin, Jr., and J. C. Patrick, *Ind. Eng. Chem.*, **28**, 1144 (1936).

moved by alkali acts as a plasticizer, being dissolved in the polymer. This sulfur is insoluble in carbon disulfide.

By the removal of two more sulfur atoms and the treatment of the nonrubberlike polymer with warm sodium sulfide, decomposition may be continued down to water-soluble ethylene dimercaptan. In alkaline solution in the presence of oxidizing agents, this substance is converted into the nonelastic polydisulfide from which, as we have seen, the rubberlike material may be obtained by treatment with sulfur.

The tetrasulfide and disulfide yield fiber diagrams in the stretched condition. Katz and Fuller[53] determined the identity period along the fiber axis in the tetrasulfide as 4.32 Å. The reflections present in the diagram can be indexed if the unit cell has the following dimensions:

$$a = 8.68 \text{ Å.} \qquad b = 4.32 \text{ Å.} \qquad c = 5.03 \text{ Å.} \qquad \beta = 87°$$

The unit cell thus contains one $C_2H_4S_4$ group. The spacing 4.3 Å. along the *b* axis indicates that the four sulfur atoms do not occur in a row, for the structure $CH_2CH_2S—S—S—S—$ would require a considerably greater spacing, while the chain:

$$—CH_2CH_2S\cdot\underset{\underset{S}{\|}}{S}—\underset{\underset{S}{\|}}{}$$

will fit exactly. According to Katz the identity period along the *b* axis in the disulfide is twice as great as that in the tetrasulfide.

By heating ethylene polysulfide with zinc oxide or copper oxide, an effect similar to heat vulcanization is produced. In this process increase in chain length may be assumed to take place by oxidation, as indicated in the following scheme:

$$—C—SH + HS—C— + O \longrightarrow H_2O + —C—S—S—C—$$

Cross linkage may also occur as a result of the formation of a complex between a metallic atom and the sulfur atoms of two chains.

The compound may also be treated with gas carbon, the action of which is similar here to that in the case of rubber.

Thiokol, which may be used like rubber for the manufacture of elastic, nonplastic objects, has considerable importance at the present as artificial rubber.[54] The vulcanizates are resistant to oils, gasoline, benzene, and to ozone; but their tensile strength and extensibility are less than those of soft rubber. The material is used for hoses, ignition leads, etc.

[53] Quoted from J. R. Katz, *Trans. Faraday Soc.*, **32**, 89 (1936). See also J. J. Trillat and R. Tertian, *Compt. rend.*, **219**, 395 (1944); *Rev. sci.*, **83**, 29 (1945).

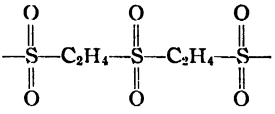
[54] See also G. Proske, *Angew. Chem.*, **52**, 344 (1939).

Products similar to Thiokol are produced by the action of sodium polysulfide on dichlorodiethyl ether and on (dichloroethyl) formaldehyde acetal.

$$—SS—CH_2—CH_2—O—CH_2—CH_2—SS—$$

$$—SS—CH_2—CH_2—O—CH_2—O—CH_2—CH_2—SS—$$

2. Polyethylene Sulfones



Many years ago Solonina[55] noted the formation of high molecular weight compounds by the action of sulfur dioxide on certain allyl ethers. Mathews and Elder[56] prepared resinous products by the addition of sulfur dioxide to simple olefins such as ethylene. The chain formula accepted at present originated with Seyer and King.[57] Later investigations were conducted by Marvel[58] and by Kharasch[59] who studied in particular the catalytic influence of peroxides, acids, etc., upon the reaction.

Nitrates soluble in the reaction mixture, such as silver and lithium nitrates, are particularly effective. Polyphenols inhibit the reaction. Light accelerates the process markedly, particularly (according to Snow and Frey[60]) light of a wave length between 3000 and 3800 Å. It is possible that the wave length 3100 Å., absorbed by sulfur dioxide is the effective band. According to the same authors, the speed of the photochemical reaction has a negative temperature coefficient; for example, illuminated isobutene-sulfur dioxide does not polymerize at room temperature, but does so at 4°C. It seems possible that an addition product which is only stable at low temperatures is an essential intermediate product.

High polymeric sulfones are thermoplastic. At temperatures only slightly above the softening temperature they begin to decompose into sulfur dioxide and olefin. No commercial use for them has so far been found.

Acetylenes display similar reactions: pentyne, hexyne, and a few others give polymers[61] with sulfur dioxide softening at 200–250°C.

[55] W. Solonina, *Chem. Zentr.*, 1899, I, 248.

[56] Mathews and Elder, Brit. Pat. 11,635 (1914).

[57] W. F. Seyer and E. G. King, *J. Am. Chem. Soc.*, 55, 3140 (1933).

[58] D. S. Frederick, H. D. Cogan, and C. S. Marvel, *J. Am. Chem. Soc.*, 56, 1815 (1934). C. S. Marvel and F. J. Glavis, *ibid.*, 60, 2622 (1938).

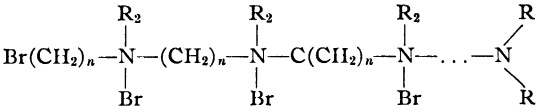
[59] M. S. Kharasch and E. Sternfeld, *J. Am. Chem. Soc.*, 62, 2559 (1940).

[60] R. D. Snow and F. E. Frey, *Ind. Eng. Chem.*, 30, 176 (1938).

[61] L. L. Ryden and C. S. Marvel, *J. Am. Chem. Soc.*, 58, 2047 (1936).

3. Polymeric Imines

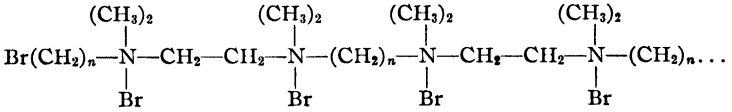
Marvel[62] and his collaborators obtained various products by auto condensing halogenalkyl dialkylamines heated *in vacuo*. If the alkyl chain consisted of less than five CH_2 groups the products were cyclic; if they consisted of more than five CH_2 groups, they were chain polymers:



These chain polymeric polyammonium salts form hygroscopic resinous or vitreous masses whose molecular weight can be estimated from the nonionic bromine attached to carbon, since each chain carries a terminal nonionically bound bromine atom. The molecular weight of the products is between 5000 and 28,000.

Chain polymers have also been formed from 1-bromo-3-dimethylaminopropane, while the corresponding diethyl and dipropyl compounds gave rise to cyclic products. *p*-Aminobenzyl chloride[63] also yields autocondensation polymers.

Chain polymeric ammonium salts are formed during the condensation of tertiary diamines with dihalogenides:



Kern and Brenneisen[64] obtained polymers from tetramethylethylenediamine and trimethylene dibromide, pentamethylene dibromide, and decamethylene dibromide. At higher temperatures they form insoluble, presumably branched polymers; below 100°C. resinous hygroscopic products of molecular weight 3000–10,000 are formed which can be pulled out into threads.

Ethylenimine polymerizes in the presence of small quantities of strong acids such as hydrobromic acid, toluenesulfonic acid, etc., to form solid, tough, hygroscopic products (Ladenburg and Abel,[65] Kern and Brennei-

[62] M. R. Lehman, C. D. Thompson, and C. S. Marvel, *J. Am. Chem. Soc.*, **55**, 1977 (1933).

[63] E. Lellmann and C. Stickel, *Ber.*, **19**, 1604 (1886). S. Gabriel and T. Posner, *ibid.*, **27**, 3509 (1894).

[64] W. Kern and E. Brenneisen, *J. prakt. Chem.*, **159**, 193 (1941).

[65] A. Ladenburg and J. Abel, *Ber.*, **21**, 758 (1888).

sen[64]). The products obtained by Kern have a molecular weight of 1000–2000. The nitrogen was in part tertiary nitrogen. It is reasonable to suppose therefore that branching has occurred by the addition of ethylenimine to the secondary nitrogen:

$$\begin{array}{l} —NHCH_2CH_2NHCH_2CH_2— \longrightarrow —CHCH_2CH_2NCH_2CH_2NH— \\ \quad +(CH_2)_2NH \qquad\qquad\qquad\qquad\qquad\quad | \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad CH_2CH_2NH_2 \end{array}$$

Undoubtedly many other representatives of this class could be prepared.

VI. POLYMERIC AMIDES

1. Products of Polymerization of Amino Acids

(*a*) *Polyglycylglycine and Derivatives*

Since these compounds are closely related to the proteins, they will be discussed in Chapter G, section III, 1.

(*b*) *Polymeric Amides from ϵ-Aminocaproic Acid*

By heating ϵ-aminocaproic acid, $NH_2(CH_2)_5COOH$, Gabriel and Maass,[66] as well as von Braun,[67] obtained (in addition to the cyclic lactam—a seven-membered ring) linear polymers:

$$—NH—(CH_2)_5—CO—NH—(CH_2)_5—CO—$$

forming solid, tough substances, insoluble in most media. Carothers and Hill included these compounds in their systematic studies of polymerization and prepared superpolymers of this structure; by heating ϵ-aminocaproic acid with hexamethylenedicarboxylic acid and trimethylene glycol, mixed esters were obtained, which were tough and pliable, and could be drawn out to form films and filaments.

According to Schlack,[68] superpolyamides of ϵ-aminocaproic acid can also be prepared by prolonged heating of the cyclic lactam; a continuous process for the commercial preparation of polyamides has been based on this observation. Superpolymers of ϵ-aminocaproic acid are of considerable commercial importance today both as synthetics and as high-grade artificial fibers. The industrial uses of superpolyamides will be discussed later.

A polymer of ω-aminoheptylic acid of melting point 215°C. was described by Manasse[69] as long ago as 1902.

[66] S. Gabriel and T. A. Maass, *Ber.*, **32**, 1266 (1899).
[67] J. von Braun, *Ber.*, **40**, 1834 (1907).
[68] P. Schlack, French Pat. 868,127 (I. G. Farbenindustrie).
[69] A. Manasse, *Ber.*, **35**, 1367 (1902).

2. Other Polyamides

(a) *Polymeric Amides from Dicarboxylic Acids and Diamines*

$CH_2NHCOCH_2...CH_2CONHCH_2...CH_2NHCOCH_2...$

Among the numerous condensation reactions discovered by Carothers[70] in the study of polymerization processes, the following has found practical application on a large scale and has opened up a new field of industrial chemistry, that of the completely synthetic textile fiber. Unbranched, saturated, aliphatic dicarboxylic acids containing at least 5 carbon atoms, condense on heating with aliphatic amines containing at least 4 CH_2 groups, to form high polymers (superpolyamides of molecular weight > 10,000). Carothers has described, for example, polymers of tetra-, penta-, hexa-, octa-, deca-, and dodecamethylenediamine, on the one hand, and azelaic acid, sebacic acid, adipic acid (tetramethylenedicarboxylic acid), and *p*-phenylenediacetic acid, on the other.

The superpolyamides are solid, stiff substances giving a not too viscous melt which can be drawn out into threads at temperatures above 250°C. Threads and fibers of the polyamide from adipic acid and hexamethylenediamine were first used commercially as bristles for toothbrushes; their use as textile fibers is of far greater importance today. They are also used as thermoplastic synthetics and, in view of their great commercial importance, we shall discuss the commercial applications of polyamides and related substances in some detail.

Po yhexamethyleneadipamide ($[\eta]$ in cresol at 25°C. = 0.974) and polyhexamethylenesebacamide ($[\eta]$ = 1.021) have been subjected to x-ray analysis by Fuller[71] and Brill.[72] The proportion of crystalline material present and the perfection of the lattice depend on the rate of cooling of the melt (melting point 280 or 260°C., respectively). The modulus of elasticity is also raised by slow cooling, since this increases with the proportion of crystalline material present. The stiffness of polyamide fibers is considerably improved by cold drawing of fibers spun from the melt.

It is clear from the x-ray diagram that the crystalline fraction is increased by this means: detailed analysis shows that neighboring chains are held together by hydrogen bonds between the CO and NH groups so that laminae are formed (see Fig. 2). The strength of the hydrogen bonds is responsible for the strength and stiffness of the polyamides and their great resistance toward most solvents.

[70] W. H. Carothers, U. S. Pat. 2,130,523 (1935); 2,130,947 (1936); 2,130,948 (1937); *Chem. Abstracts*, **32**, 9497, 9519 (1938).

[71] C. S. Fuller, W. O. Baker, and N. R. Pape, *J. Am. Chem. Soc.*, **62**, 3275 (1940).

[72] R. Brill, *Z. angew. Chem.*, **55**, 69 (1942). I. Sakurada and I. Hizawa, *J. Soc. Chem. Ind. Japan*, **43**, 348B(1940).

As Brill[73] has shown, several of these polymers occur in two crystalline forms: a form with lower symmetry, stable at low temperatures, and a form with higher symmetry, stable at high temperatures. In the case of the product obtained from adipic acid and hexamethylenediamine the low-temperature form is triclinic and the transition point lies at 140°C. In the low-temperature form the methylene groups which lie between the polar groups are distributed zigzag in parallel planes. The arrangement of the methylene groups differs therefore from that in the paraffins where the zigzag chains lie alternately in planes approximately at right angles to each other (see Chap. C, Fig. 2). The influence of the dipole groups therefore determines the fine structure.

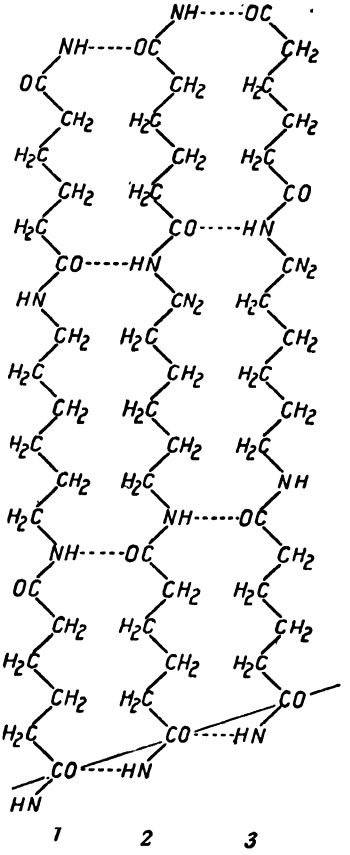


Fig. 2. Lamina formation through hydrogen bonds in a polyamide lattice (Brill).

An unusual dimorphism has been observed by Baker and Fuller[74] in the polyamide from decamethylenediamine and sebacic acid after it has been partially methylated at the nitrogen atom. In fibers tempered without tension at 170°C., and so to some extent crystallized, the chains are arranged in an incomplete lattice; the identity period is 20.2 Å. instead of 27 Å.—the length of the polyamide arrangement given in figure 2. After considerable stretching, the true polyamide lattice is established with an identity period of 25.8 Å. It seems possible that the portions of the segments consisting of methylene groups and those containing the dipole groups attempt to crystallize independently of each other. The paraffin chains attempt to form a paraffin lattice in which the zigzag chains of neighboring molecules are arranged in planes lying at right angles to each other; the dipole groups tend to form laminae as in figure 2. In the vicinity of the dipole groups, the chains are twisted and so come to be somewhat contracted and the completeness of the lattice suffers. In the

[73] R. Brill, *J. prakt. Chem.*, **161**, 49 (1943).

[74] W. O. Baker and C. S. Fuller, *J. Am. Chem. Soc.*, **65**, 1120 (1943).

course of stretching these irregularities are smoothed out and the system is compelled to adopt the polyamide structure.

Partly amorphous polyamide fibers, drawn at a high temperature, can be stretched in the cold to four or five times their original length; in the process the greater part of their substance becomes oriented and crystallizes. This behavior recalls that of many chain polymers and is to be interpreted in the same way: in the amorphous preparation the chains are largely contorted as in amorphous rubber; on stretching to five times the original length the contorted chains are straightened out and snap into position to form the lattice; further extension leads to rupture.

As we have seen already, the reason for the stiffness, high melting point, and insolubility of the polyamides is to be sought in the numerous hydrogen bonds. Softer, soluble products are obtained if the number of such bonds is reduced. This can be done by methylating the NH group so that its resonating proton is removed. In the case of polydecamethylene sebacamide. Baker and Fuller showed that with progressive methylation the substance became softer, fusible at lower temperatures, and finally rubberlike. Its water-absorbing capacity increased slightly from 1.6 to 3.1%, and whereas the unsubstituted amide was resistant to the action of chloroform and alcohol mixtures, with progressive methylation it first swelled and ultimately became soluble in these solvents. Moreover, it has been found[75] that size distribution and number of substituents of the NH groups may be varied in such a way that high-melting and stiff (no substitution), or low-melting and soft polymers (100% substitution) are formed; intermediate products are rubberlike.

The formation of hydrogen bonds is also hindered by cocondensation (Baker and Fuller) since, as a result of the different lengths of the molecules polymerized together, by no means all the NH and CO groups are able to snap together to form hydrogen bonds. Copolymers therefore soften at a lower temperature and are more soluble than homopolymers.

From osmotic determinations in cresol Staudinger[76] has shown the molecular weight of a soluble polyamide from sebacic acid and diisobutylhexamethylenediamine to be 12,000–13,000. In 90% formic acid plus 0.1 *N* sodium formate, Flory[77] found molecular weights for various highly condensed products between 10,000 and 20,000 (osmotic pressure measurements). According to Flory the molecular weight can be expressed in terms of viscosity, η (in poises), of the polyamide melt at 285°C. by eq. (1):

[75] E. L. Wittbecker, R. C. Houtz, and W. W. Watkins, *Ind. Eng. Chem.*, **40**, 875 (1948).

[76] M. Staudinger and M. Jörder, *J. prakt. Chem.*, **160**, 166 (1942).

[77] Reported by D. B. Nichols, in J. Alexander, *Colloid Chemistry*, Vol. VI Reinhold, 1946.

$$M = 113\left(\frac{\log \eta + 1.57}{0.427}\right)^2 \quad (1)$$

(*b*) *Industrial Applications of Superpolyamides*

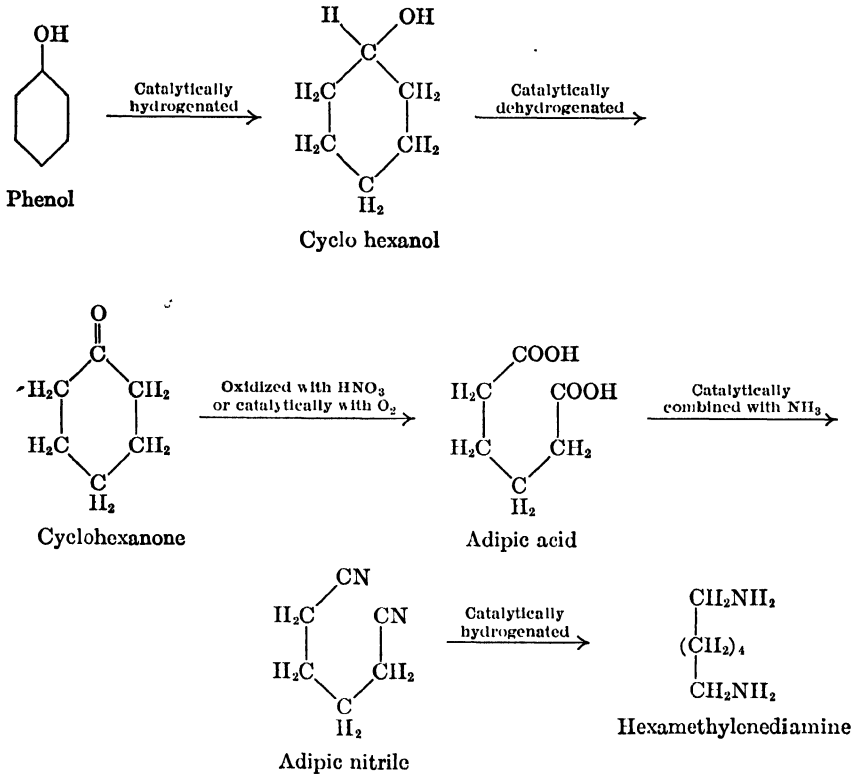
The impetus for the tremendous development of this field came from the work of Carothers and the commercial production of the most successful synthetic fiber (nylon) by Du Pont. Since that time I. G. Farbenindustrie, making use of the patents under license from Du Pont, Imperial Chemical Industries, and many other firms, have taken part in the development of this promising field. The importance of the superpolyamides lies unquestionably in their use as textile fibers. Those prepared include: the amide from adipic acid and hexamethylenediamine (nylon 66); the polyamide from ϵ-aminocaproic acid (Perlon L or nylon 6) and the polyurethan mentioned below (this last is only used for making bristles). In contrast to all other artificial fibers (spun from solutions) these are spun directly from the melt by extrusion through jets.

The initial material for the preparation of the polyamide from adipic acid and hexamethylenediamine—the most important of the polyamides from an industrial point of view—is phenol, from which both starting materials can be obtained by the reactions given at the top of page 268.

In the preparation of nylon fibers a tough, horny mass is obtained by heat condensation of adipic acid and hexamethylenediamine. This is softened by heating, converted into strips, and, after cooling, ground to a certain particle size. This material is then melted at about 250°C. and spun from jets.[78] The resulting product is to some extent microcrystalline but unoriented. The fibers are stretched in the cold to four times their initial length, giving a considerable increase in tensile strength and improving their quality as textile fibers. Stretched in this way they give a well-developed fiber diagram: the amorphous regions have crystallized and the crystallites have been so oriented that hydrogen bonds are established between all neighboring chains, thus making the whole structure more rigid. The fiber can be drawn out to one tenth of the diameter of natural silk. Stretched fibers are more elastic than silk and have a silky sheen. Fibers of 3.3 denier have a dry strength of about 5 g. per denier (*ca.* 50 kg. per mm.^{2}) and a wet strength of about 4 g. per denier and are thus superior to natural silk; the breaking extension (dry) is about 20%, wet about 30%. Resistance to kinking is extraordinarily high. It is resistant to solvents and chemical reagents.

The mechanical properties—in particular the resistance to crease—of textiles from polyamides is improved if they are exposed at a fixed length

[78] G. P. Hoff, *Ind. Eng. Chem.*, **32**, 1560 (1940).



(wound fibers or stretched fabrics) to the action of steam. This process depends presumably on the improvement in crystallization and hence on the increase in the number of hydrogen bonds formed. Polyamides can be dyed with acid, substantive, and vat dyes. Acetate rayon dyes are most commonly used; they are taken up out of neutral suspensions of the dye and "dissolve" in the fiber.

A further important use of polyamides is as thermoplastic moldings. At a fairly sharply defined temperature (*ca.* 250°C.) they fuse to give fluid melts. They do not exhibit a wide range of temperature over which they are plastic, as do other thermoplastic materials, but in spite of this they can be pressed either hot or cold and used for injection molding under certain conditions (molds must be tightly closed). The molded products obtained by simply allowing the melt to cool, in which molecules and crystallites are oriented at random, are of no great mechanical value; but the stiffness can be improved by orienting the chains, as in injection molding,

just as was described above under the stretching of fibers. A limited amount of water in the mass seems to be necessary for the maintenance of good mechanical properties; if allowed to dry out completely, molded objects become brittle.

By choosing the initial materials, for example, by introducing a bivalent alcohol, or by cocondensation, products with various properties can be obtained. The solubility—very low in the case of homogeneous superpolyamides—can be increased by methylating or acetylating the NH groups or by cocondensation.

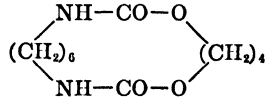
The field of application of the polyamides is wide because of their excellent mechanical properties, high heat resistance, and resistance to chemicals. Buttons, cannulas, syringes, spoons, etc., which will withstand boiling can be made by injection molding. Pressing yields plates whose properties can be improved by stretching; they can also be used as insulation and sheathing material for electric cables. If plasticizers are added the material becomes more flexible and in this condition is of great commercial value. Soluble polyamides can be used in the preparation (by dipping) of thin-walled objects or can be cast to give foils and films.

(c) *Polymeric Urethans and Ureas*

$$—NH(CH_2)_6NH—CO—O(CH_2)_4—O—CO—NH(CH_2)_6—NH—CO—O(CH_2)_4O—$$

$$—NH(CH_2)_6NH—CO—NH(CH_2)_4NH—CO—NH(CH_2)_6NH—CO—NH(CH_2)_4NH—$$

According to Bayer[79] bivalent isocyanates unite with bivalent alcohols to form polyurethans; cyclic urethans may be formed at the same time; for example, from hexamethylene diisocyanate and tetramethylene glycol the 16-membered ring compound:



may be formed. Superpolyurethans of molecular weight 5000 to 20,000 can be prepared, and these like polyamides, can be drawn out to fibers or used as molding powders.

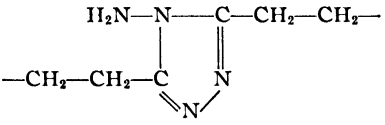
This reaction is only an addition and not a condensation; therefore no water has to be driven off. It is based on the well-known reaction, found by Wurtz, in which urethans are formed by the action of isocyanates on alcohols:

$$C_2H_5—N=C=O + HOC_2H_5 \longrightarrow C_2H_5NHCOOC_2H_5$$

[79] O. Bayer, *Ann.*, **549**, 286 (1941). O. Bayer, H. Rinke, W. Siefken, and L. Orthner, German Pat. 728,981 (1937) (I. G. Farbenindustrie).

From diisocyanates and diols linear fusible polyurethans are obtained; polyvalent alcohols yield cross-linked, nonfusible polymers.

Polymeric ureas are obtained from the condensation of diisocyanates or diurethans with diamines and by boiling diazides of the higher paraffin dicarboxylic acids with water.[79a] According to Macula and Lieser high polymers in which paraffin chains are linked together by 4-*N*-amino-1,2,4-triazole groups:



are formed by the action of hydrazine on higher aliphatic dinitriles.[79a]

(*d*) *Industrial Applications of Superpolyurethans*[80]

The linear polyurethan from *n*-hexane 1,6-diisocyanate and 1,4-butanediol is easily accessible. Polyaddition may be carried out either in solvents or in the melt. The reaction is of the second order and strongly exothermic. After boiling for one hour in chlorobenzene a powdery polymer of molecular weight 8000–9000 precipitates. As a technical process polyaddition is carried out in the melt at 200°; the hot reaction product of molecular weight 10,000–15,000 is extruded through orifices and thus obtained in the form of fibers, tapes, or bristles. The mechanical properties are improved by racking; they are not, however, superior to those of nylon fibers. The polyurethan is soluble in cold metacresol and in a mixture of 90 parts of phenol and 10 parts of water. By selecting other components rubberlike products may be obtained or tough polymers which can be used as artificial leather.

Cross-linked polyurethans may form very solid and water-resistant coatings. To this end a polyvalent alcohol (for example, an ester from one mole of adipic acid and two moles of hexanetriol) and a suitable diisocyanate are dissolved in ethyl acetate and applied as a coating; hardening is carried out at temperatures up to 150°C.

In order to obtain stable solutions addition products of diisocyanates and phenols (phenylurethans) are used in the mixture with the polyvalent alcohol instead of the free diisocyanates. The phenylurethans are stable in the cold but are decomposed by heat into phenol and free diisocyanate which reacts with the alcohol.

[79a] K. Macura and T. Lieser, *Ann.*, **564**, 64 (1949).

[80] From a monograph of O. Bayer, Leverkusen, 1948.

Diisocyanates transform, *e.g.*, incompletely acetylated cellulose acetate into an insoluble cross-linked substance. Mixtures of suitable diisocyanates and polyols with small amounts of solvents may be used as adhesives for paper, textiles, or metals.

As has been found by Wurtz, isocyanates yield, by reaction with carboxylic acids, substituted amides and CO_2:

$$C_2H_5N{=}C{=}O + HO_2C{-}R \longrightarrow C_2H_5NCOR + CO_2$$

Reactions of this type are used in the production of solid foams: a dicarboxylic acid and a triol are condensed to a polyester which contains free hydroxyl as well as carboxyl groups. If such an ester is mixed with a diisocyanate, then polyaddition takes place in the syrupy mass with evolution of heat; due to the increase of temperature the carboxyl groups react in their turn with the isocyanate groups; new cross linkings are formed and CO_2 is developed. The mass thus becomes tough and spongy and finally turns into a very hard foam. Owing to their low specific weight and valuable mechanical properties these foams can be used as material for construction and insulation.

3. Products of Condensation of Formaldehyde and Amides

(*a*) *Formaldehyde and Urea* (*Aminoplasts*)

Formaldehyde can react with amides, such as benzamide, according to scheme (*a*) or scheme (*b*)

$C_6H_5CONHCH_2OH$
Methylol compound
(*a*)

$C_6H_5CONH\diagdown$
$\qquad\qquad CH_2$
$C_6H_5CONH\diagup$
Methylene compound
(*b*)

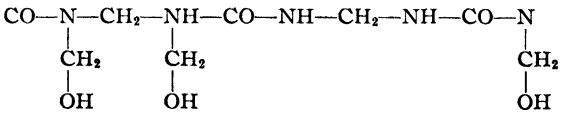
As scheme (*b*) shows, the aldehyde may link together two molecules of an amide; in the case of dibasic amides such as urea, chain polymers may be formed. The simple chain polymer:

$$-CH_2-NH-CO-NH-CH_2-NH-CO-NH-$$

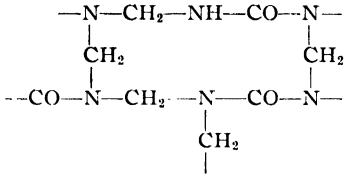
is prepared by acid condensation in the absence of excess aldehyde. Tollens[81] has named this compound methylene urea; it does not fuse, is very slightly soluble, and in its solubility resembles the higher polypeptides. It also yields a powder diagram. According to Meyer,[82] this compound may be regarded as the unbranched chain polymer.

[81] B. Tollens, *Ber.* **29**, 2751 (1896).
[82] K. H. Meyer, *Trans. Faraday Soc.*, **32**, 407 (1936).

By condensation in the presence of a higher proportion of formaldehyde (John[83]) polymers are obtained which dissolve in water, forming very viscous solutions. It is reasonable to suppose that this product consists of methylol compounds of the type:



Their solubility in water is due to the methylol groups. On evaporation, glasslike insoluble masses are obtained, which may be supposed to contain three-dimensional nets such as:



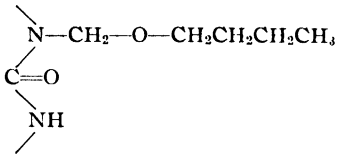
Products of a moderate degree of polymerization may be worked up with fillers and hardened under pressure by heating. This procedure, which is very widely used at the present time, was invented by Pollack.[84] Moldings (amino plastics) obtained in this way are prepared commercially and offer advantages over phenolic plastics in that they are odorless and light in color.

For the preparation of high condensation products, empirical procedures have been worked out; the product obtained depends on the composition of the mixture, that is, on the ratio of aldehyde to urea and on the *p*H during condensation. Addition of thiourea seems to have a favorable influence on the mechanical properties of the material obtained. Transparent condensation products are used as organic glass, compounded with fillers as molding powders and, in the incompletely polymerized form, in the preparation of varnishes.

The solubility can be varied by introducing alcohols, which are added on to the methylol groups, forming acetals:

[83] H. John, German Pat. 392,183 (1918). Reviews: G. Walter and M. Gewing, *Kolloid-Beihefte*, **34**, 163 (1931).

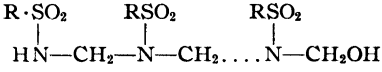
[84] F. Pollack and K. Ripper, *Chem.-Ztg.*, **1924**, 569, 582.



With glycerin alcohol-soluble resins are formed, with butyl alcohol, oil- and benzene-soluble resins.[85] On heating the lacquered object the coating hardens by further condensation and becomes insoluble. They are also used for the impregnation of cellulose fibers, thereby lessening their tendency to crease. Noncreasing cotton and rayon materials have been put on the market by Tootal, Broadhurst, Lee & Co. Ltd., Manchester, England. These are impregnated with such condensation products. The impregnation is performed (after bleaching and dyeing or printing) by treating the material with a water-soluble, low polymeric product of urea-formaldehyde condensation. In the presence of catalysts and buffers the resin is then formed in the fiber.

The use of water-soluble polymers as adhesives is of considerable commercial importance. Condensation in an acid medium is interrupted by neutralization as soon as a certain moderate degree of polymerization has been reached (this can be determined from the viscosity). The liquid is concentrated by evaporation and converted to an adhesive in powder form by spraying it as fine droplets in a stream of hot air. The product is used particularly as a thermosetting plywood glue. First trade name: Kaurit.

Sulfonamides also yield condensation polymers with formaldehyde (Walter[86]); they are also formed from amides of monobasic sulfonic acids. Condensation products so formed are soluble and fusible; they may be represented in the following way:



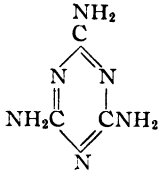
Cross-linked high polymers are formed with diamides of disulfonic acids and with substituted sulfonamides containing a second group which reacts with formaldehyde.

[85] T. S. Hodgins and A. G. Hovey, *Ind. Eng. Chem.*, **30**, 1021 (1938); **33**, 512 (1941).

[86] G. Walter, *Trans. Faraday Soc.*, **32**, 402 (1936). G. Walter and A. Glück, *Kolloid-Beihefte*, **37**, 343 (1933). G. Walter and H. Pollack, *ibid.*, **40**, 1 (1934).

(*b*) *Formaldehyde and Cyanuric Triamide* (*Melaminoplasts*)

Cyanuric triamide or melamine:



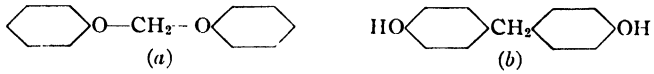
obtained by heating cyanamide or dicyandiamide, also condenses with formaldehyde to form compounds of high molecular weight, in which the formaldehyde forms bridges between different molecules. Solubility, hardness, and sheen can be varied by cocondensation with urea or phenol as well as by introducing alcohols. Products of medium and higher degrees of polymerization are prepared and are recommended as a binder for compacting cork meal, as molding powder, as a lacquer base, etc. (Trade name, Ultrapas).[87]

VII. PRODUCTS OF CONDENSATION OF ALDEHYDES AND PHENOLS

1. Condensation Products of Formaldehyde and Phenols (Phenoplasts)[88]

Of all synthetic high polymers the condensation products of phenol and formaldehyde (the so-called phenolic plastics) introduced by Baekeland[89] under the name Bakelite have by far the greatest commercial importance.

Baekeland assumed that the aldehyde reacts not only with the hydroxyl groups but also with the aromatic nucleus, and that ether linkages occur as in (*a*) as well as linkages of the type found in diphenolmethane (*b*):



(*a*) (*b*)

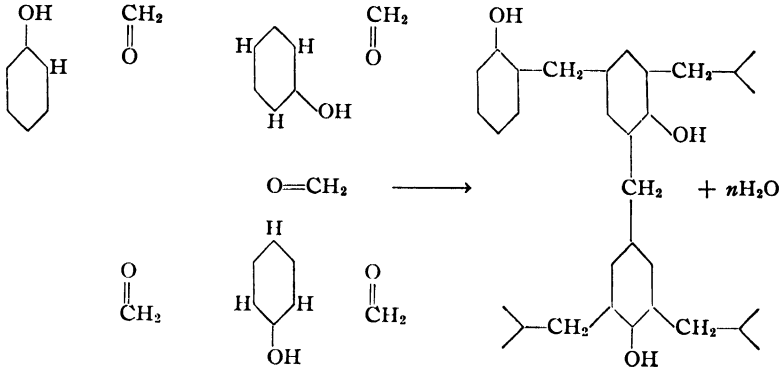
The formation of the ether linkage with the aid of formaldehyde is not, however, very probable; it seems more likely that formaldehyde links

[87] T. S. Hodgins, A. G. Hovey, S. Hewett, W. R. Barrett, and C. J. Meeske, *Ind. Eng. Chem.*, **33**, 769 (1941). A. Gams, G. Widmer, and W. Fisch, *Brit. Plastics*, **14**, 508 (1943).

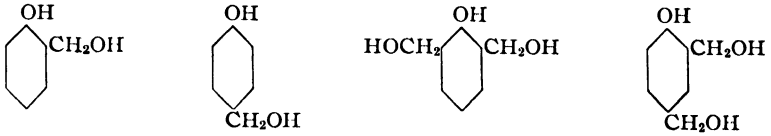
[88] See T. S. Carswell, *Phenoplasts* (*High Polymers*, Vol. VII). Interscience, New York, 1947.

[89] L. H. Baekeland, *Ind. Eng. Chem.*, **1**, 149 (1909).

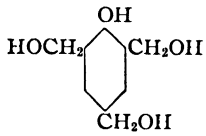
ortho and para positions together so that chain and net formation become possible (Raschig,[90] Meyer and Mark,[91] Koebner,[92] and Megson):[93]



This conception is supported by the study of the course of the reaction at the beginning of condensation. The condensation of formaldehyde and phenol in weakly alkaline solution leads, according to Meyer and Hemmi,[94] to the simultaneous formation of the compounds:



In addition, compounds belonging to the diphenylmethane series and higher condensation products are formed. The compound:



could not be detected; either the above-mentioned disubstitution products react more quickly with phenol than with formaldehyde, or the trisubstituted phenol reacts immediately with phenol to form further condensates.

[90] F. Raschig, *Z. angew. Chem.*, **25**, 1945 (1912).

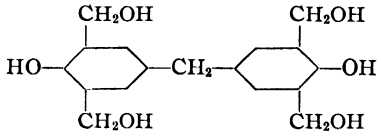
[91] K. H. Meyer and H. Mark, *Ber.*, **61**, 613 (1928); *Der Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930, p. 75.

[92] M. Koebner, *Chem.-Ztg.*, **54**, 619 (1930); *Z. angew. Chem.*, **46**, 251 (1933).

[93] N. J. L. Megson, *Trans. Faraday Soc.*, **32**, 336 (1936).

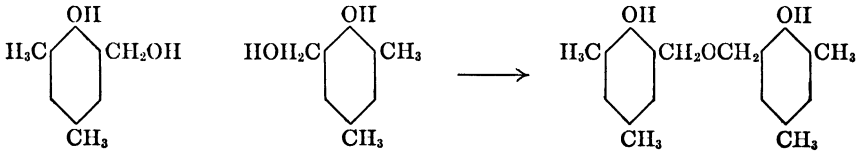
[94] K. H. Meyer, *Trans. Faraday Soc.*, **32**, 345 (1936).

Such intermediate condensation products include *p,p*-diphenolmethane, *o,p*-diphenolmethane and the compound:



all of which have been isolated by Megson[95] and Walker.[96] The higher condensation products cannot be isolated as homogeneous chemical compounds: they are, without doubt, built on the same plan. The products first formed, consisting of four to ten phenol residues, are soluble in alkali and in alcohol; on heating they condense to form masses of high molecular weight built up on the basis of the network structure described above. In the process of hardening, the product is heated under pressure in order to avoid the formation of bubbles by the water eliminated.

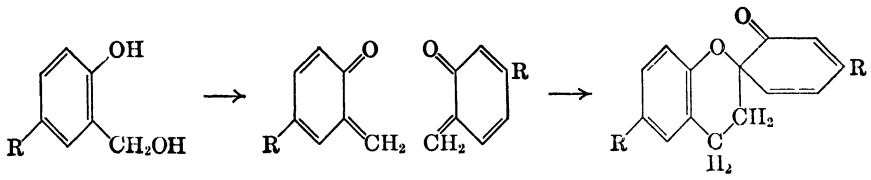
According to the work of Zinke[97] and von Euler[98] it is possible that secondary actions also occur during thermosetting with the formation of dibenzyl ether groups. If 2,4-dimethyl-6-hydroxymethyl phenol (obtained from 2,4-xylenol and formaldehyde) is heated it readily forms the corresponding dibenzyl ether:



It is possible, therefore, that this process of ether formation also occurs in the heat treatment of higher condensation products of the phenols.

In the condensation of phenols with only two reactive groups (for example, *o*- and *p*-cresol) only chains are formed, according to the reactions given above, but at higher temperatures these are cross linked by a third reaction. According to Hultzsch[99] and von Euler, dark-colored "methylene quinon'es' (quinone methides) are formed which dimerize to form quinole ethers, for example:

[95] N. J. L. Megson, *ibid.*, **32**, 336 (1936).
[96] E. E. Walker, *ibid.*, **32**, 345 (1936).
[97] A. Zinke and H. Ziegler, *Ber.*, **74**, 541 (1941).
[98] H. von Euler, *Z. angew. Chem.*, **54**, 458 (1941).
[99] K. Hultzsch, *Kunststoffe*, **32**, 69 (1942).



It is clear that phenolic hydroxyls disappear in this process, whereas in other condensation reactions the number of phenolic hydroxyls remains unchanged. Determination by acetylation of the number of hydroxyls in the finished resin may shed light on the nature of the condensation, according to Normann.[100]

The materials obtained by postcondensation show a higher specific gravity (1.25–1.34) than compounds of lower molecular weight or chain molecule compounds of the same analytical composition. This is explicable as a consequence of cross linking which leads to a diminution in volume, as the groups which were previously bound by secondary valences are now united by primary valences and the structure has contracted. Specific gravity, tensile strength, and modulus of elasticity are less, however, than the values to be expected of an ideal three-dimensional cross-linked system (de Boer[101] and Houwink[102]).

The quality of the condensation products may be varied within wide limits by changing the conditions of the reaction. In alkaline condensation, products are formed with free oxymethylene groups, while, in acid condensation, linkage formation and production of diphenolmethanes is favored. By choice of the condensation medium and by varying the proportion of phenol to formaldehyde, intermediate products with various properties may be prepared. Thus, alkaline condensation in the presence of excess formaldehyde yields products which, owing to their free methoxy groups, readily condense further; acid condensation in the presence of not too much formaldehyde yields stable, soluble products of moderate molecular weight, which can be hardened by the addition of a substance capable of splitting off formaldehyde, such as hexamethylenetetramine.

The lower condensation products may be used as varnishes which can be hardened by heating; the condensation products of moderate molecular weight are plastic while the most highly polymerized product is hard and relatively resistant to heat. The forms distinguished in the literature as

[100] W. Normann and E. Schildknecht, *Fettchem. Umschau*, **40**, 194 (1933). H. P. Kaufmann, *Fette u. Seifen*, **44**, 150 (1937).

[101] J. H. de Boer, *Trans. Faraday Soc.*, **32**, 10 (1936).

[102] R. Houwink, *ibid.*, **32**, 122 (1936).

the *A*, *B*, and *C* states cannot be sharply separated from each other; they are purely practical distinctions for the characterization of highly condensed products.

By cocondensation with substituted phenols, for example, xylenols, several properties, such as oil solubility of the intermediate products, can be favorably influenced. Products of a moderate degree of condensation display elastic properties; presumably large portions of the chains are still able to move as this seems to be a necessary condition for rubberlike elasticity. On warming, the elastic properties rapidly disappear as a consequence of progressive condensation leading to a very fine-meshed net (Houwink).

m-Cresol, which, like phenol, possesses three reactive positions (*o*, *o*, *p*), yields similar solid masses. In aqueous alkaline condensation *o*-cresol yields chain polymers of a moderate degree of polymerization, the sodium salts of which are only very slightly soluble (Koebner[103]).

2. Condensation Products of Furfural and Phenols[104]

Furfural, obtained on a commercial scale by acid treatment of the pentosans in oat husks and corn cobs, yields resins through the action of alkali. The formation of these may be due to polymerization involving the double bonds, as well as to condensation. The products are of no practical importance. Condensation products with phenols are used as thermosetting plastics. The intermediate condensation products maintain their flow characteristics over relatively long periods at temperatures slightly above those required to induce plasticity. They can therefore be used for injection molding.

3. Manufacture and Applications of Formaldehyde-Urea and Formaldehyde-Phenol Plastics

It has been mentioned that the condensation products of medium molecular weight of formaldehyde and urea (aminoplasts) and of formaldehyde and phenol (phenoplasts) are used very largely as molding materials which can be hardened. The products, prepared in powder form, flow together on warming to form a plastic mass which, by subsequent condensation, is transformed into a compact solid mass. Shaping and hardening take place in heated molds under pressure.

[103] M. Koebner, *Angew. Chem.*, **46**, 251 (1933).

[104] Review: A. P. Dunlap and F. N. Peters, in J. Alexander, *Colloid Chemistry.* Vol. VI, Reinhold, New York, 1946, p. 1048.

The greater part of the condensation products are not prepared pure but are mixed with fillers. They are then compressed in heated molds and left to harden. The fillers serve to give the finished product strength and color; for this purpose fibrous materials and pigments are used. In aminoplasts the strengthening materials are saturated with an aqueous solution of the condensation products in mixing machines, and are then dried and ground to molding powder. In phenoplasts, the resinous condensate is mixed with powdered fillers (in particular, wood meal) in heated kneading machines. In this process, pigments as well as metallic oxides and hexamethylenetetramine are added; stearic acid may be used as a softener. The mixture is allowed to cool and is then ground to molding powder. From this powder are manufactured plates, tubes, rods, and blocks which can be worked up still further by mechanical means (drilling, milling, sawing, etc.), or the molded objects are given the final shape required (frequently very complex) by pressing in molds or by laminating under high pressure. Aminoplasts are employed when white or brightly colored objects are required. The number of thermoset moldings in everyday life, as well as parts of machines and apparatus, increases continually as does the use of phenoplasts for lacquers and as adhesives for plywood.

4. Natural Condensation Products of Phenols

Many phenolic condensation products occur in nature. The tannins will, however, not be treated here, since their molecular weight does not bring them within the high polymer range. The melanins, which are probably oxidation and condensation products of tyrosin, are omitted for the same reason. On the other hand, lignin, a true high polymer containing phenolic residues, will be dealt with in chapter F together with other polymer substances associated with cellulose.

VIII. CONDENSATION PRODUCTS OF FORMALDEHYDE AND ANILINE (ANILINOPLASTS)

Aniline condenses with formaldehyde to form compounds of high molecular weight. Of recent years Ciba in Basel has paid particular attention to these compounds and has developed a practicable method for the production of thermoplastic moldings. Resin-like polymers are formed, according to Böhler,[105] on heating anhydroformaldehyde-aniline, $C_6H_5N{=}CH_2$; in all probability the aniline residues are united to the nitrogen atoms only

[105] C. C. Böhler, German Pat. 335,984 (1917). See also I. G. Farbenindustrie, German Pat. 452,009 (1925), and supplement 453,276 (1925).

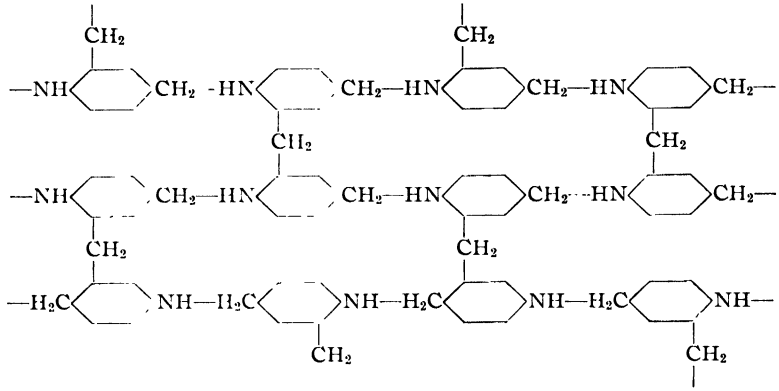
by means of CH_2 bridges since aniline first reacts with formaldehyde at the nitrogen atom:

$$R—HN—CH_2—NH—R$$

The so-called azomethine resins are soluble, brittle, and fusible, and are not, therefore, of very high molecular weight. In the presence of acid catalysts, formaldehyde also attacks the benzene nucleus. A substance described by Kalle & Co.[106] as anhydro-*p*-aminobenzyl alcohol has been given a chain formula by Frey:[107]

$$—NH—C_6H_4—CH_2—NH—C_6H_4—CH_2—NH—C_6H_4—CH_2—$$

By condensation of one mole of aniline with more than one mole of formaldehyde in dilute hydrochloric acid, high polymeric substances may be obtained which, after neutralization of the acid, washing, and drying, may be molded to form dense homogeneous, translucent products by heating under pressure.[108] An exact formula cannot be given. Frey suggests, by analogy with the formulas for other similar polymers given by Meyer and Mark, the following netlike structure:



When formaldehyde reacts with phenol in solution, it behaves to some extent as a methylol group, capable of forming further bridge linkages, but when it condenses with aniline, only $—CH_2$ bridges appear to be formed, whether as $—N—CH_2—N$ or $—N—CH_2—C_6H_4—N$ bridges. The high polymeric condensation product with aniline behaves as a completely con-

[106] Kalle & Co., German Pat. 93,540, 95,184, 95,600, 96,851.

[107] K. Frey, *Helv. Chim. Acta*, **18**, 491 (1935).

[108] P. Haller and H. Kappeler, German Pat. 487,654 (1927), and supplement 496,981 (1929). Ges. f. Chem. Industrie, Basel, German Pat. 559,324 (1928), 562,518 (1931), 594,926 (1932); Brit. Pat. 283,965 (1928).

densed product; it does not harden on heating but is thermoplastic. The temperature of molding and the heat resistance of the molded material depend on the way in which condensation is conducted, and, in particular, on the ratio of aniline to formaldehyde; products with 1.2 moles of formaldehyde require a molding temperature of 150–160°C., those with 1.5 moles of formaldehyde, a temperature of over 200°C. (Frey). Heat resistance and molding temperature are thus higher than in the case of polystyrene and most other thermoplastic masses.

IX. CONDENSATION PRODUCTS AS ION EXCHANGERS[109]

If phenolsulfonic acid or another substance with acid groups is condensed with phenol and formaldehyde, net polymers are obtained whose acid group can dissociate into anions, bound to the net, and mobile cations. If such products are prepared in a suitably granular and porous form, they are able, like clays or zeolites, to exchange their cations for other cations. They can then be used in the form of their sodium salts for softening water, the calcium ions in the hard water being exchanged for sodium ions. Copolymers capable of exchanging anions can be obtained by introducing residues with basic groups, such as phenylenediamine, into anilinoplasts.* Furthermore, polymers which exchange cations and anions can be used for accumulating ions from very dilute solutions. It is also possible to remove salts almost completely, that is, as completely as they are removed in ordinary distilled water, from water of low salt content by allowing it to react first with a cation exchanger in the form of the free acid, and subsequently with an anion exchanger in the form of the free base, as in the following scheme:

$$NaCl + H^+ \text{ exchanger} \longrightarrow HCl + Na^+ \text{ exchanger}$$
$$HCl + OH^- \text{ exchanger} \longrightarrow H_2O + Cl^- \text{ exchanger}$$

The organic chemist may note with interest that solutions of sugars and similar substances can be freed from salts, acids, and bases by means of synthetic ion exchange resins, and that in naturally occurring calcium salts the calcium can be easily replaced by sodium.

Ion exchange resins were discovered by Adams and Holmes and this important field was opened up on a commercial scale by I.G. Farbenindustrie

* Their basicity may be increased by addition of methyl iodide to the free amino groups.

[109] B. A. Adams and E. L. Holmes, *J. Soc. Chem. Ind.*, **54**, 1 (1935). R. Griessbach, *Z. angew. Chem.*, **52**, 215 (1939). R. J. Myers *et al.*, *Ind. Eng. Chem.*, **33**, 697 (1941). R. J. Myers, in *Advances in Colloid Science*, Vol. I, Interscience, New York, 1942, p. 317. W. C. Baumann and J. Eichhorn, *J. Am. Chem. Soc.*, **69**, 2830 (1947).

A.-G. (Wofatite) and by the Resinous Products and Chemical Company in Philadelphia (Amberlite). A number of special brands for different industrial purposes are on the market. The kinetics of ion exchange have been investigated by Boyd and Myers.[110]

Primary hydroxyl groups at the surface of cellulose can be oxidized by nitrogen peroxide to carboxyl groups. Cellulose treated in this way and containing a high percentage of superficial hydroxyls ("celluronic acid") can be used as a cation ion exchanger.

[110] G. E. Boyd, A. W. Adamson, and L. S. Myers, *J. Am. Chem. Soc.*, **69**, 2836 (1947). See also S. W. Mayer and E. R. Tompkins, *ibid.*, **69**, 2866 (1947).

E. CELLULOSE AND ITS DERIVATIVES[1]

I. CONSTITUTION AND TEXTURE OF CELLULOSE

The name cellulose is often used to denote a fibrous product obtained by subjecting vegetable material to certain mechanical or pulping processes. This product contains several different chemical substances; the main constituent is a water and alkali insoluble polysaccharide: β-1,4-polyanhydroglucose or rather a mixture of homologous polymer polyanhydroglucoses. In the interest of clarity it is preferable to refer to the raw material as "raw cellulose" or "cellulose pulp" and to reserve the name cellulose or pure cellulose for the chemically defined polysaccharide.

Even pure cellulose may vary with respect to chain length, crystallinity, and arrangement of the chains; only in a qualified sense it is legitimate to speak of a material as cellulose.

Cellulose forms the principal constituent of the walls of nearly all plant cells; only in the fungi is it replaced by chitin. Cellulose is also produced by certain bacteria, in the form of submicroscopic filaments, and occurs in the animal kingdom as tunicin in the mantle of tunicates.

We shall first discuss the most important properties of cellulose and the constitution associated with these properties. We shall then consider the differences between various types of cellulose, *i.e.*, native and regenerated celluloses.

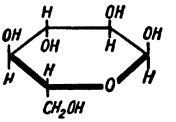
1. Constitution of Cellulose

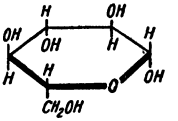
(*a*) *Constitutional Formula*

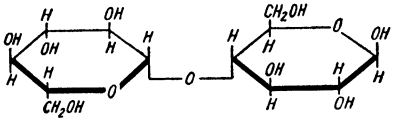
All celluloses are decomposed quantitatively to form glucose when treated with strong acids. This fact has been known for more than one hundred years but it leaves unanswered the question of the nature and position of the linkage of the glucose residues; the discovery by Franchi-

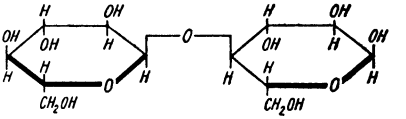
[1] General accounts: H. Mark, *Physik und Chemie der Zellulose*, Springer, Berlin, 1932. A. G. Norman, *The Biochemistry of Cellulose, the Polyuronides, Lignin, etc.*, Oxford Press, London, 1937. High Polymers, Vol. V, E. Ott, editor, *Cellulose and Cellulose Derivatives*, Interscience, New York, 1942.

mont[2] and Skraup[3] that a disaccharide (cellobiose) may be obtained in the acetolysis of cellulose was therefore an important step forward. In this reaction the conditions are such that cellobiose cannot arise from glucose; this was further emphasized by the work of Willstätter and Zechmeister,[4] who found that during the decomposition of cellulose with concentrated hydrochloric acid cellobiose is formed as an intermediate product, which decomposes to form glucose as the reaction proceeds. The explanation of the constitutional formula of cellobiose, for which we are chiefly indebted to Haworth,[5] was therefore of fundamental importance to the chemistry of cellulose. According to Haworth, cellobiose is β-glucosidoglucopyranose; one glucose residue is connected to the fourth carbon atom of the second ring by means of a β-glucosidic linkage:


α-Glucose


β-Glucose


β-Cellobiose


α-Maltose

Maltose is an isomer in which both rings are connected by means of an α-glucosidic linkage between carbon atoms 1 and 4. Cellobiose is thus a β-glucoside and as such a derivative of β-glucose; maltose is an α-glucoside.

It was natural at this stage to suppose cellobiose to be already present in cellulose, with all the glucose residues united by the same linkage as the two glucose residues in cellobiose. The wide acceptance at an early date of this point of view is clear from the textbook of Meyer and Jacobson (1913),[6] in which the idea is put forward very definitely. It was supported

[2] A. P. N. Franchimont, *Ber.*, **12**, 1938 (1879).

[3] Z. H. Skraup, *Monatsh.*, **26**, 1415 (1905).

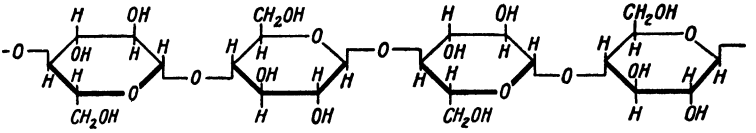
[4] R. Willstätter and L. Zechmeister, *Ber.*, **62**, 722 (1929).

[5] W. N. Haworth, *Helv. Chim. Acta*, **11**, 534 (1928).

[6] V. Meyer and P. Jacobson, *Lehrbuch der organischen Chemie.* Vol. I, Part 2, 2nd ed., De Gruyter, Berlin, 1913, page 1024, *et seq.*

by quantitative experiments on the cellobiose yield by Klein[7] and later by Haworth[8] and Freudenberg.[9] In 1928 this view was put forward in more precise terms by various men; by Haworth[10] in connection with his work on cellobiose, by Meyer and Mark[11] in their work on the three-dimensional model of cellulose, and by Freudenberg[12] in connection with work on 2,3,6-trimethylglucose anhydride. Further evidence was provided by the isolation of cellotriose, cellotetraose, and cellohexaose from the decomposition products.[13]

The structure of cellulose, regarded as a chain of glucose residues linked as in cellobiose, is expressed by the following formula:



The results of x-ray analysis have confirmed this formula and have led to the construction of a three-dimensional model (page 302, *et seq.*).

Purely chemical methods leave the question undecided as to whether a small proportion of other linkages may not be present in addition to β-glucoside linkages. For the crystalline fraction of cellulose (which is by far the most important constituent present in purified preparations) this question has been answered by x-ray analysis, as it has been shown that the unit cell is small. Cellulose must therefore be built up from similar structural units, without any foreign intermixtures, such as maltose residues (consisting of two glucose residues joined by an α-linkage) or gentiobiose residues. The structural principle (all cellobiose linkages) is implicit in an element of symmetry revealed by x-ray analysis—the digonal screw axes parallel to the fiber axis. Their presence shows that in directions parallel to the fiber axis only one type of linkage exists between the glucose residues. Since the presence of cellobiose linkages has been demonstrated chemically,

[7] F. Klein, *Z. angew. Chem.*, **25**, 1409 (1912).

[8] W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*, **119**, 193 (1921).

[9] K. Freudenberg, *Ber.*, **54**, 767 (1921).

[10] W. N. Haworth, *Helv. Chim. Acta*, **11**, 534 (1928).

[11] K. H. Meyer and H. Mark, *Ber.*, **61**, 593 (1928); *Z. physik. Chem.*, **B2**, 115 (1929)

[12] K. Freudenberg and E. Braun, *Ann.*, **460**, 288 (1928).

[13] R. Willstätter and L. Zechmeister, *Ber.*, **62**, 722 (1929). See also G. Bertrand and S. Benoist, *Bull. soc. chim.*, **33**, 1451 (1923). L. Zechmeister and G. Tóth, *Ber.*, **64**, 854 (1931).

the digonal screw axis shows that only such linkages occur (Meyer and Mark, 1928[11]). This result is confirmed by the value obtained for the optical rotation of cellulose (Mark and Meyer, 1929[11]) and further by the results of the investigation of the kinetics of acid hydrolysis made by Freudenberg and Kuhn.[14]

(*b*) *Molecular Weight of Native Cellulose and Various Cellulose Preparations*

Cellulose dissolves in cuprammonium solution without undergoing any degradation if the solution is carried out in the complete absence of oxygen and with addition of a small amount of glucose. From the viscosity of this solution Staudinger[15] deduced (by extrapolation of his equation) a molecular weight of 162,000 for native cotton cellulose, corresponding to a degree of polymerization (D.P.) of 1000. Later he doubled and finally even tripled this value. Gralén[16] and Svedberg[17] obtained much higher values by direct measurements in the ultracentrifuge (Table I).

TABLE I

Cellulose	M.W. × 10^{-3}	D.P.	$[\eta]$
Flax	5900	36,000	16.8
Ramie	2000	12,400	10.6
Nettle	1900	11,600	13.6
Cotton	1750	10,800	12.6
Bacterial cellulose	700	4,300	6 2[18]

Experiments of Golowa[19] seem to prove, however, that these values are still much too low. Native cotton carefully freed from oxygen by repeated treatment with helium exhibits a viscosity $[\eta]$ several times higher than 12.6. According to Gralén's results such a viscosity corresponds to a degree of polymerization of at least 100,000, a molecular weight of about 20 million, and a length of the molecule of about 0.05 mm.

Cellulose from milled wood dissolves to a small extent in cuproammonium solution. The solution contains one high molecular component, M.W. = 800,000 (cellulose), and a second one of lower M.W. (hemicellu-

[14] K. Freudenberg, W. Kuhn, W. Dürr, F. Bolz, and G. Steinbrunn, *Ber.*, **63**, 1510 (1930).

[15] H. Staudinger and B. Ritzenthaler, *Ber.*, **68**, 1226 (1935).

[16] N. Gralén, *Dissertation*. Uppsala, 1944.

[17] T. Svedberg, *J. Phys. & Colloid Chem.*, **51**, 1 (1947).

[18] H. Fikentscher, *Cellulosechemie*, **13**, 58 (1932).

[19] O. P. Golowa and V. I. Ivanov, *Bull. acad. sci. U.R.S.S., Classe sci. chim.*, **1945**, 279.

lose). The value of 800,000 may be considered as the minimum value for wood cellulose; in sulfite and sulfate pulps the cellulose is slightly degraded (Gralén). For cellulose derivatives (by measurement of the osmotic pressure[20]) values between 5000 and 500,000 have been obtained.

Estimation of the end groups has also been used for the determination of the molecular weight. An open cellulose chain has a free aldehyde group at one end and a free hydroxyl group attached to the fourth carbon atom at the other end. The presence of this hydroxyl group may be shown by complete methylation followed by hydrolysis; if it is free, the degradation products include 2,3,4,6-tetramethylglucose. By this method Freudenberg[21] and Hess,[22] as well as Haworth and his collaborators,[23] were not able to demonstrate any end groups in native cellulose which was prepared with particular care. This negative result can be readily understood if the extremely low percentage of end groups (0.01% for cotton) is taken into account. Methylated preparations made under less carefully controlled conditions from cellulose which was already somewhat degraded showed one end group to every few hundred sugar residues (Haworth,[24] Hess[25]). Bergmann and Machemer[26] have tried to determine the free aldehyde groups in preparations of cellulose, using the well-known titration method of Willstätter and Schudel for the measurement of the aldehyde sugar by means of hypoiodite. With cellulose, however, this method does not give the true number of free aldehyde groups present, as the reaction cannot be taken to completion without the formation of some new reducing groups.[27]

[20] Experiments by the following authors may be cited as examples: J. J. Duclaux and E. Wollmann, *Compt. rend.*, **152**, 1582 (1911); E. H. Büchner and P. J. P. Samwell, *Proc. Acad. Sci. Amsterdam*, **33**, 749 (1930); E. H. Büchner, P. J. P. Samwell, and H. E. Steutel, *Trans. Faraday Soc.*, **29**, 32 (1933); E. H. Büchner and H. E. Steutel, *Proc. Acad. Sci. Amsterdam*, **36**, 671 (1933); R. O. Herzog and A. Deripasko, *Cellulosechemie*, **13**, 25 (1932); W. Herz, *ibid.*, **15**, 95 (1934); R. Obogi and E. Broda, *Kolloid-Z.*, **69**, 172 (1934); A. Dobry, *ibid.*, **81**, 190 (1937); C. G. Boissonnas and K. H. Meyer, *Helv. Chim. Acta*, **20**, 783 (1937); H. Staudinger and G. V. Schulz, *Ber.*, **68**, 2320 (1935); **70**, 1577 (1937); H. Staudinger and G. Daumiller, *Ann.*, **529**, 219 (1937); K. H. Meyer and E. Wolff, *Kolloid-Z.*, **89**, 194 (1939).

[21] K. Freudenberg and E. Braun, *Ann.*, **460**, 288 (1928).

[22] K. Hess and F. Neumann, *Ber.*, **70**, 721, 728 (1937). K. Hess and K. P. Jung, *ibid.*, **73**, 980 (1940).

[23] W. N. Haworth, *Chemistry & Industry*, **58**, 917 (1939). W. N. Haworth, E. L. Hirst, L. N. Owen, S. Peat, and F. J. Averill, *J. Chem. Soc.*, **1939**, 1885.

[24] W. N. Haworth and H. Machemer, *J. Chem. Soc.*, **1932**, 2270.

[25] K. Hess, D. Grigorescu, E. Steurer, and H. Frahm, *Ber.*, **73**, 505 (1940).

[26] M. Bergmann and H. Machemer, *Ber.*, **63**, 316, 2304 (1930).

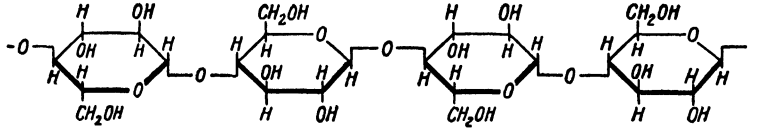
[27] See also K. Hess, K. Dziengel, and H. Maas, *Ber.*, **63**, 1922 (1930).

The dependence of the physical properties of cellulose preparations on chain length is fundamentally similar to that displayed by synthetic high polymers. As an example the solubility may be quoted: cellobiose, cellotriose, and cellotetraose are soluble in water; chains of 10–20 glucose residues are no longer soluble in water but dissolve in dilute alkalis; still longer chains are insoluble even in alkalis. The tensile strength also increases as some function of the chain length.

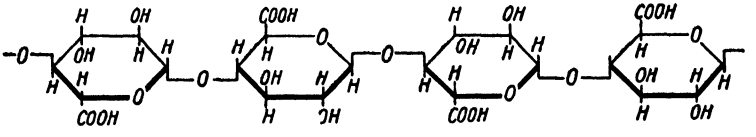
Different kinds of cellulose or even different preparations of the same kind may vary not only in mean chain length but also in the degree of polydispersity (polymolecularity), that is, in the distribution of their molecular weights. According to Svedberg's measurements of the rate of sedimentation, native celluloses are reasonably homogeneous, whereas celluloses degraded by technical processing show themselves to be polydisperse to a greater or less extent. In general, celluloses with a wide distribution of molecular weights are not suitable for technical purposes.

(c) *Carboxyl Groups in Cellulose Fibers*

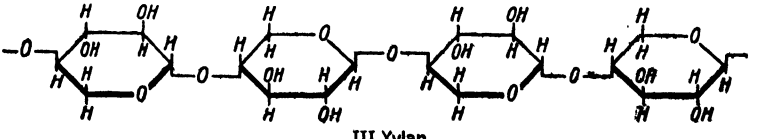
Native cellulose is always found in association with other substances including some compounds whose composition is entirely different from that of cellulose, and others which are chemically similar. The most important member of the first group is lignin, which may be removed by suit-



I Cellulose



II Polyglucuronic acid



III Xylan

Chain formulas of cellulose and related substances

able chemical treatment, involving, presumably, rupture of covalent bonds (Hägglund[28]). Cellulose freed from lignin and containing hemicelluloses *e.g.*, xylan, as well as other less well-defined polysaccharides is known as holocellulose. Many celluloses contain polyglucuronic acids as associated substances; some of the cellulose chains may even include a few glucuronic acid residues incorporated among the glucose units. A glance at the constitutional formulas of these substances (see opposite page) shows their close relationship to cellulose.

Presumably the plant is able to oxidize cellulose to glucuronic acid, from which xylan may be derived by decarboxylation.

Cotton fibers, pulp, and, more particularly, all bleached preparations of cellulose contain a small proportion of carboxyl groups; ever since the investigations of Schmidt[29] these groups have been considered an integral part of the cellulose molecule. Recent experiments of Studer and van der Wyk[30] have shown however that a carboxyl-free cellulose can be prepared from cotton as well as from pulp in more than 90% yield by precipitation from cuprammonium solution, leaving in solution a material richer in carboxyl groups. The carboxyl-rich chains should therefore properly be included among the hemicelluloses, and the molecule of true native cellulose should be considered as free of carboxyl groups.

Their peculiar properties make the carboxyl groups in technical products very noticeable in spite of their small numbers; cations (Na^+ and Ca^{++}, for example) are adsorbed in equivalent amounts, that is, are taken up from salt solutions by double decomposition (exchange adsorption); basic dyes and organic bases are adsorbed. The negative charge on cellulose particles (which is revealed in electrokinetic experiments) and the permeability of cellulose membranes to cations are also due for the most part to the presence of carboxyl groups, although the alcoholic hydroxyl groups of cellulose have often been held, erroneously, to be responsible.

Determination of the percentage of carboxyl groups is a valuable method of assessing the extent to which treated cellulose preparations have been chemically degraded; it may be used, for example, to detect damage to cotton or other textiles from bleaching. As the carboxyl groups in commercial preparations are mostly neutralized by combination with alkali or calcium, they can only be estimated quantitatively if they are first set free by treatment with dilute hydrochloric acid, followed by careful wash-

[28] E. Hägglund, *Holzchemie.* 2nd ed., Akadem. Verlagsgesellschaft, Leipzig, 1939, page 254.

[29] E. Schmidt *et al.*, *Ber.*, **67**, 2037 (1934); **68**, 542 (1935); **69**, 366 (1936).

[30] J. A. van der Wyk and M. Studer, *Helv. Chim. Acta.*, **32**, 1698 (1949).

ing or electrodialysis. Conversely, if the neutral salt is prepared and dispersed in water, its cations will be exchanged for hydrogen ions from the water, thus giving rise to an alkaline reaction on the part of the latter: the salt is said to undergo *Donnan hydrolysis*. For this reason, a direct titration of an insoluble acid dispersed in pure water leads to values which are too low. Insufficient attention seems to have been given to this point by several authors; among others we may cite the work of Schmidt on the potentiometric titration of cellulosic acids.

Donnan hydrolysis can, however, be completely suppressed in practice if the cellulose is saturated with sodium chloride solution before titration (the method used by Neale[31]). The method recommended by Lüdtke,[32] which consists of titration in a strong aqueous solution of calcium acetate, appears to the author to be less suitable. Neale and Stringfellow[31] have calculated, from the results of titrations, that the dissociation constants of the acids concerned are about 3×10^{-4}. As Heymann,[33] however, has pointed out, their calculations involve so many arbitrary assumptions that we cannot accept the results. In general the dissociation constant of cellulosic acids, such as occur in hemicelluloses, is probably of the order of 10^{-5}. Reaction with salts of heavy metals, such as Ba, Pb, and Ag, which yield insoluble products with celluloses containing acid groups, and the adsorption of basic dyes[34] are similar phenomena.

Determination of the uptake of methylene blue (*methylene blue number*) is often used as a means of assessing oxidative damage to cellulose. The values obtained from technically processed cellulose preparations and from typical oxycelluloses (cellulose containing oxidized layers at the surface of the fibers) by various different methods agree fairly well with one another; there is approximately one carboxyl group for every 50 to 500 glucose residues, the proportion varying with the degree of bleaching.

Rebek[35] (by exchange with basic crystal violet) and Husemann and Weber[36] (by estimation of "reversibly bound" methylene blue) have attempted to determine the extremely low proportion of carboxyl groups present in undamaged fibers and obtained one group for about 2000 glucose units. A complete reaction of the basic dye with all the carboxyl groups

[31] S. M. Neale and W. A. Stringfellow, *Trans. Faraday Soc.*, **33**, 881 (1937).

[32] M. Lüdtke, *Z. angew. Chem.*, **58**, 650 (1935).

[33] E. Heymann and G. Rabinov, *Trans. Faraday Soc.*, **38**, 209 (1942).

[34] L. Brissaud, *Mém. poudres*, **26**, 93 (1934/35); **28**, 43 (1938); H. Rath and H. Rademacher, *Melliand Textilber.*, **20**, 749 (1939). H. Rath and H. Dolmetsch, *Klepzig's Textil-Z.*, **41**, 475 (1938).

[35] M. Rebek, *Kolloid-Z.*, **92**, 217 (1940). O. H. Weber, *J. prakt. Chem.*, **158**, 33 (1941).

[36] E. Husemann and O. H. Weber, *ibid.*, **159**, 334 (1941).

present is, however, unlikely for steric reasons; moreover, Studer[30] has recently shown that even carboxyl-free cellulose adsorbs some methylene blue, so that little significance can be attached to these results. Measurements by Sookne and Harris[37] showed that raw cotton was capable of binding one equivalent of base for every 1000 glucose residues, of which 80% was bound by pectins or hemicelluloses and only 20% by the cotton itself. Heymann and Rabinov[33] found equally low values for the proportion of acidic groups; and recently van der Wyk and Studer[30] found that cellulose from either cotton or wood, if freed from hemicelluloses, contained no carboxyl groups at all, the extremely small uptake of alkali being due entirely to hydroxyl groups.

(*d*) *Further Deviations from Ideal Structure*

The x-ray diagram shows clearly that bundles of pure cellulose chains are present in untreated fibers; these may be linked chemically at their edges with substances such as xylan, other hemicelluloses, or lignin. Larger or smaller quantities of associated substances may lie between the ordered regions, but the opinion upheld by many authors that each cellulose chain is chemically united with accompanying substances by a considerable fraction of its glucose residues cannot be reconciled with the results of x-ray analysis. In addition to the chains of associated substances other than cellulose, short chains of the true cellulose type, but soluble in alkali, are present in raw cellulose.

In order to explain the observation that cellulose is insoluble in water several authors have made the assumption that the chains are cross linked by some hypothetical primary bonds. Since cellulose reprecipitated from solution where no cross linkings are possible is also insoluble we must reject this hypothesis. Other observations of Haworth[38] and Pacsu[38a] have also been interpreted by these authors as indicating the presence of cross links, but their arguments are by no means conclusive. Another assumption concerns the presence in the cellulose chain of a few xylose or other residues whose glucosidic bonds are more rapidly hydrolyzed than those of the glucose units; but neither the kinetic experiments of Ekenstam[39] nor the more

[37] A. M. Sookne and M. Harris, *J. Research Natl. Bur. Standards*, **25**, 47 (1940); **26**, 205 (1941).

[38] W. N. Haworth, *Chemistry & Industry*, **1939**, 917.

[38a] E. Pacsu, *Textile Research J.*, **17**, 405 (1947).

[39] A. af Ekenstam, *Ber.*, **69**, 549 (1936).

recent results of Schulz and Husemann[40] provide sufficient evidence for this assumption.

Critical treatment of all the experimental evidence available leads to the conclusion that by far the major part of all cellulose fibers is formed by the unbranched, non-cross-linked, and carboxyl-free linear polymer: β-1,4-polyanhydroglucose. It is suggested this substance be denoted pure cellulose.

2. Preparation and Methods of Testing

(a) *Preparation and Purification*

The preparation and purification of cellulose are confined to the removal of the substances with which it is associated in nature, and both commercial and scientific methods depend therefore upon the composition of the material in question. Fibers which are largely free from association with other substances, such as cotton fibers, are usually treated with dilute alkalies under pressure and bleached with hypochlorite. The cellulose of bast fibers is freed from pectins and other associated substances by fermentation (retting).

The most important raw materials for technical pulp—wood, straw, and the like—must be subjected to a more drastic chemical treatment because of their high lignin content. Wood is usually digested by boiling with caustic alkali or bisulfite, or more recently by the action of nitric acid; straw is usually treated with chlorine; any of these processes may be followed by chlorine bleaching which is fundamentally a process of oxidation. Space does not permit a detailed consideration of the various methods involved; it may be mentioned, however, that it appears unlikely that the accompanying substances can be removed without the cellulose being attacked (at least at the surface) by the harsh reagents used. This applies also to the method recommended by Schmidt,[41] using chlorine dioxide, although, compared to that caused by other technical oxidizing agents,[42] the degradation of cellulose and hemicelluloses by chlorine dioxide is small.

In the technical preparation of cellulose (pulp), the choice of raw material and the method of digestion are conditioned by the degree of purity

[40] G. V. Schulz and E. Husemann, *Z. physik. Chem.*, **B52**, 23 (1942).

[41] E. Schmidt, E. Geisler, P. Arndt, and F. Ihlow, *Ber.*, **56**, 23 (1923).

[42] H. Staudinger and J. Jurisch, *Papier-Fabr.*, **35**, 462 (1937). E. Husemann, *J. prakt. Chem.*, **155**, 13 (1940). See also C. V. Holmberg and E. C. Jahn, *Paper Trade J.*, **111**, No. 1, 33 (1940).

required in the final pulp. In paper making the most important factor is the length of the fibers, and so, for paper pulp, no very high degree of chemical purity is required. Recently there has even arisen a tendency to leave in most of the hemicelluloses in order to improve the yield. Paper pulp is, in fact, coming more and more to have the characteristics of a *holocellulose*, a tendency which is encouraged by the fact that hemicelluloses do function as cements and so improve the mechanical properties of the paper.[43]

In pulps destined for further chemical processing (*i.e.*, conversion to rayon or cellulose esters or ethers) the most important property is chemical purity. The rayon industry demands as complete a removal as possible of all impurities, a point which is regarded as essential for further improvement in the quality of the fibers obtained.[44]

(*b*) *Conventional Methods of Testing*

A series of conventional test methods is used in industry to assess the physical and chemical properties of cellulose. These have recently been standardized in Germany[45]; those used in America are well described in Dorée's book.[46]

The lignin content is measured by determination of the residue after solution and hydrolysis of celluloses and hemicelluloses with 87% H_2SO_4 (see Chap. F, sect. II 1*a*). Parallel to determination of the lignin content, and even under certain conditions proportional to it, is the grading as determined by the consumption of oxidizing agents such as permanganate (Johnsen-Noll[47]), a figure which gives a measure of the amount of chlorine required for bleaching.

The proportion of constituents soluble in dilute alkali (5%) is known as the wood gum figure. The greater part of the less readily soluble hemicelluloses is removed by 17.5% sodium hydroxide. The fraction insoluble in 17.5% sodium hydroxide is known as α-cellulose; it is of importance for the yield in the rayon industry. The pentosan content is obtained, according to Tollens, as furfural by distillation with 12% hydrochloric acid.

Two methods are available to estimate the extent of degradation: (*1*) determination of the *copper number* (the weight of cupric oxide precipitated

[43] See, for example, R. Runkel, *Papier-Fabr.*, **31**, 75 (1933).

[44] R. E. Dörr, *Z. angew. Chem.*, **53**, 13, 292 (1940).

[45] See the *Merkblätter der Faserstoff-Analysen-Kommission des Vereins der Zellstoff und Papier-Chemiker und Ingenieure.*

[46] C. Dorée, *Methods of Cellulose Chemistry.* Van Nostrand, New York, 1933.

[47] A. Noll, *Papier-Fabr.*, **31**, 581 (1933).

after boiling with Fehling's solution under standard conditions), and (*2*) measurement of the viscosity. The first method gives a figure for the terminal aldehyde groups and should therefore be inversely proportional to the mean (number average) molecular weight. It is, however, not a reliable index of the number of end groups because reducing aldehyde radicals may arise in acid solution by oxidation of the CH_2OH group on carbon atom 6; it is therefore only used as an empirical index of quality for technical products. The second method gives approximately the weight average.

In Table II are given the compositions of some refined pulps prepared from beech and spruce in 1940, and for comparison that of a typical rayon pulp prepared in 1930.

TABLE II

Composition of Rayon Pulps[48]

	Spruce sulfite		Beech sulfite			Spruce sulfite (1930)
	Highly refined	Refined	Highly refined	Refined	Not refined	
α-Cellulose, %	99.2	96.4	98.5	96.1	87.2	87.6
Wood gum	2.5	1.5	4.6	2.0	5.5	4.5
Pentosan	1.0	1.3	1.6	1 6	3.9	—
Copper number	—	—	—	—	—	1.8
Resin (dichloromethane extract	0 03	0 03	0.04	0 12	0 15	0.72
Ash	0.03	0.16	0.21	0.23	0.21	0.26–0.30
Viscosity[a]	25 5	26	19	16	15	—

[a] $\eta_{rel.}$ of a 1% solution in cuprammonium.

(*c*) *Testing Degree of Degradation and Homogeneity by Means of Viscosity*

Viscosity measurements have been used for many years in the commercial testing of cellulose esters, as it is known that the viscosity falls as the extent of degradation increases. The use of viscosity measurements on material dissolved in cuprammonium solution for the characterization of cellulose materials was introduced comparatively recently.

The first systematic examination of an extensive series of industrial celluloses was made by Fikentscher[49] in the laboratories of the I. G. Farbenin-

[48] From H. Müller-Clemm, *Z. angew. Chem.*, **54**, 119 (1941).

[49] H. Fikentscher, *Cellulosechemie*, **13**, 58 (1932).

dustrie A.-G. in 1927 and 1928, and published in 1932. At the same time, Staudinger and his collaborators have also determined the viscosity of celluloses in cuprammonium solution. Since then the viscosity in cuprammonium solution has been used in science and industry as a test for the degree of degradation of cellulose preparations.

Staudinger calculated the mean degree of polymerization, D.P., from the intrinsic viscosity by multiplying by an empirical factor. In his original work he used a factor of 100, but he later used 200, and finally 300. The true values of D.P. as obtained by Gralén[50] in the ultracentrifuge are computed in Table III; the values for straw pulp and acetate rayon are interpolated from $[\eta]$ according to Gralén's results. It appears clear from the comparison of Staudinger's values with the true D.P. values that the former are not even of the right order. It seems, therefore, preferable to indicate $[\eta]$ alone and not to give incorrect numbers for the D.P. Furthermore, this corresponds with the well-tried practice of quoting the results of technological tests, *e.g.*, viscosity of lubricants or boiling points of gasolines as such, unencumbered by superfluous deductions.

TABLE III

Cellulose	$[\eta]$	M W.	D.P. (found)	D.P. (interpolated)	D.P. (Staudinger)
Flax	17	5900×10^3	36,000	—	3400
Raw cotton	10	1800	11,000	—	2000
Sulfite pump (spruce)	5.5	500	3,100	—	1100
Sulfate pulp (pine)	4 7	400	2,500	—	940
Straw pulp	4	—	—	2100	800
Acetate rayon	1 5	—	—	750	300
Rayon viscose	0.95	75	460	—	190
β-Cellulose	0.2	27	170	—	40

It is, therefore, a matter worthy of congratulations that in specifications issued by the American and German Pulp and Paper Chemists' Associations only the viscosity is required as an index of quality; in Germany the relative viscosity of a 1% solution in cuprammonium prepared in an atmosphere of nitrogen is used; in the United States, the absolute viscosity in centipoises of a similar solution is used. Both numbers agree well. It is to be wished that rayon chemists too would adopt this standard and abandon the misuse of the "degree of polymerization."

Because of the inconvenience of working under oxygen-free conditions

[50] N. Gralén, *Dissertation*, Uppsala, 1944.

the nitrate viscosity is often used as an index of quality, although for bleached pulps this may lead to errors for reasons to be described below.

One gram of pulp is nitrated for 1 hour at exactly 20°C. with 100 g. of nitrating mixture made up from 27 g. of phosphorus pentoxide, 33 g. of phosphoric acid crystals, and 40 g. of nitric acid ($d = 1.52$). It is then drained on a sintered glass filter and stabilized by boiling for four periods of 0.5 hour in water, to saponify any OPO_4H_2 groups. Water is then removed with methanol and the material is dried by drawing a stream of air through it.

In the course of careful nitration about 1 out of 3000 linkages is broken. This means that the degradation of high polymer cellulose is more important than that of a lower one. Cotton with a true D.P. of 11,000 gives by hydrolysis of 3 linkages a nitrocellulose with a D.P. of 2700 but a sulfite pulp of D.P. 3000 yields a nitrate of D.P. 1500, only one linkage being broken. In Table IV the grading numbers of various preparations have been collected.

TABLE IV

Grading Numbers

	η_{rel}		
	1% in cuprammonium	1% in acetone	$[\eta]$
Cotton	406[a]	—	7.75
Cotton (nitrated)	—	7500[a]	12.20
Gun cotton linters	9.75	—	2.63
Nitrated linters	—	24.6	3.76
Sulfite pulp	28.8	—	3.98
Viscose rayon	4.32	—	1.64

[a] Measured in the Ubbelohde viscometer No. 5 at 50 cm. pressure of H_2O.

Viscometric determinations of the chain length of cellulose in cuprammonium solution or of the corresponding nitrate in organic solvents often show poor agreement with each other, the values given by the latter method being too high. Davidson[51] was the first to notice the discrepancy between the viscosity of bleached cellulose determined in cuprammonium solution or as the nitrate, and he also provided the explanation: oxidation causes points of weakness in the chains which survive the procedure of nitration but are broken by alkali or cuprammonium (see Sect. V2*c*).

Viscosity measurements can also be used to investigate the relative proportion of long and short chains, *i.e.*, the homogeneity (Polydispersity, poly-

[51] G. F. Davidson, *J. Textile Inst.*, 29, T195 (1938).

molecularity), a knowledge of which is essential before the worth of a sample can be assessed. For this the cellulose is best nitrated according to the methods described above and then dissolved in 50 to 60 parts of acetone according to its viscosity, filtered, and fractionally precipitated by the addition of ligroin drop by drop.[52] The last fraction is best recovered by distilling off the solvent to a small volume and pouring the remaining fluid into water. As an example we may consider three fractions obtained from a typical viscose rayon; in 1% solution the fractions had relative viscosities of 29, 20, and 15. Straus and Levy[53] recommend fractional precipitation of the cellulose from solution in cupric oxide in dilute ethylenediamine by sulfuric acid.

Differential solubility in caustic soda at various concentrations and temperatures[54] can also be used for fractionation. It has, however, been shown that with this method the amount going into solution under any conditions depends not only upon the chain length, but also upon the micellar fine structure of the material; with fractional precipitation of the nitrate, on the other hand, partition according to chain lengths is obscured by the carrying down of fractions of lower molecular weight. With either method local differences in temperature and concentration make it hard to obtain reproducible results. A really sharp analysis of cellulose material according to chain length is not in fact possible by any of the methods so far described, which are only useful in giving an approximate idea of the proportional content of the lower molecular weight fractions; in rayon pulps this should be as low as possible.

In the viscose rayon industry the viscosity of cellulose which has been converted into xanthate is determined in accordance with the practical conditions prevailing in the manufacture of viscose. There is no simple relationship between viscosity in cuprammonium solution and viscosity as xanthate, particularly as various cellulose products undergo a very characteristic fall in viscosity during ripening (see sect. III2). If, therefore, it is required to know the viscosity of the viscose solution obtainable from given cellulose material, there is no alternative to the long and tiresome determination of the viscosity as xanthate.

[52] R. E. Dörr, *Z. angew. Chem.*, **53**, 13 (1940). H. Staudinger and J. Jurisch, *Melliand Textilber.*, **20**, 693 (1939). H. Dolmetsch and F. Reinecke, *Zellwolle u. Deut. Kunstseiden-Ztg.*, **5**, 219 (1939).

[53] F. L. Straus and R. M. Levy, *Paper Trade J.*, No. 3, **114**, 31 (1942); **114**, No. 18, 33 (1942).

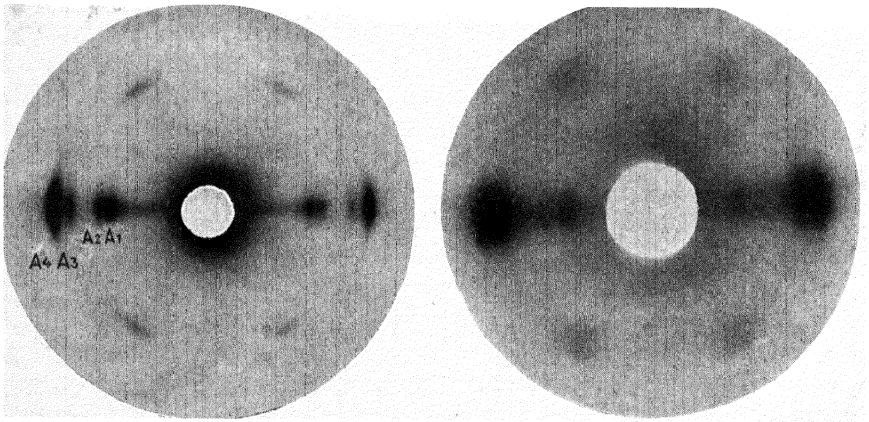
[54] H. Rath and H. Dolmetsch, *Klepzig's Textil-Z.*, **41**, 475 (1938). W. Schieber, *Z. angew. Chem.*, **52**, 561 (1939). O. Eisenhut, *Cellulosechemie*, **19**, 45 (1941).

3. Crystalline Structure of Cellulose

In view of the biological importance of cellulose, as well as of the fact that the elucidation of its structure and texture may serve as a pattern for the investigation of other substances, this branch of our subject will be dealt with in some detail. Since the lattice of native cellulose differs from that of mercerized or regenerated cellulose it is convenient to denote the native form "cellulose I."

(*a*) *Unit Cell and Space Group*

The first experiments on the diffraction of x-rays by cellulose were made by Nishikawa and Ono[55] in 1913 but escaped the notice of other workers in this field. These authors concluded from their diagrams that small crystallites were present, oriented parallel to the fiber axis. Scherrer[56] began the



(*a*) (*b*)

Fig. 1. Fiber diagrams of ramie: (*a*) native, cellulose I; (*b*) after mercerization, cellulose II.

x-ray examination of cellulose in 1918, and in 1920 obtained a fiber diagram. In 1919 Herzog and Jancke[57] began their investigation of cellulose by means of x-ray analysis, and this was continued for many years. Polanyi[58] undertook the evaluation of the Herzog-Jancke diagrams and published the following result as early as 1921: unit cell rhombic, $a = 8.6$ Å., $b = 10.3$ Å.

[55] S. Nishikawa and S. Ono, *Proc. Phys. Math. Soc. Japan*, Sept. 20, 1913.
[56] P. Scherrer, in R. Zsigmondy, *Kolloidchemie.* 3rd ed., Spamer, Leipzig, 1920.
[57] R. O. Herzog and W. Jancke, *Z. Physik*, **3**, 196 (1920); *Ber.*, **53**, 2162 (1920).
[58] M. Polanyi, *Naturwissenschaften*, **9**, 288 (1921).

(fiber period), $c = 7.8$ Å. Later (1926) Sponsler[59] attacked the same problem; he decided on an orthorhombic unit cell with the dimensions 10.3, 6.10, and 5.4 Å., and concluded, from the symmetry of the cell, that any two adjacent glucose residues in the chain are oriented in opposite senses with reference to the b axis. Polanyi[60] pointed out, however, that these conclusions are not reconcilable with the optical asymmetry of glucose residues. A series of detailed investigations by Mark, Hengstenberg, and von Susich,[61] made from 1927 onward, led them to suggest the monoclinic unit cell, which was confirmed by the painstaking work of Gross and Clark[62] and is still accepted.

TABLE V

Equatorial Reflections of Native Cellulose (Cellulose I)

Reflection	Glancing angle	Net plane spacing, Å.	Index (*hkl*)
A_1.....	7.3°	6.05	(101)
A_2.....	8.1°	5.45	(10$\bar{1}$)
A_4.....	11.3°	3.92	(002)
A_5.....	13.0°	3.42	(10$\bar{2}$)
A_6.....	16.5–17.7°	2.71–2.53	(20$\bar{2}$), (301), (103), (30$\bar{1}$)
A_7.....	18.5–18.8°	2.42–2.39	(10$\bar{3}$), (302)
A_8.....	20.9–21.3°	2.155–2.12	(30$\bar{2}$), (20$\bar{3}$)
A_9..... ...	23.1°	1.96	(004)

We shall now consider the data in greater detail. Figure 1*a* shows a fiber diagram of cellulose; from this diagram the identity period along the fiber axis may be determined as 10.3 ± 0.1 Å. The possible existence of reflections indicating greater periods along the fiber axis has recently been investigated with great care by Clark and Parker[63]; in native cellulose they find no trace of such reflections. Reflections arising from the crystal lattice certainly do not occur between A_1 and the primary beam. Table V gives the equatorial reflections and their indices.

The indexing has been confirmed by photographs made from more highly oriented preparations. According to Mark and von Susich,[64] highly

[59] O. L. Sponsler, *J. Gen. Physiol.*, **9**, 221, 677 (1926); *Nature* **120**, 767 (1927).

[60] N. Polanyi, *Naturwissenschaften*, **16**, 263 (1927).

[61] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930, page 94, *et seq.*; *Z. physik. Chem.*, **B2**, 115 (1929).

[62] S. T. Gross and G. L. Clark, *Z. Krist.*, **99**, 357 (1938).

[63] G- L. Clark and E. A. Parker, *Science*, **85**, 203 (1937).

[64] H. Mark and G. von Susich, *Z. physik. Chem.*, **B4**, 431 (1929).

oriented preparations of bacterial cellulose or of tunicin may be obtained by stretching thin films. The cell wall of the alga *Valonia ventricosa* contains cellulose in an almost perfectly oriented condition, and Sponsler[65] obtained the photograph reproduced in figure 2 (see below) by irradiating a block of regularly superimposed fragments of the cell wall in the direction of the *b* axis (the chain axis). The points marked *T*, *R*, and *D* correspond to A_1, A_2, and A_4. It is immediately obvious that A_1 and A_2 arise from planes standing practically at right angles to each other, while A_4 arises from planes diagonal to these.

The unit cell has the following dimensions:

$$a = 8.35 \text{ Å.} \quad b = 10.3 \text{ Å. (fiber period)} \quad c = 7.9 \text{ Å.} \quad \beta = 84°$$



Fig. 2. X-ray diagram obtained from the cell wall of *Valonia ventricosa* showing higher orientation. Beam parallel to the fiber axis (Sponsler).

From these data and from the specific gravity of cellulose (1.57) the number of glucose residues in the unit cell, *Z*, is found to be 4. Of the possible monoclinic crystal classes all but P2 are excluded, since cellulose displays optical activity. Of the three space groups of the crystal class P2 the most probable is $P2_1$. Two independent sets of digonal screw axes occur as symmetry elements in this space group; the reflections (0*k*0) are extinguished when *k* is odd. Hengstenberg[66] and later Kiessig[67] found the reflection (030); Meyer and Misch[68] and van der Wyk and Meyer[69] showed that this reflection is much weaker than (020), which in turn is much weaker than (040), while (040) is much fainter than (002).

It is therefore justifiable to accept the space group $P2_1$ as a good approximation to the symmetry relationships in cellulose although the prohibited reflections, (030) and (010)—the latter even fainter than (030)—appear. The cellulose fiber is not a perfect crystal, and any distortion of the lattice—for example, a bending of the chains—may lead to the appearance of prohibited reflections.

[65] O. L. Sponsler, *Protoplasma*, **12**, 241 (1931).
[66] W. Hengstenberg, in K. H. Meyer and H. Mark, *Z. physik. Chem.*, **B2**, 115 (1929).
[67] H. Kiessig, *Z. physik. Chem.*, **B43**, 79 (1939).
[68] K. H. Meyer and L. Misch, *Helv. Chim. Acta*, **20**, 234 (1937).
[69] K. H. Meyer, and A. J. A. van der Wyk, *J. Polymer Sci.*, **1**, 46 (1946).

(*b*) *Three-Dimensional Model of Cellulose Chain*

It is impossible in practice to determine the position of the atoms in cellulose from the symmetry of the unit cell and the intensities of the spots alone because there are so many atoms in the cell (with approximately the same scattering power); the intensity of the reflections cannot be determined with sufficient accuracy due to the coincidence of reflections. We must, therefore, take advantage of existing data on interatomic distances

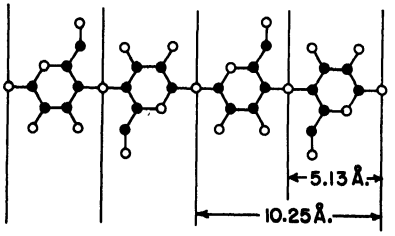


Fig. 3. Three-dimensional model of the cellulose chain (Sponsler and Dore).

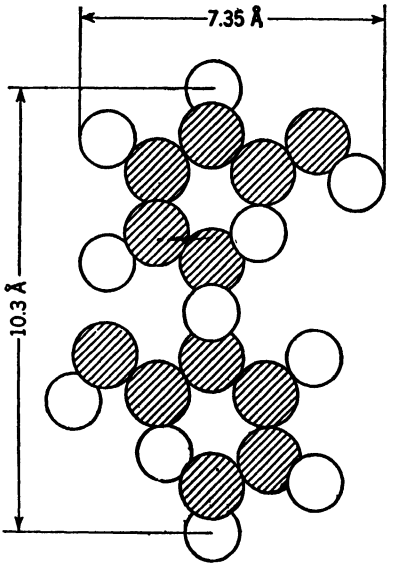


Fig. 4. Three-dimensional model of a cellobiose residue.

and valence angles in carbon compounds, in order to construct a model of the crystalline structure. The first suggestion is due to Sponsler and Dore[70] and was based on their own previously mentioned x-ray data.

Figure 3 shows their chain model and the glucose formula upon which it is based, in which the residues are linked together alternately by 1,1-glucose linkages or 4,4-ether linkages. But this model is inconsistent with chemical facts and must be replaced by one based on a chain of cellobiose units.

The identity period along the fiber axis is 10.3 Å. If we now assume that the cellobiose chains are arranged parallel to this direction and that two

[70] O. L. Sponsler and W. H. Dore, *Colloid Symposium Monograph*, **4**, 174 (1926).

residues occupy the fiber period, it follows that the chain must be very nearly straight; only a slight inclination of the rings with respect to the fiber axis is possible. Taking into consideration the values for interatomic distances and valence angles, we obtain the model shown in figure 4. In this, each ring of the chain is rotated through 180° with respect to its neighbors. The lateral oxygen atoms 2 and 3 possess a slight possibility of movement in a direction at right angles to the plane of the paper while the position of oxygen atom 5 (attached to carbon atom 6) is indeterminate, as there is no reason to suppose any considerable limitation of free rotation about the linkage between carbon atoms 5 and 6.

Chains which are not associated to form crystallites need not retain the extended form shown here, for, although the framework of the glucose rings must be assumed to be fairly rigid, rotation or vibration about the oxygen bridges between carbon atoms 1 and 4 seems to be possible. It may, therefore, be assumed that all possible twisted configurations occur in chains in solution.

(c) *Crystalline Structure of Native Cellulose (Cellulose I)*

The chains must now be arranged in the unit cell in such a way that the distance between the atoms of neighboring chains agrees with our knowledge

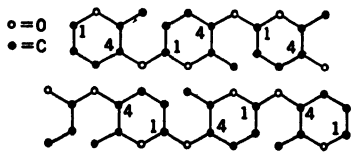


Fig. 5. Three-dimensional model of cellulose (Haworth).

of what such spacing ought to be, and is consistent with the observed intensities of the reflections in the x-ray diffraction pattern. In addition, the three-dimensional structure must be in agreement with other properties of cellulose: tensile strength, elasticity, birefringence, etc.

Sponsler and Dore proposed an arrangement based on their hypothetical chain molecule; a lattice built up from primary valence chains which fulfilled the mechanical requirements, but did not agree with either the chemical properties or with the x-ray data. This model was rejected by Haworth[71] on chemical grounds. He proposed another three-dimensional model shown in figure 5. Haworth's model assumed that the incomplete

[71] W. N. Haworth, *Helv. Chim. Acta*, 11, 534 (1928).

hexagonal rings between the closed hexagonal rings of the glucose residues might have the same effect on the x-ray beam as the glucose residue itself. This suggestion, however, was irreconcilable even at that time with the state of knowledge of the smallest possible distance between carbon atoms not united with one another. The distance between the carbon atoms 6 and 2 of the neighboring rings, which can never be less than 3.0 Å., is assumed in Haworth's model to be 1.5 Å. Haworth subsequently dropped this suggestion.

In 1928 Meyer and Mark[72] postulated a monoclinic unit cell and space group $P2_1$ in which digonal screw axes are present. The arrangement of tension-free cellobiose chains along the *b* axis implies a digonal screwing of the chains; Meyer and Mark therefore placed the cellobiose chains of their model along the screw axes as in figure 6.

As the two sets of digonal screw axes, which traverse the unit cell in the space group $P2_1$ are independent of each other, several arrangements of the cellulose chains are possible: (*a*) the two sets of chains run in the same direction; (*b*) one set runs in the opposite direction to the other; (*c*) chains run in either direction at random in every crystallite; and (*d*) the crystallites consist of bundles of fibers running the same direction and each fiber contains an equal number of crystallites running in either direction. Meyer and Mark based the continuation of their argument on hypothesis (*a*), which appeared to accord best with the known mode of growth in the plant.

In the light of the following facts, which were not all known at the time, Meyer[73] was led to abandon hypothesis (*a*) in favor of hypothesis (*b*). Cellulose II formed directly from ramie fibers by treatment with sodium hydroxide possesses the same crystal lattice as Cellulose II precipitated from solution. On statistical grounds it appears probable that in precipitated or spun fibers equal numbers of chains will be directed in either direction, from which it follows that the crystallite of Cellulose II contains two sets of chains running in opposite directions. Cellulose II may be formed from native cellulose without disturbing its external form, and as it is inconceivable that a complete reversal of one set of chains takes place in this process, it must be supposed that the same arrangement of the chains occurs in native cellulose (Fig. 6).

The next alternative is that chains running in one direction alternate regularly with those running in the other. We must then arrange two chains directed in opposite senses in such a way that the calculated intensities

[72] K. H. Meyer and H. Mark, *Ber.*, **61**, 593 (1928); *Z. physik. Chem.*, **B2**, 115 (1929).
[73] K. H. Meyer, *Ber.*, **70**, 266 (1937).

agree with the observed intensities of the spots of the diagrams. The very high intensity of (002) indicates that this plane is densely populated, that is, one may assume that (002) coincides with the plane of the rings of the chains. This determines the structural model (with the exception of a slight mobility of oxygen atoms 2 and 3), but for the displacement of the two sets of chains relative to each other and the position of oxygen atoms V (Fig. 8).

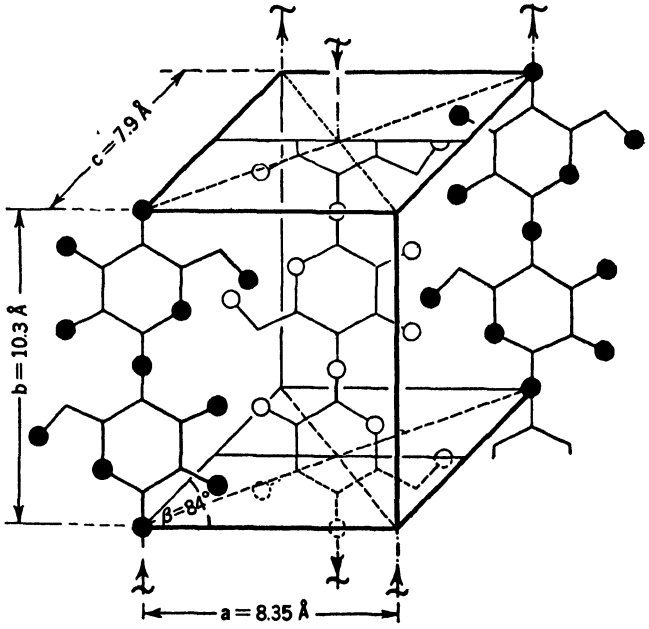


Fig. 6. Diagrammatic representation of the unit cell of native cellulose (Meyer and Misch).

Attempts to determine the position of the atoms based solely on models, for example, Stuart's hemisphere model (*e.g.*, Hermans[74]) are of no value and must be regarded as a step in the wrong direction. Not only must the spacing of the atoms obey the usual rules governing interatomic distances, but it must be consistent with the physical properties of the material. Of these, the most important is its behavior under x-rays; whether or not a suggested structure agrees with theory can only be verified by measurements of the intensity. It is not possible to deduce the polymorphic character of cellulose solely from a consideration of wooden models, nor can one invent models for each of the different modifications, which must however differ in structure, because they behave differently with x-rays. Arbitrary modifications of the crystal structure proposed by Meyer and Misch are valueless if they cannot be justified by intensity measurements.

[74] P. H. Hermans, *Kolloid-Z.*, **102**, 169 (1943).

Misch[75] has made calculations for a number of different arrangements and finds good agreement of the calculated with the observed intensities for the structure shown in Figures 7 and 8.

Not only the regular but also a statistical arrangement of the chains running in opposite directions is possible; the calculation of the intensities for the statistical model is, however, practically impossible. For a defini-

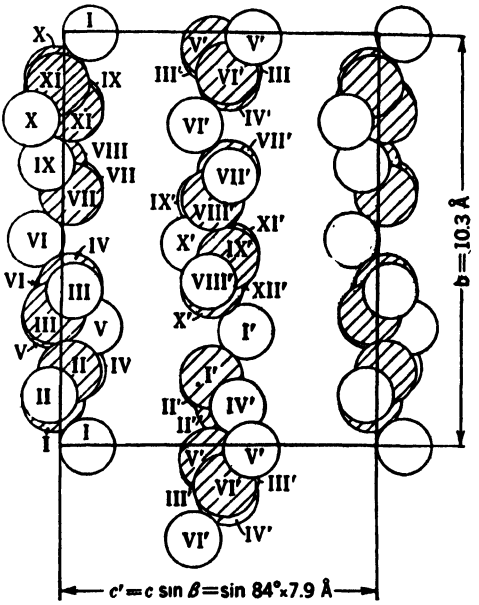


Fig. 7. Projection of the unit cell viewed along a on the plane $bc' \perp a$.

tive calculation of the atomic arrangement by the Fourier method (Chap. A, sect. II 2*g*) the number of the observed interferences in relation to the number of atoms is not nearly sufficient. The methods at present available do not permit further progress.

Along the fiber axis then, we have primary valence linkages of the cellobiose type. In the direction of the a axis the primary valence chains are connected to one another to form a plane network by means of strong secondary valences, of which the most important are hydrogen bonds between hydroxyl groups V and II. These networks lying in planes parallel to a and b hang together in sheets by means of secondary valences.

[75] K. H. Meyer and L. Misch, *Helv. Chim. Acta*, **20**, 232 (1937).

The great difference between the types of linkage along and at right angles to the fiber axis also shows itself in a number of other properties. Hengstenberg[76] has shown, by x-ray analysis, that thermal expansion is much less in the direction of the fiber axis than at right angles to it. The modulus of elasticity (Meyer and Lotmar[77]), the optical properties (rotation and birefringence), and finally the topochemical mode of reaction

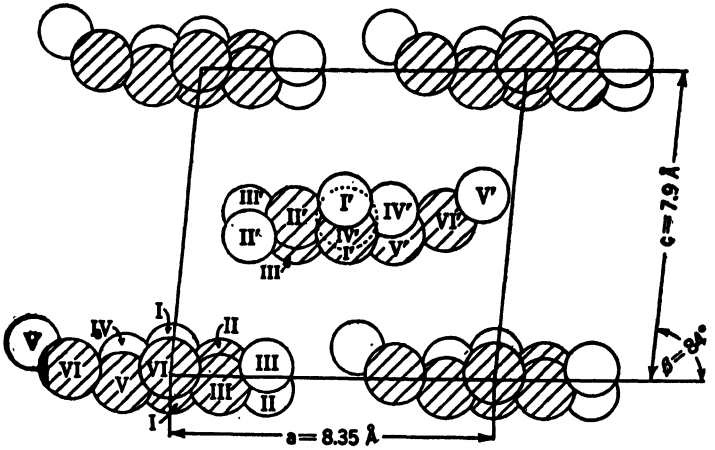


Fig. 8. Projection of the unit cell viewed along b on the place $ac \perp b$.

(absorption of reagents between the chains) all support the assumption that the model corresponds with reality.

(*d*) *Polymorphy of Cellulose*

The form of cellulose described above has been found in all native vegetable celluloses and also in the tunicin of the tunicates. The definition of the diagram may be obscured by accompanying materials, but if these materials are removed the same diagram is always obtained.

It is, therefore, all the more remarkable that cellulose as precipiated from solution or regenerated from compounds has not yet been obtained in the native form. Such cellulose may possess a number of different molecular arrangements of which three: cellulose II, cellulose III, and the so-called cellulose T, are crystalline and plainly distinguishable from one another. There are also forms in which the lattice is grossly distorted, as

[76] See K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*. Akadem. Verlagsgesellschaft, Leipzig, 1930, page 97.

[77] K. H. Meyer and W. Lotmar, *Helv. Chim. Acta*, 19, 68 (1938).

well as intermediates between the different forms, so that it is scarcely justifiable to regard all of these forms as definite allotropic modifications of cellulose. If cellulose is precipitated from solution or regenerated from some addition compound of native cellulose, for example, soda cellulose or Knecht's compound, the chains do not assemble themselves into the lattice characteristic of native cellulose but into that of an allotropic modification which has been given the unfortunate name of hydrated cellulose. This form is, however, not a hydrate but has the same composition as cellulose I and should therefore rather be denoted cellulose II. All artificial silks, plastic sheets, and moldings made from cellulose, as well as mercerized cottons are hydrated celluloses in this sense. The diagram of a well-oriented cellulose II is shown in figure 1b.

The unit cell of this modification of cellulose is monoclinic[78] with axes:

$$a = 8.14 \text{ Å.}$$
$$b = 10.30 \text{ Å.}$$
$$c = 9.14 \text{ Å.}$$
$$\beta = 62°$$

The space group is the same as that of native cellulose. It may, therefore, be assumed that here too the cellobiose chains are arranged along the digonal screw axes. The relative intensity of $(10\bar{1})$ is greater than in native cellulose, which suggests that the planes of the rings are twisted out of the plane *ab*. A comparison of the projection of the unit cell of hydrated cellulose on the *ac* plane with that of native cellulose shows that the sets of chains are displaced relative to one another along *a*. As a consequence of the rotation of the glucose ring out of the plane *ab* in the diagonal plane $(10\bar{1})$ (*cf.* Fig. 9), the coherence of the layer in *ab* appears to be diminished; hence new planes appear whose primary valence chains are probably connected to one another by hydrogen bonds in the $(10\bar{1})$ plane. As Kratky[79] has observed there are also a number of other properties which suggest a layer structure parallel to $(10\bar{1})$; these layers come to lie in the plane of the film when a sheet is stretched or calendered.

Hydrated cellulose is converted, by heating with glycerine or water under pressure at a temperature between 140° and 300°, to a modification so similar to native cellulose that it was in fact originally mistaken for it. This was first demonstrated by Meyer and Badenhuizen[80] and confirmed soon afterward by Kubo and Kanamaru. According to Hutino and

[78] K. Andress, *Z. physik. Chem.*, **B4**, 190 (1929). A. Burgeni and O. Kratky, *ibid.*, **B4**, 401 (1929). K. Weissenberg, *Naturwissenschaften*, **17**, 181 (1929).

[79] B. Baule, O. Kratky, and R. Treer, *Z. physik. Chem.*, **B50**, 255 (1941).

[80] K. H. Meyer and N. P. Badenhuizen, *Nature*, **140**, 281 (1937). See also T. Kubo and K. Kanamaru, *Z. physik. Chem.*, **A182**, 341 (1938).

Sakurada,[81] Kubo,[82] and Hess and Kiessig[83] this modification has a rhombic (almost tetragonal) lattice, with $a = 8.11$ Å., $b = 10.3$ Å., and $c = 7.9$ Å.; Hess has given it the name cellulose T. According to Kubo the arrangement of the chains is similar to that of native cellulose, except that the groups of chains are slightly displaced relative to one another along the *b* axis.

Kubo claimed that this variety of cellulose occurred in many plants, such as coltsfoot, agave, rice straw, beech, etc., and that naturally occurring cellulose could be divided into two kinds: the ramie and the coltsfoot types. Hess and Kiessig have since shown, however, that the diagram of the coltsfoot variety is not really identical with that of cellulose T, but can be interpreted as the diagram of an impure ramie fiber. The true ramie diagram can be obtained after the removal of impurities.

Cellulose T may also be formed together with hydrated cellulose by the decomposition of alkali cellulose with hot water. In the cold only cellulose II is formed, from which it appears that this cellulose is the first product, but that it is unstable and at 100° is transformed into the stable form as soon as it arises; this transformation does not take place when cellulose II is formed at lower temperatures. The formation of cellulose II as the first product of decomposition is explicable in view of the arrangement of the chains and substituents in derivatives, as for example in alkali cellulose I and Knecht's compound.

Insofar as the lattices of these compounds have been investigated, they show that the reagent penetrates between the chains at the points marked with circles in figure 9, and that, as a result, the chains are separated from each other in the *a* direction. At the same time, the planes of the rings are twisted out of the *ab* plane and the sets of chains are displaced relative to each other along *a*. The arrangement of cellulose II is, therefore, preformed in the derivatives. If the reagent is now carefully removed, the chains will approach each other again and the spaces become smaller, but the displacement and rotation will remain. This interpretation is supported by the observations on the destruction of ammonia cellulose I, the diagram of which, according to Hess and Gundermann,[84] indicates an arrangement in which the rings are markedly twisted out of the *ab* plane. If the compound is carefully decomposed, a cellulose is obtained in which this displacement remains. This cellulose, described as cellulose III by Hess

[81] K. Hutino and I. Sakurada, *Naturwissenschaften*, **28**, 577 (1940).
[82] T. Kubo, *Z. physik. Chem.*, **A187**, 297 (1940).
[83] K. Hess and H. Kiessig, *Z. physik. Chem.*, **B49**, 235 (1941).
[84] K. Hess and J. Gundermann, *Ber.*, **70**, 1788 (1937).

and Gundermann, forms cellulose T on heating with water to 200°C. Cellulose precipitated from solution without any mechanical treatment, for

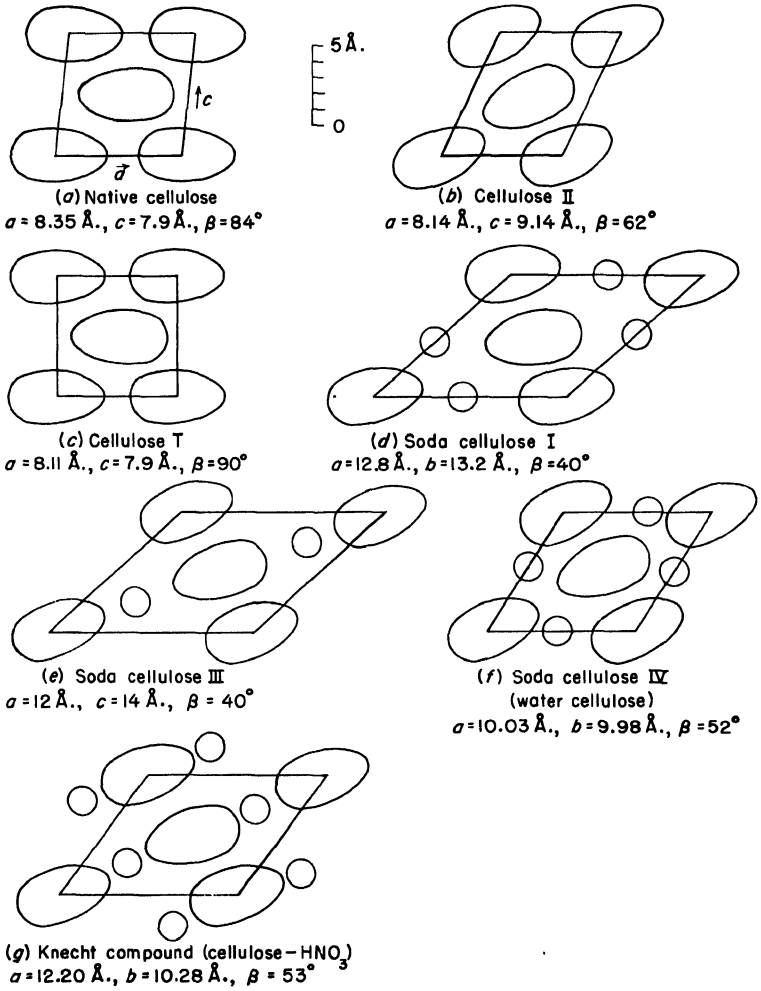


Fig. 9. Schematic projections of cellulose chains on the plane $ac \perp b$. The glucose rings are shown as ovals, molecules of reagent as circles.

example, when ammonia is allowed to evaporate from ammonia cellulose II, or when nitrocellulose is denitrated, is amorphous. As Hess[85] has

[85] K. Hess, E. Steurer, and H. Fromm, *Kolloid-Z.*, **98**, 148 (1942).

lately shown, intensive mechanical disintegration (beating) has the same results. Such amorphous fibers preserve some traces of orderly arrangement in that their chains still lie parallel, and by the action of hot water they can be transformed into crystalline fibers of cellulose II without disturbance of their external shape. In this process all possible intermediates occur, so that it is not possible to distinguish sharply between amorphous and crystalline fibers. Even in native fibers the degree of orientation of contiguous regions may vary very considerably, so that it would be rash to assume the existence of sharply differentiated "crystalline" and "amorphous" elements.

It is therefore not justified to express the degree of order in percentage of crystalline substance as used by Hermans.[86] Numbers such as he gives, based either on the density of fibers or on the half-width of the principal equatorial X-ray interferences, can only be considered as empirical data related to the degree of crystalline order. His value of 69% crystalline substance in ramie, for example, has no basis in fact.

4. Size, Shape, and Arrangement of Micelles

(*a*) *Micellar Theory*

That cellulose fibers are composed of elongated submicroscopic structures, crystalline in character, was first recognized by Nägeli, who introduced the term *micelle* for such units (from *mica*, a crumb). He had already made use of this term to describe crystalline, submicroscopic, elongated particles in starch grains, whose existence he inferred from the study of their birefringence.

Ambronn[87] subsequently elaborated and extended the micellar theory by a long series of investigations. Separating the brefringence into two components—form birefringence, which provides information about the shape and arrangement of the particles, and intrinsic birefringence (*Eigendoppelbrechung*), which indicates internal anisotropy and (as was then supposed) the crystalline character of the particles—he was able to demonstrate the presence of elongated, submicroscopic "crystalline" particles in cellulose, stretched nitrocellulose, and gelatin. By the same method the presence of "crystalline" micelles in chitin (Möhring[88]), muscle fibers (Stübel)[89], and

[86] P. H. Hermans and A. Weidinger, *J. Applied Phys.*, **19**, 491 (1948). *J. Polymer Sci.*, **4**, 135, 317 (1949).

[87] H. Ambronn, *Kolloid-Z.*, **18**, 90, 273 (1916); **20**, 173 (1917). H. Ambronn and A. Frey, *Das Polarisationsmikroskop*, Akadem. Verlagsgesellschaft, Leipzig, 1926.

[88] A. Möhring, *Kolloidchem. Beihefte*, **23**, 162 (1926). *Dissertation*, Jena, 1922.

[89] H. Stübel, *Arch. ges. Physiol. Pflügers*, **201**, 629 (1923).

plant cell walls (Frey[90]) was demonstrated. At Ambronn's suggestion Scherrer[91] made an x-ray photograph of cellulose fibers, and thus confirmed the internal crystalline arrangement predicted by Nägeli and Ambronn.

The concept of the micelle was originally put forward for structures in the solid state. Except as applied to solutions the term now denotes submicroscopic, crystalline, supermolecular units, that is, particles built up from a more or less complete crystal lattice. In speaking of chain molecules it comes to mean bundles of parallel chains.

Noncrystalline particles in solution, consisting of several molecules, are often spoken of as micelles and a solution containing such particles is known as a micellar solution. Since the concept of crystalline micelles refers only to the solid state, while that of noncrystalline micelles applies to dissolved substances, the possibility of confusion is slight.

(*b*) *Micelles or Fibrillae of Native Cellulose*

As explained in Chap. A, sect. II 3*e*, the dimensions of the crystalline regions of a polycrystalline object can be determined by measuring the width of the x-ray interference spots. It appears, however, from the explanation on page 76, that cellulose fibers cannot be treated as a mosaic of small crystals with definite intercrystalline boundaries such as occurs in a metal wire. All observers agree about this. Sponsler and Dore[92] deny entirely the existence of discrete crystalline particles and instead assume a certain degree of orientation of all chains in the fiber, which leads to the appearance of interferences. Meyer and Mark[93] subscribed to Nägeli's micellar hypothesis according to which the fiber consists of micelles built up of bundles of primary valence chains; in the inside of the micelle the chains are well oriented, whereas at the edges there is a gradual transformation to the disorderly condition characteristic of the amorphous interstitial material, so that the individual chains may be longer than the crystalline regions.

In later papers by Frey-Wyssling[94] and Kratky[95] the assumption is made that primary valence chains may take part in more than one crystalline

[90] A. Frey, *Jahrb. wiss. Botan.*, **65**, 195 (1926).

[91] P. Scherrer in R. Zsigmondy, *Kolloidchemie.* 3rd Ed., Leipzig, 1920.

[92] O. L. Sponsler and W. H. Dore, *Colloid Symposium Monograph*, **4**, 174 (1926).

[93] K. H. Meyer and H. Mark, *Ber.*, **61**, 593 (1928).

[94] A. Frey-Wyssling, *Protoplasma*, **25**, 261 (1936).

[95] O. Kratky, *Z. Papier, Pappe, Zellulose, Holzstoff*, **56**, 149 (1938); *Kolloid-Z.*, **70**, 14 (1935).

region. Herzog[96] and, later, Hengstenberg,[97] have estimated the dimensions of the crystalline regions from the width of the x-ray interferences. Both authors arrive at figures of about 60–100 Å. for the thickness of the region in which a perfectly regular arrangement prevails (the corresponding interference points are considerably extended) and at least 600 Å for the length (the horizontal interferences are scarcely extended at all). Since we are not in this instance dealing with a truly polycrystalline material however, these figures can only be taken as indicating the order of magnitude.

There are many observations to show that these crystalline, fibrillar micelles are no mere geometrical abstractions but represent the basic units of both chemical and morphological structure, so that it is permissible to speak of them as submicroscopic fibrillae.

In the first place, cellulose has a very great internal surface, as is shown by its powers of absorption. When liquid water is taken up, the system suffers a diminution of volume accompanied by evolution of heat and decrease of entropy (Stamm and Loughborough[98]). The water is apparently adsorbed at particular points, presumably on glucose residues whose hydroxyl groups are not saturated by the formation of hydrogen bonds with their neighbors. Native cellulose free from impurities takes up less water than regenerated cellulose, in which the internal surface is greater because the fibrillae are thinner. These conclusions have been confirmed by an independent method: deposition of gold in the intermicellar spaces followed by x-ray investigation of the products (Kratky and Schossberger[99]).

Cellulose fibers may be split up by a micromanipulator into fibrils of 1.4 μ diameter (Farr[99a]), these in turn are composed of ultrafibrils 0.1–0.3 μ thick which are themselves bundles of micelles. It may therefore be assumed that the micelles also have the form of long slender threads.

The crystalline fibrillae of cellulose are not, therefore, entirely comparable with the crystallites in rubber, which cannot be truly said to possess a "surface" at all, as the crystalline regions pass into the amorphous without any definite boundary.

The fibrillae are not circular in cross section, but are thicker in a direction parallel to the net plane (101) than in a direction at right angles. They

[96] R. O. Herzog, *J. Phys. Chem.*, **30**, 457 (1926).

[97] J. Hengstenberg and H. Mark, *Z. Krist.*, **69**, 271 (1928). H. Mark and K. H. Meyer, *Z. physik. Chem.*, **B2**, 115 (1929). K. Hess, C. Trogus, L. Akim, and I. Sakurada, *Ber.*, **64**, 408 (1931).

[98] A. J. Stamm and W. K. Loughborough, *J. Phys. Chem.*, **39**, 121 (1935).

[99] O. Kratky and F. Schossberger, *Z. physik. Chem.*, **B39**, 145 (1938).

[99a] W. Farr, *Contrib. Boyce Thompson Inst.*, **6**, 189 (1934).

can be oriented by pressure; the net plane (101) then comes to lie parallel to the plane of the film (Kratky and Mark[100]) (see also figure 12).

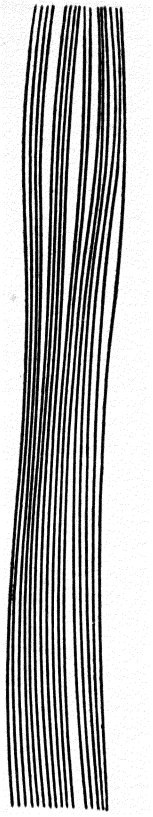


Fig. 10. Schematic diagram of a cellulose micelle (Meyer).

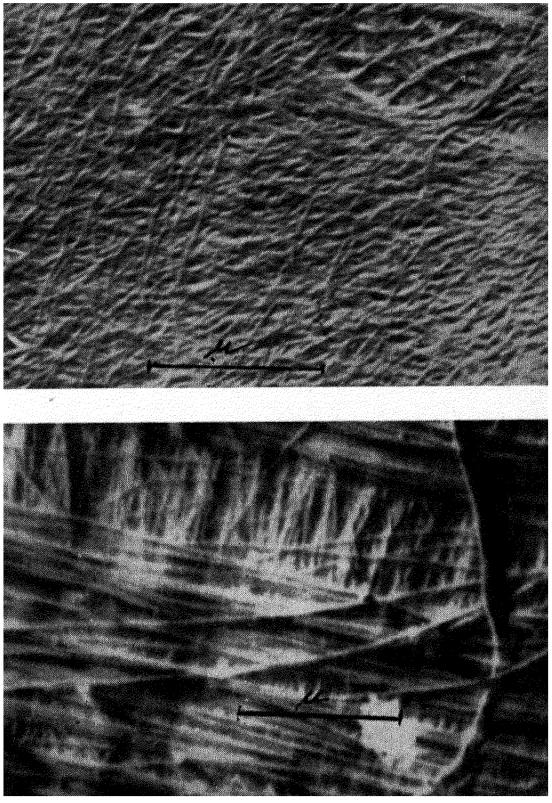


Fig. 11. *Top*, Gold-shadowed electron micrograph of the cell wall (primary wall) of *Avena* coleoptile, ×24000 (Mühlethaler). *Bottom*, Cell wall (secondary wall) of *Valonia*, ×22000 (Preston *et al.*).

There are no reasons to suppose that the crystalline structure of a fibrilla is interrupted by amorphous regions, or that the crystallite contains free ends of chains. The crystalline arrangement is comparable to that of an

[100] O. Kratky and H. Mark, *Z. physik. Chem.*, **B36**, 129 (1937). B. Baule, O. Kratky, and R. Treer, *Z. physik. Chem.*, **B50**, 255 (1941).

asbestos fiber, in which deformation gives rise to a gradual, continuous deformation of the lattice (Fig. 10). Imperfections of the diagram may be explained by local strains or flaws; it is unlikely that there are any truly amorphous regions or independent chains which are not incorporated in the lattice, although there may be considerable variation in the perfection of the lattice as between fibers of different origin.

These conclusions obtained indirectly[101] seemed at first to be disproved by electron micrographs. Husemann[102] and Frey-Wyssling[103] obtained, by mechanical disintegration of cellulose fibers, fibrils of varying diameter which they considered to be artefacts. But later, gold-shadowed electron micrographs of thin intact cell walls of *Valonia* (Preston)[104] (Fig. 11) and of *Avena* coleoptiles (Frey-Wyssling, Mühlethaler, and Wyckoff)[104a] (Fig. 11*b*) clearly showed the existence of individual fibrils of uniform width of 250 Å. and undeterminable length. These fibrils form an irregular felt in the primary cell wall and are aligned in parallel in the layers of the secondary cell wall (Fig. 11). It cannot be seen from the electron micrographs if the fibrils are cylindric or flattened. It is, however, not improbable that they are flattened because x-ray pictures show that the 101 plane of all fibrils in Valonia lies parallel to the plane of the wall, which is easily understood if the fibers have the form of ribbons. The scheme shown in Figures 10 and 12a, obtained in 1940 from indirect considerations, therefore appears to be confirmed. Cellulose forms in the growing cell at first an irregular felt; on growing, the fibrils are aligned in parallel, forming regular sheets in the secondary walls.

Bacterial cellulose from *Acetobacter xylinum* forms an irregular network[104b] of long fibrils slightly less thick (100–150 Å.). They must be flattened because they can be oriented by pressure so that finally all the 101 planes lie vertical to the direction of stress.

(*c*) *Texture of Native Cellulose*

Fibrillae can be combined to give different textures, in which both the type and perfection of the orientation can vary very widely. When the long axes of the micelles lie parallel to the fiber axis the texture is said to

[101] K. H. Meyer, *Natural and Synthetic High Polymers*, 1st ed., Interscience, New York, 1942, page 251.

[102] E. Husemann, *J. makromol. Chem.*, **1**, 118 (1943).

[103] A. Frey-Wyssling and K. Mühlethaler, *Textile Research J.*, **17**, 32 (1947).

[104] R. D. Preston, *et al.*, *Nature*, **162**, 665 (1948).

[104a] A. Frey-Wyssling, K. Mühlethaler, and R. W. G. Wyckoff, *Experientia*, **4**, 475 (1948).

[104b] K. Mühlethaler, *Biochim. Biophys. Acta*, **3**, 527 (1949).

be fibrous. This is found in its most perfect form in bast fibers such as ramie and flax. The individual fibers have the form of a hollow cylinder, sometimes flattened, with a very small lumen and thick walls built up from concentric layers (Sponsler and Dore[105]; Lüdtke[106]). The x-ray diagram as well as the mechanical properties show that in these fibers all the fibrillae and the primary valence chains lie parallel to the fiber axis. The extraordinarily high modulus of elasticity can only be understood if one assumes that all chains are extended parallel, so that when the material is subjected to tension they share the load equally with consequent deformation of the valence angles. Experiments show that the very high modulus which this mechanism would lead one to expect is in fact realized (Meyer and Lotmar[107]).

In a completely dry fiber the packing must of necessity be very close and regular, because the specific gravity measured in helium according to Davidson[108] reaches 1.57 ± 0.02 and is therefore nearly as great as the density (1.60 ± 0.05) of the crystallites (calculated from the volume of the unit cell as measured by x-rays and from the weight of all the atoms contained in it). The chains are therefore packed everywhere as tightly as they are in the crystalline region.

This simple fact has never been questioned, but it has not yet attracted the attention it deserves in discussions of the texture of cellulose. It has several important consequences. Truly amorphous regions in which there is an entirely random arrangement of the chains cannot be present in native bast fibers, because for rigid chains any arrangement in which they did not all lie parallel to the axis would lead to a lower density than that found in the crystalline region; the density of completely dry but disoriented preparations of cellulose is, in fact, considerably lower. Flaws might arise by a failure in the regular arrangement of the chains along the fiber or by their being twisted slightly out of position, phenomena which might occur either during growth or as a consequence of mechanical strains.

Another deduction which can be made from the high density is that in the dry fiber there can be practically no vacant spaces. This does not imply that the structural elements of all neighboring chains are united to form a single lattice; the most complete desiccation does not improve the x-ray diagram, showing that the crystallites are not thereby increased in size.

There must then be regions in the outer parts of the micelles in which

[105] O. L. Sponsler and W. H. Dore, *Colloid Symposium Monograph*, **4**, 174 (1926).
[106] M. Lüdtke, *Papier-Fabr.*, **28**, 129 (1930).
[107] K. H. Meyer and W. Lotmar, *Helv. Chim. Acta*, **19**, 68 (1938).
[108] G. F. Davidson, *J. Textile Inst.*, **18**, 175, 275 (1927).

the regular crystalline arrangement is disturbed. The saturation of the hydroxyl groups must be less complete there than it is in the interior of the crystallites, because if two hydroxyl groups attached to neighboring chains are prevented by the rigidity of the chains from approaching to within 3.6 Å. of one another they will behave as if they were completely free; for a hydrogen bond to be established the groups must approach to within 2.6 Å., a condition which is in fact fulfilled in the interior of the crystallites.

In the presence of water such hydroxyl groups become fully hydrated, and the gaps increase in size and finally become cavities filled with water. The existence of such a system of cavities has been demonstrated both by Frey-Wyssling[109] and by Kratky and Schossberger[110] with the gold or silver impregnation method of Ambronn.[111] The form and size of the impregnating metallic crystals can be determined by measurements in polarized light or by x-rays. Together with exceedingly small crystals of 10 to 15 Å. thickness there occur larger inclusions of diameter approximately 100 Å. Frey-Wyssling[112] deduces from this the presence of a system of irregular cavities and canals, but we prefer to regard the larger cavities as artifacts.

The dimensions of various intact fibers are as follows:

Fiber	Length, cm.	Lateral dimensions, μ
Cotton	1–5	6 × 40
Wood fibers	0.1–1.4	20 × 50
Bast fibers (flax, ramie)	4–25	10 × 50

The diameters of the various structural units are: fiber, 10,000 mμ; ultimate fibrilla (micelle), 20 mμ; primary valence chain, 0.6 mμ.

Fibrous texture in the strict meaning of the term includes only arrangements in which the crystallites lie parallel to one another in the long axis but are variously distributed with regard to both remaining axes. There is, however, reason to suppose that a higher degree of orientation exists in the concentric layers of fibers. In such layers it is probable that both the remaining axes are also determined so that a molecular arrangement similar to that found in single crystals results. A texture of this kind was

[109] A. Frey-Wyssling, *Protoplasma*, **27**, 372, 563 (1937).

[110] O. Kratky and F. Schossberger, *Z. physik. Chem.*, **B39**, 145 (1935).

[111] H. Ambronn, *Ber. Verhandl. sächs. Akad. Wiss. Leipzig, Math. phys. Klasse*, **48**, 613 (1896).

[112] A. Frey-Wyssling, *Protoplasma*, **25**, 261 (1936).

found by Sponsler[113] in the cell wall of the large unicellular alga *Valonia ventricosa* and by Nicolai and Frey-Wyssling[114] in the alga *Chaetomorpha*. It also occurs in the sporangia of fungi (*Phycomcyes blakesleeanus*[115]) with

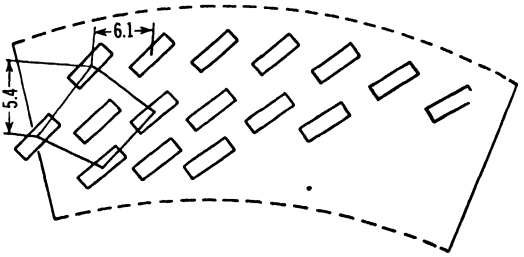


Fig. 12. Arrangement of chains in the cell wall of the alga *Valonia ventricosa*. The rectangles indicate sections through the glucose units.

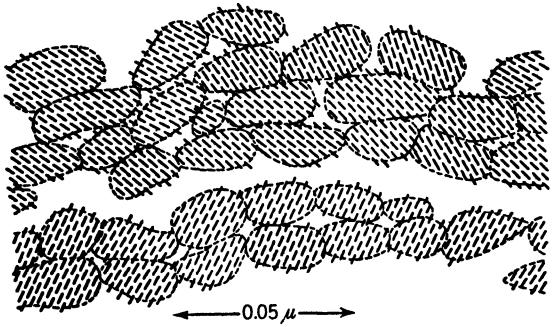


Fig. 12a. Part of a schematic cross-section of two successive laminae of the cell wall of a cellulose fiber. The dashes indicate the position of the glucose rings; the scale refers to the micellar ropes (the glucose rings are about four times too large). The micelles should therefore contain more glucose units. The spaces contain water, air, or cementing materials (Meyer).

the difference that we are dealing here with chitin instead of cellulose. Figure 12 shows a diagrammatic cross section through a layer of the cell wall of *Valonia ventricosa* taken at right angles to the long axis of the chains.

It is still impossible to determine experimentally the presence or absence

[113] O. L. Sponsler, *Protoplasma*, **12**, 2 (1931). See also R. D. Preston and W. T. Astbury, *Proc. Roy. Soc. London*, **B122**, 76 (1937).

[114] E. Nicolai and A. Frey-Wyssling, *Protoplasma*, **30**, 401 (1938).

[115] A. N. J. Heyn, *Protoplasma*, **25**, 372 (1936).

of an orientation like that prevailing in a single crystal in cellulose fibers. As this type of arrangement seems to be very widespread in biological material, it is to be assumed that it applies also to cellulose fibers, an assumption which is confirmed by the macerated preparations obtained when wood-destroying fungi attack fibers (Frey-Wyssling).[116] Probably in bast fibers, as well as in the *Valonia* cell, the plane of the glucose rings makes an angle of approximately 45° with the cell wall, and the (101) plane lies tangential to the wall of the cylinder (Sponsler and Dore[117]; Frey-Wyssling). Reviewing the information available the scheme which emerges for the structure of the cell wall is that shown in figure 12a. It is generally admitted that a high degree of orientation entails a kind of lamellar structure, and Kratky[118] claims that a lamellar structure can be demonstrated even in regenerated cellulose, with the (101) plane lying in the plane of the lamellae.

Many fibers, for example, wood fibers of conifers, have a thin outer layer in which the fibrillae and micelles lie tangentially and seem to be twisted helically around the fiber.[119] If the micelles lie with their longitudinal axes distributed at random in a plane the resulting texture is said to be foliar. It is found in the walls of cells which have no well-defined morphological axis (parenchyma cells) and in the tunicin of the mantle of tunicates.

The term screw texture is used to describe the arrangement in which the micelles are inclined at an angle to the longitudinal axis, so that they run helically around this axis. This texture is found in cotton fibers, where the pitch of the helix is about 30°; a flatter helix with the angle 45° is shown by palm fibers, such as those of coconut. Such fibers are extensible and are therefore known as ductile fibers.

In bast and wood fibers which are heavily impregnated with impurities, the various components of the cell wall—cellulose, hemicellulose, and lignin —are arranged in separate lamellae which run through the fiber in the longitudinal and perhaps also in the cross direction (Lüdtke[120]; Freudenberg[121]; Ritter[122]; etc.).

[116] A. Frey-Wyssling, *Papier-Fabr.*, **36**, 212 (1938).

[117] O. L. Sponsler and W. H. Dore, *Colloid Symposium Monograph*, **4**, 174 (1926).

[118] B. Baule, O. Kratky, and R. Treer, *Z. physik. Chem.*, **B50**, 255 (1941).

[119] G. W. Scarth, R. D. Gibbs, and J. D. Spier, *Trans. Roy. Soc. Can.*, **23**, V, 269 (1929). See also A. Frey-Wyssling, *Die Stoffausscheidungen der höheren Pflanzen*, Springer, Berlin, 1935, page 59.

[120] See the general account of M. Lüdtke in *Protoplasma*, **22**, 457 (1934).

[121] K. Freudenberg, F. Sohns, W. Dürr, and C. Niemann, *Cellulosechemie*, **12**, 263 (1931).

[122] G. J. Ritter, *Ind. Eng. Chem.*, **20**, 941 (1928). G. J. Ritter and G. H. Chidester, *Paper Trade J.*, **87**, No. 17, 131 (1928). G. J. Ritter and R. M. Seborg, *Ind. Eng. Chem.*, **22**, 1329 (1930).

(*d*) *Texture of Mercerized and Reprecipitated Cellulose*

In the process of solution the native micelles of cellulose are completely destroyed. It is not known, however, if they survive the process of mercerization (treatment, under stress, with strong alkali) or if they are disintegrated and re-form a new system when the alkali is removed. In either case they are certainly profoundly changed, as is evidenced by the x-ray interferences, which are broadened and indicate a different lattice (cellulose II), and by the considerable increase in water absorption.

Cellulose precipitated from solution without any mechanical treatment shows no crystalline interferences, and can therefore be regarded as completely amorphous. Cellulose in this condition has peculiar chemical properties; it may, for example, be soluble in dilute alkalis and lose this solubility when dried. If the water is replaced in the cold by pyridine, the system may be converted by the action of acetic anhydride into a soluble triacetate of a high degree of polymerization, whereas dry cellulose yields a triacetate which will swell but not dissolve (Staudinger and Daumiller[123]).

Amorphous cellulose, in fact, reacts completely, whereas in the material which will only swell, some points of linkage must be present in the raw material as small, compact crystalline regions which survive the process of acetylation. In freshly precipitated cellulose, while it is still impregnated with fluid, there are evidently no such regions present. In contrast to native fibers which contain no truly amorphous regions, we have here cellulose which contains no crystalline constituents at all. Such material is metastable; if it is dried, warmed in water, or stretched, an irreversible change occurs with the formation of crystalline regions and the separation of water.

In this "crystallization process" the first stage is the formation of very small regions of a fair degree of orderly arrangement whose existence cannot be demonstrated by x-ray methods; these we may call submicelles. These seed nuclei gradually increase in size to form crystallites which can be recognized on the x-ray diffraction diagram, a process which is accelerated by stretching or steaming.

If amorphous cellulose is dried under high vacuum it becomes "horny," that is to say, it is converted into a tough, homogeneous, structureless mass which is impermeable to nonpolar solvents and will not therefore react with mixtures of pyridine and acetic anhydride. Mercerized or regenerated celluloses, which contain a fair proportion of amorphous regions lose their capability of reacting with pyridine–acetic anhydride after drying under

[123] H. Staudinger and G. Daumiller, *Ann.*, **529**, 219 (1937).

high vacuum (Staudinger[124]): this clearly involves transformation of the strongly hydrated amorphous regions into continuous films which surround and protect the micelles. In order to obtain any considerable degree of acetylation it is essential to wet such nonreactive cellulose with water, and then to replace the water with pyridine. The water breaks the hydrogen bonds between the chains thus allowing the acetylation mixture to penetrate.

If the water is replaced by alcohol or acetone, and this in turn is replaced by cychohexane or paraffin, it is found that this type of cellulose obstinately retains considerable volumes of the hydrocarbon even under high vacuum ("inclusion celluloses" of Staudinger).

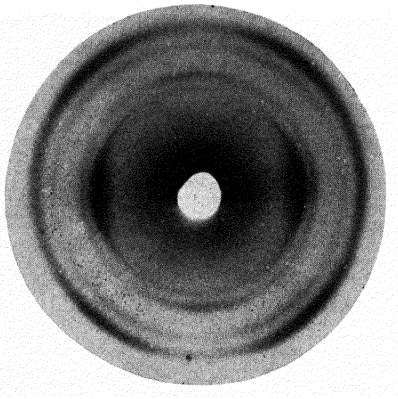


Fig. 13. Fiber diagram of cellophane.

As Lieser[125] has shown, aqueous solutions of cellulose may be obtained if very dilute solutions of certain soluble compounds, *e.g.*, xanthated tetraethylammonium addition compounds, are dialyzed against water at a low temperature. The solutions of cellulose obtained in this way are unstable and slowly precipitate.

If solutions of cellulose are precipitated under slight pressure in the form of sheets the preparations yield a diagram like that in figure 13. This shows only a moderate degree of orientation, and the inner interferences are absent altogether. The birefringence shows that all the chains lie in the plane of the film, giving a texture known as foliar.

[124] H. Staudinger, W. Döhle, and O. Heick, *J. prakt. Chem.*, **161** 191 (1943). H. Staudinger and W. Döhle, *ibid.*, **161**, 219 (1943).

[125] T. Lieser, *Cellulosechemie*, **18**, 121 (1940); *Kolloid-Z.*, **98**, 142 (1942).

If precipitation takes place under conditions in which there is a considerable tension on the half-coagulated mass, as in spinning artificial silk, the crystallites become oriented, their position and size depending upon the conditions under which solvent is removed. The texture is most easily imagined by considering the structure of the spinning solution. This is not like the solution of a substance of low molecular weight but must be considered rather as a felt of thin flexible threads each about 0.1–0.2 μ long (corresponding to 200–400 glucose residues), the spaces between being filled with fluid. If a coagulant is introduced at any particular place it will first affect those parts of the chains with which it first comes in contact. When a coagulated particle is drawn out of the solution it brings with it parts of the chain which are still surrounded by solvent as well as neighboring chains, and so forms a thread. The chains come to lie more or less parallel and the result is a diffuse system full of water, which has rubberlike properties. Free lengths of chain, still strongly hydrated, lie among distorted crystallites; any one chain may take part in one or more crystallites, passing from one to the other through spaces filled with water.

The water content of freshly coagulated cellulose is considerable; in unpublished experiments by van der Wyk on the precipitation of viscose, films were obtained with a water content of 800%; similar values (20–30% content of dry matter) were obtained by Hubert, Matthes, and Weisbrod[126] on viscose threads.

Crystalline regions are oriented according to the degree of tension. Orientation during the spinning process has been followed by Krebs[127] using x-ray technique; water is given off during stretching (Kratky). The salt or acid content of the precipitation bath has a very strong effect on the water content of the fibers (Hubert, Matthes, and Weisbrod[126]).

The anastomosis of the molecular threads gives rise to a network built up from micelles joined together by flexible threads. As Kratky and Hermans[128] have shown, the mechanical properties of hydrated cellulose are best explained by such a model. The shape of the cavities formed depends on the spatial arrangement of the micelles, being narrower in threads which have been stretched than in disoriented precipitates. If the material is strongly dried the cavities collapse and the free hydroxyl groups at the micellar surfaces "condense" with one another as far as the rigidity of the system will allow. If water is present the cavities may open again to some

[126] E. Hubert, A. Matthes, and K. Weisbrod, *Kolloid-Z.*, **98**, 173 (1942).

[127] G. Krebs, *Kolloid-Z.*, **98**, 200 (1942).

[128] See B. Baule, O. Kratky, and R. Treer, *Z. physik. Chem.*, **B50**, 255 (1941).

small extent under the influence of the restoring force exerted by valences distorted by the "condensation." The association of two alcoholic hydroxyls liberates 14,000 calories, an amount of energy which is sufficient to cause considerable deformation of valences or parts of chains. In preparations which have not been stretched, however, the packing remains comparatively loose even when dry because of the random arrangement of the micelles, as is shown by the specific gravity, which only reaches a value of 1.3, as against 1.57 for native cellulose.

Further conclusions about texture may be drawn from experiments on the modulus of elasticity of various products (Meyer and Lotmar[129]). This is very high (11,000 kg. per sq. mm.) in ramie and flax, but much lower in artificial fibers. The modulus also changes during the stretching of moderately oriented artificial fibers; any considerable extension results in an irreversible stiffening of the texture. This is in agreement with our picture of irregularly spaced micelles which may be rearranged by stretching.

The opening and closing of the pores is clearly shown by the experiments of Trillat[130] on the diffusion of air through cellulose films, which are permeable to air when damp, but impermeable when dry. The changes produced by drying are only very slowly reversible, so that it is often supposed that they are essentially irreversible processes. An irreversible change in the sense of a permanent reduction in permeability may be produced in various ways, for example, by strong pressure.

In conclusion, it may be said that differences between various cellulose preparations may be due to differences in mean chain length, variation in homogeneity with respect to chain length, associated substances, and, finally, the crystal structure, the degree of orientation, the breadth of the crystallites, the proportion of crystalline material present, the extent of flaws and cavities, the degree of swelling, and the density. To these we must add gross differences in the arrangement of the micelles and chains in particular preparations, since the arrangement may well be different at the surface of the fiber from what it is in the interior. Differences of this kind may occur in native fibers—witness the surface membrane mentioned above —as well as in spun fibers. In the latter they are determined, among other things, by the conditions prevailing during coagulation.

5. Birefringence and Optical Activity of Cellulose

Cellulose, in the form of fibers or oriented threads and films exhibits positive birefringence, with the optical axis parallel to the direction of the

[129] K. H. Meyer and W. Lotmar, *Helv. Chim. Acta*, **19**, 68 (1938).

[130] J. J. Trillat and M. Matricon, *J. chim. phys.*, **32**, 101 (1935).

chains. Anisotropy of refractive index is associated, therefore, with the anisotropy of tensile strength, of swelling, and of the temperature coefficient of expansion.

The birefringence of cellulose is of historic interest, as it was on observations of the refractive index of cellulose and of starch that Nägeli based his micellar theory. Much later Ambronn[131] placed this theory on a more certain experimental basis by his classical investigations on the birefringence of cellulose. He showed (using Wiener's technique) that the birefringence of cellulose is, to a very large extent, rodlet birefringence; moist cellulose fibers behave optically as if composed of parallel rodlets of submicroscopic dimensions embedded in a medium of lower refractive index, in this case water. On soaking in liquids of increasing refractive index, the birefringence diminishes to a minimum and then increases again; the minimum value represents the birefringence of the micellar substance itself (intrinsic birefringence). The refractive indices have been determined by Frey-Wyssling[132]: n_γ (plane of vibration parallel to the fiber axis) at 20°C., for the D line = 1.599. The indices $n_\alpha \sim n_\beta$ in the two directions at right angles to the chain axis are equal to 1.532. The birefringence is eight times that of quartz and about equal to that of strongly anisotropic anatase.

By means of the birefringence, it is possible to follow the progressive orientation of a film during stretching. But the importance of change in birefringence is still greater in botanical studies, since it is possible to determine by this means the position of the micelles in microscopic objects.

Derivatives of cellulose are also birefringent when oriented. Solutions of cellulose and its derivatives display birefringence of flow (Signer[133]). The analysis of this phenomenon shows that the cellulose chains which are not normally extended in solution become partially extended, and at the same time oriented, under the influence of the shear forces.

The optical activity of cellulose in solutions of concentrated acids is slight; this is in agreement with the chemical formula (Mark and Meyer[134]). From the rotation of cellobiose and cellotriose the rotation of a glucose residue in β-glucosidic linkage may be estimated; the value is found to lie between 0° and 30°, which is small in comparison with the high values for

[131] See H. Ambronn and A. Frey, *Das Polarisationsmikroskop.* Akadem. Verlagsgesellschaft, Leipzig, 1926.

[132] A. Frey-Wyssling, *Die Stoffausscheidungen der höheren Pflanzen*, Springer, Berlin, 1937; *Helv. Chim. Acta*, **19**, 900 (1936); **22**, 981 (1939).

[133] R. Signer, *Z. physik. Chem.*, **150**, 257 (1930). C. Sadron and H. Mosimann, *J. phys. radium*, **9**, 381, 384 (1938).

[134] H. Mark and K. H. Meyer, *Z. physik. Chem.*, **B2**, 115 (1929).

rotation of most sugars. Presumably the rotations of the individual asymmetric elements compensate each other in these residues. Freudenberg[135] comes to a similar conclusion from a study of the rotation of methyl cellulose and of methylated oligosaccharides.

TABLE VI

Physical Constants of Cellulose

Specific gravity in helium	1.54–1.58
Refractive index in the fiber direction	1.599
Refractive index at right angles to this	1.532
Specific heat	0.37
Heat of combustion, cal.	4200
Tensile strength (see Sect. IV1)	
Modulus of elasticity (see Sect. IV2)	
Dielectric constant	5.7
Breakdown voltage, kv./cm.	500

II. MODES OF REACTION OF CELLULOSE

With this picture of the micellar structure of cellulose in mind, we shall now consider what modes of reaction are to be expected. We shall then discuss the present state of our knowledge of the course of reactions in cellulose preparations, and try to interpret them in the light of the general conclusions which we have drawn.

The structure—slender crystalline fibrillae separated by cavities full of water—leads one to expect two types of reaction: (*1*) Reactions confined to the surface of the crystallites, which do not affect the lattice structure. These are known as micellar surface reactions (frequently called intermicellar). (*2*) Reactions which penetrate into the interior of the lattice and are known as intramicellar or permutoid reactions.

Katz[136] used the method of x-ray analysis to determine whether swelling takes place in an intra- or intermicellar manner. If, in a given case (such as the swelling of cellulose in water or dilute alkalis), neither the position nor the intensity of any of the observed reflections varies, the swelling process is said to be intermicellar. This classification and method of distinguishing the two types of reaction is by no means limited to swelling phenomena but may be applied to all types of reaction. Adsorption, involving the deposition of foreign material on the surface of the micelles, is

[135] K. Freudenberg, K. Friedrich, and I. Bumann, *Ann.*, **494**, 41 (1932).

[136] J. R. Katz, *Ergeb. exakt. Naturw*, **3**, 316 (1924); **4**, 154 (1925).

an example of the first type; solution, which involves complete destruction of the lattice, may be regarded as an extreme example of the second.

It is clear that intramicellar reactions are always accompanied by reactions at the internal surface as well; conversely, surface reactions cannot be regarded as limited entirely to the surface, since reaction will also occur in cracks and places where the crystallites are less regularly built or where they are frayed. It is to be expected that both types of reaction will be superimposed, and in fact there is often a continuous transition from one type to the other.

1. Micellar Surface Reactions

(*a*) *Internal Surface*

The process of adsorption on internal surfaces and the reactions taking place there are influenced by three factors: (*1*) the nature of the system of cavities through which reagents penetrate to the internal surfaces; (*2*) the extent of the internal surfaces; and (*3*) the attractive forces exerted on the reagent by the groups lying at the surface. The relative importance of these factors may be quite different in different cellulose preparations. The internal surface and the pore size are increased, for example, by the removal of associated substances during bucking; their extent in spun rayon is very different from what it is in the native fiber. In any one preparation the extent of the internal surface will vary with the degree of humidity, as the forces between the hydroxyl groups of neighboring crystallites are weakened by the penetration of water and new surfaces are formed. These changes take place slowly and may thus give rise to the phenomenon of hysteresis.

No exact measurements of the internal surface, that is of the number of hydroxyl groups available to reagents which do not penetrate through the lattice, are yet available The adsorption of methylene blue or of cobalt chloride[137] gives no true measure of the surface, as both reagents are fixed at carboxyl groups by a process of adsorption involving ion exchange. The uptake of direct dyes up to the saturation point, which has been determined for cotton[138] and for pulp macerated in a vibrating ball mill,[139] does not give a reliable measure of the surface because for one thing the large molecules of the direct dyes do not penetrate into the finest pores, and for another

[137] O. R. Howell and A. Jackson, *J. Chem. Soc.*, **1937**, 979.

[138] S. M. Neale and W. A. Stringfellow, *J. Soc. Dyers Colourists*, **56**, 19 (1940).

[139] K. Hess and W. Gramberg, *Kolloid-Z.*, **97**, 87 (1941).

because there is a tendency for the dye to precipitate as multimolecular aggregates within the fiber. Measurements of the adsorption of dilute gases (SO_2, HCl, and NH_3) have not as yet given any final answer; more fundamental conclusions may be possible from the study of the reaction kinetics of the decomposition of cellulose by periodic acid[140] and of the exchange of hydrogen against deuterium in heavy water[140a]; in pulp and in cotton linters *ca.* 50% of hydroxyl groups react very rapidly with D_2O and must therefore lie at the surface of the crystallite or at least very near to the surface. By all these methods it can be shown that the surface is doubled in extent by the action of caustic soda. Apart from this we are then forced, for an estimate of the internal surface, to a calculation of the dimensions of the micelles. Flat bundles 200 Å. broad and 80 Å. thick consist of about 450 primary valence chains, of which about 120, that is, one quarter, lie at the internal surface; in thinner bundles, such as are presumed to occur in mercerized cellulose, about half the chains would lie at the surface. The result of these geometrical relationships is that the uptake of certain substances appears to obey stoichiometrical laws, and to indicate the formation of a "compound" $(C_6H_{10}O_5)_2X$, although the process may only involve the saturation of groups lying at the surface. Pseudostoichiometrical adsorption may be recognized by the fact that the x-ray diagram of cellulose remains unchanged.

Pseudostoichiometrical ratios may also occur when excess reagent is removed from true cellulose compounds (for example, compounds with alkalis, bases, salts, nitric acid, or perchloric acid). In this case the reagent may disappear much more rapidly from the outer portions of the micelles than from within the lattice, so that analyses made at intermediate stages may lead to deceptive values. Thus the ratio 2 glucose residues to 1 reagent may be found, whereas in reality the ratio within the crystallite is 1 glucose residue to 1 reagent, while the glucose residues on the edges of the crystallites are free from reagent.

Finally adsorption is determined by interaction between the adsorbing groups and the substance adsorbed. In cellulose the adsorbing groups are primary and secondary hydroxyl groups and a relatively small proportion of carboxyl groups. The action of the latter will be discussed in the chapter on exchange adsorption. The adsorption potential of hydroxyl groups is influenced by geometrical factors, such as position at corners, along edges, or at the surface of cavities.

The isotherms of micellar surface reactions follow the mass action law for

[140] G. Goldfinger, H. Mark, and S. Siggia, *Ind. Eng. Chem.*, **35**, 1083 (1943).

[140a] V. J. Frilette, J. Hanle, and H. Mark, *J. Am. Chem. Soc.*, **70**, 1107 (1948).

homogeneous reactions. If the adsorbing groups are uniformly active, and if the number of groups remaining free is much larger than the number of those already occupied, adsorption obeys Henry's law. It is, therefore, inadmissible to conclude that a process of solution is taking place if, during the adsorption of small quantities of materials, the quantity of material adsorbed is found to be proportional to the external concentration. If the process of sorption is followed at higher concentrations, and if it is found that the number of groups of the absorbing material taking part in adsorption is greater than the number of such groups at the internal surface, permutoid penetration or solution may be assumed to have occurred. In this case the x-ray diffraction pattern changes as a result of sorption.

(*b*) *Reactions of Carboxyl Groups* (*Exchange Adsorption*)

As was shown in Sect. II*c*, technical cellulose contains carboxyl groups in a proportion depending on the nature of the raw material and on its previous treatment. It is usually saturated by metallic cations, such as Na^+, K^+, and Ca^{++}, which may be removed by treatment with dilute acids, yielding ash-free cellulose. Cellulose freed from all electrolytes by electrodialysis takes up metallic ions from dilute alkalis until the carboxyl groups are saturated; with neutral salt solutions the reaction follows the law of mass action for homogeneous reactions. The replacement appears macroscopically as an exchange of Na^+ for H^+, and on this account the process is known as exchange adsorption.

Many of the adsorption phenomena which have been observed in cellulose, particularly the adsorption of basic dyes such as methylene blue, are to be referred to the activity of the carboxyl groups. By the action of suitable salts, such as aluminum chloride, the carboxyl groups can be blocked to form insoluble complexes. As Kanamaru[141] found, cellulose which has been treated with 0.05% aluminum chloride can be shown by measurements of the electrokinetic potential to be neutral toward water and to have a higher breakdown voltage than the initial material, which was negatively charged, or, to put it chemically, acid.

(*c*) *Sorption of Water*

It would lead us too far to consider here in detail the problem of the sorption of water by cellulose, although it is of great technical importance;

[141] K. Kanamaru and T. Nakamura, *Kolloid-Z.*, **78**, 83 (1936). K. Kanamaru, A. Shiratori, and T. Nakamura, *ibid.*, **84**, 50 (1938).

reference may be made to the comprehensive account of Valkó.[142] Water uptake is dependent on the previous treatment of the cellulose; mercerization, in particular, increases the water uptake from 30 to 50%. The water does not penetrate into the cellulose lattice (Katz and Mark[143]) (see, on the other hand, Sect. III1*b*, on water cellulose).

No marked change takes place in the diffraction pattern, but it is observed that the diffuse scattering gradually increases, owing to the increase in the mass of adsorbed liquid. It is to be noted that on swelling in water the amorphous liquid ring of water does not immediately appear, and, in fact, remains invisible until the quantity of water present is considerable. This is due to the fact that the liquid taken up by the fiber is adsorbed on the surface of the micelles, and does not occupy regions of sufficient size to permit the appearance of a liquid diffraction pattern. The diffraction ring of water does not appear in its normal position and at its normal intensity until the fiber is macroscopically wet.

The water-uptake isotherms follow an S-shaped course, which may be ascribed to the action of two factors. A certain quantity of water (from 3 to 6%, according to the material) enters into combination with dry cellulose, the process being accompanied by the evolution of heat.[144] This water undergoes a perceptible diminution in volume, and does not exhibit the x-ray diffraction pattern of liquid water. The uptake of this water, adsorbed on the hydroxyl groups of the cellulose, determines the course of the isotherms up to the point of inflection. An uptake of 3% corresponds to a ratio of 1 molecule of water to 4 glucose residues; 6% to a ratio of 1:2; this is reminiscent of the relations already discussed.

Water absorbed after this point is bound by capillarity, and may amount to 20–25% by weight of the cellulose. From the well-known relation between vapor pressure and surface curvature, it may be calculated that the vapor pressure of water in capillaries 100 Å. in diameter will be lowered by about 20%; in capillaries 30 Å. wide, the vapor pressure will be about half the normal value. The whole course of the sorption curve is in agreement with the assumption that the greater part of the water is in fact bound in capillaries or cracks 30 -200 Å. in diameter. It is by no means necessary for the effect of this capillary system that it should exist in dry cellulose

[142] E. Valkó, *Kolloidchemische Grundlagen der Textilveredlung*, Springer, Berlin, 1937. See also A. J. Stamm, *Colloid Chemistry of Cellulosic Materials, Misc. Pub. U. S. Dept. Agr.*, **240** (1936).

[143] J. R. Katz and H. Mark, *Z. physik. Chem.*, **115**, 385 (1925).

[144] Recent determination of the heat of sorption: K. Lauer, R. Döderlein, C. Jäckel, and O. Wilde. *J. makromol. Chem.*, **1**, 76 (1943).

with the same shape and the same dimensions; it is only necessary that it exist at sorption equilibrium.

A dried sample of cellulose will not again take up the amount of water it originally contained; the desorption and absorption curves do not coincide. Apart from a retardation in the attainment of equilibrium (hysteresis), there is an irreversible change, probably due to progressive formation of crystalline regions whose water uptake is less than that of amorphous regions.[145] An experimental curve is shown in figure 14; similar isotherms are given by graphite oxide and bentonite (Chap. B, sects. II 1*a* and II 5*b*).

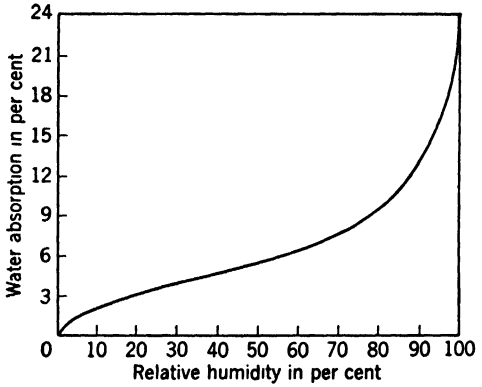


Fig. 14. Water uptake of bleached cotton at 25°C. (Urquhart and Williams).

Many natural celluloses—particularly bacterial cellulose from *Acetobacter xylinum*, and tunicin—contain considerable quantities of water bound in the intermicellar spaces. If the preparations are dried they do not swell again in water. Similar swollen celluloses may be obtained from nitrocelluloses by coagulating films of nitrocellulose, containing solvent, with acetic acid and denitrating the swollen, water-containing nitrocellulose film. Such cellulose preparations, used in ultrafiltration, lose their imbibition water on drying and do not regain their porous structure if subsequently placed in water.

(*d*) *Uptake of Metallic Salts*

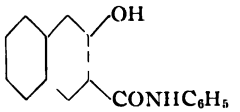
It may be assumed that in the uptake of mordants (such as aluminum, chromium, or iron salts) these electrolytes form saltlike complexes like those

[145] A. R. Urquhart and A. M. Williams, *J. Textile. Inst.*, **15**, 138, 433, 599 (1924); **16**, 155 (1925); **17**, 38 (1926).

formed with polyalcohols and sugars of low molecular weight. In general, reaction takes place only in the outer layers of the fiber, since the pores become blocked by the salts as they penetrate, with the result that diffusion of the ions with their large solvent atmospheres into the interior proceeds very slowly.

(*e*) *Dyeing with Substantive and Vat Dyes*[146]

We shall now attempt to interpret the process of substantive dyeing in the light of our knowledge of the structure of cellulose and, in particular, of the internal surface and the intermicellar spaces. This process includes not only dyeing by means of direct cotton dyes—mostly alkali salts of sulfonic acids belonging to the dis- and polyazo groups— but also the treatment of cellulose fibers with the leuco salts of vat dyes, which also exhibit an affinity for cellulose fibers, as Pummerer and Brass[147] have shown. Some phenols, in particular β-hydroxynaphthoylanilide in alkaline solution, also possess substantive properties:



X-ray analysis shows that none of these substances penetrates into the lattice.

The process of dyeing is reversible, provided that secondary reactions (such as oxidation of the adsorbed leuco compound) do not occur. After long exposure to the dye, equilibrium is eventually established between the dye in the fiber and that in the aqueous solution.[148] Equilibrium is not reached in the short period customary in commercial dyeing practice, so that the result obtained depends not only on the affinity but also on the rate of penetration. The equilibrium may be influenced in favor of the fiber by the addition of salts ("salting out"), or in favor of the solution by the addition of pyridine, formamide, glycerol, alkalis, and other suitable substances in which the dye is soluble. Raising the temperature always shifts equilibrium in favor of the solution. If dyeing takes place in a hot bath, penetration is more rapid and excessive sorption in the outermost

[146] Review: H. A. Standing, *Trans. Faraday Soc.*, **41**, 410 (1945).

[147] R. Pummerer and K. Brass, *Ber.*, **44**, 1647 (1911). See also K. Brass and G. Torinus, *Kolloid-Z.*, **45**, 256 (1928).

[148] See J. Boulton, A. E. Delph, F. Fothergill, and T. H. Morton, *J. Textile Inst.*, **24P**, 113 (1933). L. H. Griffiths and S. M. Neale, *Trans. Faraday Soc.*, **30**, 395 (1934).

layers of the fiber is prevented; the equilibrium uptake is not, however, increased.

The result of the superposition of the two effects—increase of the speed of adsorption and diminution of the equilibrium adsorption with rising temperature—is that, over a limited dyeing period, such as is customary in practice, the uptake of rapidly penetrating dyes passes through a maximum at moderate temperatures. Curves relating the dye uptake to the temperature of the bath have been obtained for a number of dyes by Whittaker[149] and have been used for the classification of substantive dyes.

Neale[150] has determined both the velocity of uptake (by determining the apparent diffusion coefficient in cellophane) and the dye uptake at equilibrium under fixed conditions for a great number of substantive dyes. Since the uptake of a number of typical dyes is influenced in a similar way by factors such as salt concentration and temperature, these results, though obtained under special conditions, probably possess more general significance. A rough correlation exists between the values for adsorption at equilibrium and the apparent diffusion constants, since the latter diminish as the adsorption increases.

The first equivalents of dye are more firmly adsorbed than those taken up later, in which a tendency to the formation of multimolecular layers is probably more marked.[151] Both the rate of uptake and the stability of the combination formed depend upon the nature of the cellulose, uptake being greater, for example, in moderately oriented than in well-oriented rayon, and in mercerized than in native fibers. Oxidation of cellulose in alkaline solution decreases the uptake of substantive dyes.[152]

The large differences in dye uptake observed in commercial practice (in which it is not usual to dye to equilibrium) between different samples of rayons or flock do not depend primarily on differences in the equilibrium adsorption but on differences in the rate of uptake (Boulton and collaborators[153]). This is determined by the extent of the orientation of the primary valence chains and by the size and distribution of the intermicellar spaces,

[149] C. M. Whittaker, *J. Soc. Chem. Ind.*, **51**, T.66 (1932). See W. Weltzien and K. Götze, *Monatsh. Seide u. Kunstseide*, **34**, 136 (1929); W. Weltzien and K. Windeck-Schulze, *ibid.*, **35**, 360 (1930).

[150] S. M. Neale, *J. Soc. Dyers Colourists*, **52**, 252 (1936).

[151] S. M. Neale and W. A. Stringfellow, *J. Soc. Dyers Colourists*, **56**, 19 (1940).

[152] S. M. Neale and W. A. Stringfellow, *J. Soc. Dyers Colourists*, **56**, 17 (1940).

[153] J. Boulton, A. E. Delph, F. Fothergill, and T. H. Morton, *J. Textile Inst.*, **24**, 113 (1933). W. Weltzien and K. Windeck-Schulze, *Angew. Chem.*, **51**, 729 (1938). *Zellwolle, Kunstseide, Seide*, **44**, 399 (1939). W. Weltzien and H. Froitzheim, *Monatsh. Seide u. Kunstseide Zellwolle*, **44**, 185, 238, 269, 314 (1939).

which in turn depend on chemical and physical conditions in the spinning process. In stained cross sections concentric zones of different fine structure can be demonstrated in a fiber and the influence of the degree of ripeness, composition of the precipitation bath, etc., on the development of these zones can be studied (Schramek[154]).

(*f*) *Nature of Linkage between Fiber and Dye*

The free hydroxyl groups of the internal surface are to be regarded as the groups by which the dye molecules are held.[155] It cannot be that it is the carboxyl groups which are concerned, as experience has shown that oxidation of the cellulose reduces its affinity for substantive dyes. Blocking the hydroxyl groups altogether suppresses the power to take up substantive dyes. It is in fact found that substantive dyestuffs are concentrated at crystalline surfaces containing alcoholic hydroxyl groups. For example, if a solution of congo red saturated with pentaerythrite is allowed to run through a layer of powdered pentaerythrite, as for chromatographic analysis, the first drops come through colorless while the pentaerythrite is stained red. It seems reasonable to suppose that the hydroxyl groups of cellulose play a similar part in the adsorption of dyestuffs. The dye itself must possess certain groupings with an affinity for hydroxyl groups, *e.g.*, —CO NHR— (which according to Krzikalla and Eistert[156] should be in the enol form: —COH=NR—) or phenolic hydroxyls. The presence of conjugated double bonds also appears to be necessary (Schirm[157]); they may intensify the activity of the other groups.

The shape of the dye molecule, which must apparently adapt itself to the structure of the internal surface, is also of importance. Long, straight molecules, such as the derivatives of benzidine, are suitable, but some platelike molecules, such as the leuco compound of flavanthrene, may also act as substantive dyes. Systematic investigations of the relation between molecular structure and affinity have been made by Ruggli[158] and Hodg-

[154] W. Schramek and J. Helm, *Kolloid-Z.*, **85**, 291 (1938); *Cellulosechemie*, **18**, 1 (1940). W. Weltzien and K. Windeck-Schulze, *Angew. Chem.*, **51**, 729 (1938); *Zellwolle, Kunstseide, Seide*, **44**, 399 (1939). W. Weltzien and H. Froitzheim, *Monatsh. Seide u. Kunstseide Zellwolle*, **44**, 185, 238, 269, 314 (1939).

[155] K. H. Meyer, *Melliand Textilber.*, **9**, 573 (1928).

[156] H. Krzikalla and B. Eistert, *J. prakt. Chem.*, **143**, 50 (1935).

[157] E. Schirm, *J. prakt. Chem.*, **144**, 69 (1935). See also H. H. Hodgson and P. F. Holt, *J. Soc., Dyers Colourists*, **53**, 175 (1937).

[158] P. Ruggli, *Kolloid-Z.*, **63**, 129 (1933). See L. H. Griffiths and S. M. Neale, *Trans. Faraday Soc.*, **30**, 395 (1934).

son.[159] In general, the straight molecular form does not appear to be indispensable for substantivity, as Ruggli[160] was able to show for dyes prepared from the *cis* and *trans* forms of *p,p'*-diaminostilbene, of which the bent *cis* dye proved to be more strongly substantive than the *trans* form.

In the process of dyeing, a monomolecular layer of dye is first adsorbed; larger quantities are adsorbed later, and often assume the size of submicroscopic crystallites recognizable by x-ray analysis. Further uptake, after the internal surface of a pore or crack has become completely saturated with molecules of the dye united to the hydroxyl groups, is presumably due to capillary forces. A solid body with a concave surface—such as is formed by the dye in the pore—has a lower vapor pressure and lower solubility than a similar body with a plain or convex surface. If L is the solubility of a solid body with a plane surface, L_w the solubility of the same compound in a cavity on the internal surface of which the body has been adsorbed, σ the interfacial tension, M the molecular weight, ρ the density, and r the radius of the cavity, the following relation, developed by Ostwald, holds:

$$L_w = Le^{-2\sigma M/RT\rho r}$$

As far as is known, the interfacial tension, solid/liquid, may amount to a value of 50 dynes or more; taking into consideration the relatively high-molecular weight of the dye, and postulating a pore diameter of 200 Å., we obtain a reduction of solubility to a small fraction of the normal value.

Values for the molecular heat of sorption, which could be determined by comparison of the sorption equilibrium at various temperatures, are not available. From measurements by Neale[161] on the sorption equilibrium of Fast Red K on cellophane at 25° and 90°C., we obtain $Q = 5000$ cal. per mole. For chrysophenin, a stilbene dye, on cellophane, values of about 14.00 cal. have been found.[162] These values seem high, and it may be supposed that one molecule of the dye enters into association with several hydroxyl groups, an arrangement which is quite possible geometrically. To summarize, it must be admitted that, although the process of substantive dyeing can be explained qualitatively, there is still no quantitative theory which takes into consideration the forces concerned and the shapes of the molecules.

[159] H. H. Hodgson, *J. Soc. Dyers Colourists*, **49**, 213 (1933). H. H. Hodgson and P. F. Holt, *ibid.*, **53**, 175 (1937).

[160] P. Ruggli, *Bull. soc. chim.*, **3**, 2166 (1936). P. Ruggli and F. Lang, *Helv. Chim. Acta*, **19**, 996 (1936).

[161] W. M. Garvie, L. H. Griffiths, and S. M. Neale, *Trans. Faraday Soc.*, **30**, 271 (1931).

[162] H. F. Willis, J. O. Warwicker, H. A. Standing, and A. R. Urquhart. *Trans. Faraday Soc.*, **41**, 506 (1945).

According to Krüger and Rudow[163] the absorption spectrum of dyes is shifted toward the red by sorption from aqueous solution on to cellulose; the form of the extinction curve may also change (formation of a second maximum). These changes may be due to the fact that in solution the dye molecule is surrounded by water molecules, whereas in the fiber, in consequence of aggregation, it comes in contact with its own kind. This interpretation is supported by the fact that a similar if less striking change in the curve appears in very concentrated solutions of dye,[164] in which the particles have already begun to associate into micelles. The effective color may be influenced by this change, but so many other factors (such as luster, thickness of the fibers, transparency, and nature of the tissue) are also concerned that it is impossible to account for the final color in a quantitative manner.

(g) *Intermicellar Swelling*

In the cases which have hitherto been considered, dissolved substances become adsorbed on the internal surfaces without the volume of the fibers undergoing any considerable change. The uptake of a number of electrolytes proceeds in an essentially different manner, and is accompanied by marked swelling. Such electrolytes include both strong acids (HCl, HNO_3, and H_2SO_4), lithium, calcium, and zinc halides and thiocyanates, and double salts of mercury, such as K_2HgCl_4, as well as alkalis, organic bases, and complexes of copper or silver with ammonia or other nitrogen-containing bases. In order to understand the reaction, it is necessary to consider the behavior of alcohols of low molecular weight which react with the reagents mentioned above to form the following compounds:

$$-CHOH + H^+ + Cl^- \longrightarrow -CHOH_2^+ + Cl^- \text{ (oxonium salt)}$$

$$-CHOH + Li^+ + Br^- \longrightarrow -CHOHLi^+ + Br^- \text{ (metallic complex)}$$

$$-CHOH + Na^+ + OH^- = -CHOH_2O^-Na^+ \text{ (formation of alcoholates)}$$

It may be supposed that cellulose reacts in an analogous way, and that the cellulose hydroxyls are amphoteric, like the hydroxyls of water or alcohol; with strong proton donors they form oxonium cations. The addition of lithium ions and other strongly polarizing cations (such as Ca^{++} and Zn^{++}), which tend to form complexes, is a process analogous to the addition of protons. On the other hand, the hydroxyl group may dissociate with the liberation of a hydrogen ion, while the portion remaining attached to the

[163] D. Krüger and H. Rudow, *Ber.*, **71**, 707 (1938).
[164] D. Krüger, *unpublished work.*

cellulose is converted into an anion. When this occurs water may be bound, forming a hydrated anion.

According to the reagent used, therefore, the hydroxyl groups of cellulose may be transformed into cations or anions. The ions formed in this way are attached to the cellulose by primary valences; the ions of opposite sign are mobile, but are prevented from diffusing away from the fixed ions by the interionic forces, and therefore remain within the intermicellar spaces.

The content is controlled by two factors. Electrostatic forces tend to produce a uniform distribution of the charges in the liquid as a whole so that there is a certain tendency for the cellulose salt to expand. This effect predominates in the presence of concentrated solutions of acids and salts. In more dilute solutions the following effect is also observed. Since the cellulose ions are unable to diffuse into the solution, a distribution of mobile ions according to the conditions of the Donnan equilibrium is set up between the cellulose (containing bound ions of opposite sign)and the ions in the surrounding solution. According to the Donnan equilibrium, the ionic product of the diffusible ions must be the same in both phases. This is possible only if a certain amount of the electrolyte, y, penetrates into the cellulose phase. If A is the concentration of the nondiffusible cellulose ions and of the associated ions of opposite sign, x is the final concentration of the diffusible ions in the external solution, and y is the concentration of the ions which have penetrated into the cellulose, we have the relation.

$$y\,(A + y) = x^2$$

When the ionic products are equal, the concentration of diffusible ions inside, $(A + y) + y$, is greater than that outside $(2x)$. An excess osmotic pressure arises within the fiber and leads to distention of the ion-filled meshes of the cellulose network until the tension in the network compensates the osmotic pressure difference. This type of swelling was first investigated by Procter and Wilson[165] in gelatin, and we shall return to their results in the chapter on proteins. These authors also showed that with increasing electrolyte concentration the difference in osmotic pressure passes through a maximum, recognizable by a swelling maximum. The swelling theory of Procter and Wilson has been applied by Pauli and Valkó[166] and by Neale[167] to the swelling of cellulose in alkali.

In the equation given above, the concentration (more correctly the activity) of the sodium and hydroxyl ions referred to the intermicellar liquid

[165] H. R. Procter and J. A. Wilson, *J. Chem. Soc.*, **109**, 307 (1916).

[166] W. Pauli and E. Valkó, *Elektrochemie der Kolloide.* Springer, Wien, 1929.

[167] S. M. Neale, *J. Textile Inst.*, **20**, 373T (1929); **21**, 225T (1930).

is x, A is the mean concentration of the ions attached to the cellulose, $(A + y)$ is the concentration of the ions of opposite sign to cellulose (here Na^+), and y is the concentration of hydroxyl ions in the intermicellar liquid:

Inside	Outside
A Cellulose$^-$	x OH^-
y OH^-	x Na^+
$(A + y)$ Na^+	

. As Neale showed, the amounts of alkali taken up by cellulose (in the form of cellophane) under various external concentrations can be accounted for quantitatively, if it is assumed that cellulose behaves as a monobasic acid with a dissociation constant of 2×10^{-14} and a molecular weight of 162; this constant is of the same order as the dissociation constant of alcohols. The alkali which has entered into combination with cellulose is known as truly bound alkali, in contrast to the sodium hydroxide present in the intermicellar spaces, the apparently bound alkali.

The processes of swelling and electrolyte uptake depicted here take place at the micellar surfaces and in the intermicellar spaces; this type of limited swelling is determined by intermicellar processes. If swelling is to lead to solution, on the other hand, intramicellar reactions are required; reagents must penetrate the lattice itself. Intramicellar reactions may also lead to the formation of a new lattice structure, thus providing new points of linkage and preventing separation of the chains. We shall first consider various types of intramicellar reactions and then review the individual reactions and the compounds formed.

2. Intramicellar Reactions

(*a*) *Heterogeneous Type of Reaction*

Several possible types of intramicellar reactions must be considered; we begin with the heterogeneous type of reaction, involving transformation of the crystallites into a new crystalline phase without the formation of mixed crystals.

In well-developed crystals, reactions in which a solid phase reacts with a dissolved substance to form a second solid phase present a highly characteristic picture. At a given temperature there is only one concentration of the dissolved substance at which both solid phases coexist; below this concentration only the initial material and the solution exist; above it only the product of reaction. The surface reacts at concentrations below the

critical value, but for well-developed crystals the amount adsorbed at the surface is so small as to be negligible. If, as the concentration is increased, the critical concentration is reached, the concentration remains unchanged during the addition of more reagent until the reaction is complete, thus giving a stepped isotherm.

In natural or regenerated cellulose the importance of the surface reaction is greater because of the higher ratio of surface to volume, and the simple stepped curve is smoothed into an S curve. It is therefore scarcely permissible to regard these very small crystallites as a crystalline phase in the sense of the phase rule, as was pointed out in 1930[168]; the requirement

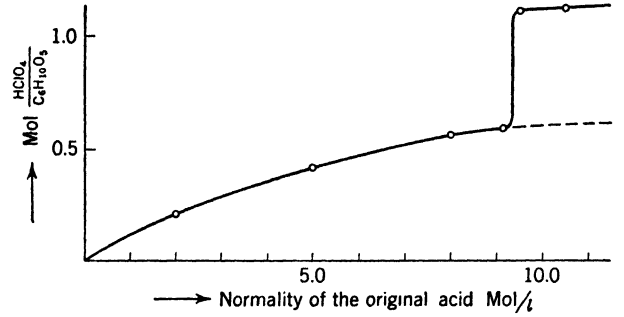


Fig. 15. Uptake of perchloric acid by cellulose (Andress).

for the application of this rule, that all the groups in the crystallite should behave similarly and in the same way as groups in a normal crystal, is not fulfilled here.

The experimental results available show that a sigmoid curve, indicating the heterogeneous type of reaction, has been found only in a few exceptional cases. Vieweg[169] found indications of such a curve in the uptake of alkali by native cellulose; on the other hand, in the liberation of alkali every trace of the vertical portion disappears and the reaction proceeds homogeneously. A well-marked step was found by Andress and Reinhardt[170] in the uptake of perchloric acid (Fig. 15). The acid is taken up at first according to an "adsorption isotherm"; saturation of the surface is reached when one acid molecule is taken up for every two glucose residues (pseudo-stoichiometrical proportion). At high concentration the crystallites react

[168] K. H. Meyer and H. Mark, *Aufbau der hochpolymeren organischen Naturstoffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, page 129.

[169] W. Vieweg, *Z. angew. Chem.*, **37**, 1008 (1924). For further literature see E. Valkó, *Kolloidchemische Grundlagen der Textilveredlung*, Springer, Berlin, 1937.

[170] K. Andress and L. Reinhardt, *Z. physik. Chem.*, **A151**, 425 (1930).

as if the reaction were heterogeneous. In the formation of Knecht's compound the uptake of nitric acid follows a similar course (Fig. 20, p. 366).

(*b*) *Homogeneous Type of Reaction*

The transformation of the micelles is continuous. As has been mentioned above, this type of reaction is always found in strongly swollen preparations of cellulose in which the crystalline arrangement has been practically destroyed. It does, however, also occur in celluloses with a micellar arrangement if the lattice structure has been distorted by some secondary reaction, as, for example, by the penetration of sulfuric acid during nitration. For these reasons this is the most common type of reaction. In nitration, in the uptake of alkalis by viscose foils, in acetylation, and during the uptake of swelling agents by cellulose esters, for any given concentration in the external medium there is a corresponding concentration of reagent throughout the interior of the cellulose phase.

A similar type of reaction is shown by permutites (zeolites) in their exchange reactions and, following a suggestion made by Freundlich, we shall describe all reactions of cellulose and other high polymers which proceed in this manner as "permutoid." In permutites, as in cellulose, there exists a stable, primary valence scaffolding, but this does not prevent the attached groups from reacting as in a homogeneous solution.

(*c*) *Solution*

When cellulose forms an addition compound with any reagent in aqueous solution, whether the cellulose will go into solution depends upon the solubility of the newly formed compound. The great differences between the solubility of addition compounds of cellulose with sodium, potassium, or lithium hydroxides, which are practically insoluble in water, as compared with those formed with ammoniacal copper oxide or tetraalkylammonium hydroxide, which are water-soluble, is analogous to the great differences which may occur in the solubility of any one anion in combination with different cations.

Many reagents which dissolve cellulose are most effective at a particular concentration of the aqueous solution. Below a certain concentration the reagent does not penetrate the lattice, so that the solid phase consists of pure cellulose; whereas at higher concentrations the solid phase consists of the addition compound. Presumably at higher concentrations the solubility limits of the cellulose–alkali combination are exceeded so that cellulose in the solid phase is transformed directly into the compound

without any extensive solution. It is scarcely necessary to emphasize the fact that the solubility of these addition compounds increases with the degree of degradation of the cellulose, and that many solvents, such as calcium thiocyanate, dissolve cellulose only after considerable preliminary degradation. The degree of degradation and the texture are important factors. Large, well-oriented crystallites are much more difficult to dissolve than smaller crystallites. Precipitated cellulose, for example, shows a very considerable dependence of the alkali solubility on the conditions under which it was precipitated; conditions for the formation of crystalline regions are probably more favorable if precipitation is slow and is accompanied by progressive orientation.

(*d*) *Topochemistry of Permutoid Reactions of Cellulose*

In all addition reactions of cellulose in which the fiber structure is retained, a thickening of the fiber is observed, but never a lengthening. If minor causes are excluded, the fiber length remains constant; it is to be concluded that the reagent penetrates between the chains. From a chemical point of view this is significant, since the reactive hydroxyl groups are placed laterally along the chains. This conclusion has been confirmed by x-ray analysis of a large number of derivatives. The constancy of the fiber length corresponds to the presistence of a fiber period $n \times 5.15$ Å. in all cellulose derivatives. That is to say, all derivatives show identity periods of $10.3 = 2 \times 5.15$, or $15.4 = 3 \times 5.15$, or finally $25.6 = 5 \times 5.15$ Å. Camphor nitrocellulose alone appears to have a still larger period. Tabel VII summarizes the identity periods of the cellulose derivatives known up to the present. As is clear from the table, the derivatives may be divided into three main families whose identity periods are 10.3, 15.4, and 25.6 Å.; camphor nitrocellulose I alone occupies a peculiar position.

We shall consider first the family of identity period 10.3 Å. The addition products belonging to this family contain less of the penetrating substance than the corresponding members of the family of identity period 15.4 Å. This follows from the conditions of formation of ammonia cellulose I and II, soda cellulose I and II, and the perchloric acid compounds I and II. In the 10.3 Å. family the digonal screw axes of native cellulose are retained, and the form of the chains remains unchanged. From this it follows that all the glucose residues of a chain take part in the same way in the addition of the foreign substances. Since it is very improbable that an unchanged cellulose chain could occur in a crystallite next to a chain of the addition compound, it must be supposed that at least one group is added to each glucose group.

TABLE VII

Identity Periods of Cellulose Derivatives

Family I 10.3 Å.	Family II 15 4 Å.
Ammonia cellulose I	Ammonia cellulose II
Methylamine and other primary amino celluloses	Soda cellulose II
Hydrazine cellulose I and II	Copper alkali cellulose II
Ethylenediamine cellulose I and II	Perchloric acid cellulose II
Various tetraalkylammonium hydroxide celluloses[171]	Cellulose ethyl oxalate
Lithium cellulose I	*p*-Methylcyclohexanone nitrocellulose I
Soda cellulose I and III	**Family III 25.6 Å.**
Potash cellulose	Nitrocellulose I
Copper alkali cellulose I	Acetone nitrocellulose I and II
Nitric acid compound (Knecht)	Camphor nitrocellulose III and IV
Perchloric acid compound I	Cyclohexanone nitrocellulose
Lithium thiocyanate compound	Methylcyclohexanone nitrocellulose II
Cellulose acetate I and II	Fenchone nitrocellulose
Propionyl cellulose	**Family IV 38.3 Å.**
Methyl cellulose	*d*-Camphor nitrocellulose I
m-Xylol nitrocellulose	

In a few simple cases it is possible to determine the nature of the change in the lattice. In ammonia cellulose, Knecht's compound, and certainly also in soda cellulose I, the spacing (001) is not changed by the penetration of the substance to be added. The a axis is lengthened and the lamellae lying along a are displaced relative to one another. The angle β is much more acute than in native cellulose. A scheme for the topochemistry of this reaction has already been given in figure 9, Sect. 13*d*. The substituent penetrates between the hydroxyl groups attached to carbon atom 6 of one chain and those attached to carbon atoms 2 and 3 of the neighboring chain, so that the chains are rotated more or less out of the plane. At the same time, one lamella is displaced relative to the other so that the angle β becomes more acute. If we compare this scheme with the lattice of cellulose II, the formation of this compound becomes understandable. The holes close, but the displacement of the lamellae relative to one another remains.

The members of the family of identity period 15.4 Å. contain more of the substituent than the corresponding members of the 10.3 Å. family. With this circumstance is associated the fact that the chains themselves are distorted in this family; the change in the lattice is different from and more profound than that in the 10.3 Å. family. Moreover, trigonal screw axes are present, parallel to the fiber axis (Gundermann[172]). Successive

[171] W. A. Sisson and W. R Sauer, *J. Phys. Chem.*, **43**, 687 (1939).

[172] J. Gundermann, *Z. physik Chem.*, **B37**, 387 (1937).

glucose residues of a chain make an angle of 120° with each other instead of one of 180°; this is possible only if a slight deformation of the valence angle at the oxygen atom takes place. Figure 16 shows the projections of the superposed glucose units in the two families.

Finally, the last type with an identity period of five glucose residues along the fiber axis is confined to the trinitro derivatives and their addition



(*a*) (*b*)

Fig. 16. Projection of the chain on the *ac*-plane: (*a*) family 10.3 Å.; (*b*) family 15.4 Å.

compounds with ketones. Only two chains traverse the unit cell in opposite senses (see also trinitrocellulose).

(*e*) *Course of Reaction Followed by X-Ray Analysis*

While views on the arrangement of adsorbed reagents are now, for the most part, generally accepted, various opinions exist as to the course of reaction. During esterification, x-ray diagrams can only be interpreted unequivocally either in the first part of the reaction, where the unchanged diagram indicates that the interior of the crystallites is unchanged, or at the end of esterification, when the diagram of the completely esterified product emerges distinctly. This holds both for nitration and acetylation. At intermediate stages the diagrams are more vague; the oblique reflections are lacking and the equatorial reflections are drawn outand may migrate.

A series of such x-ray photographs is shown in figure 17. Hess and Trogus[173] interpret them as mixed diagrams arising from the simultaneous presence of both unchanged initial material and end product. In the transitional stage the crystallites are supposed to be transformed at their surfaces; the heterogeneous reaction proceeds gradually into the micelle interior. Many observations[174] are irreconcilable with any general application of this conception, and it is opposed by the more plausible interpretation of the x-ray diagram of nitrocelluloses of varying nitrogen content, put

[173] K. Hess and C. Trogus, *Z. physik. Chem.*, **B15**, 157 (1932). *Ergebnisse der techn. Röntgenkunde*, Vol. IV, Akadem. Verlagsgesellschaft, Leipzig, 1934, page 21.

[174] See D. Krüger, M. Lüdtke, and F. Oberlies, *Z. angew. Chem.*, **47**, 806 (1934).

forward by Mathieu and Desmaroux.[175] According to this theory the reagent penetrates between the chains and displaces them somewhat irregularly in the direction of the *a* axis, while the spacing of the chains in

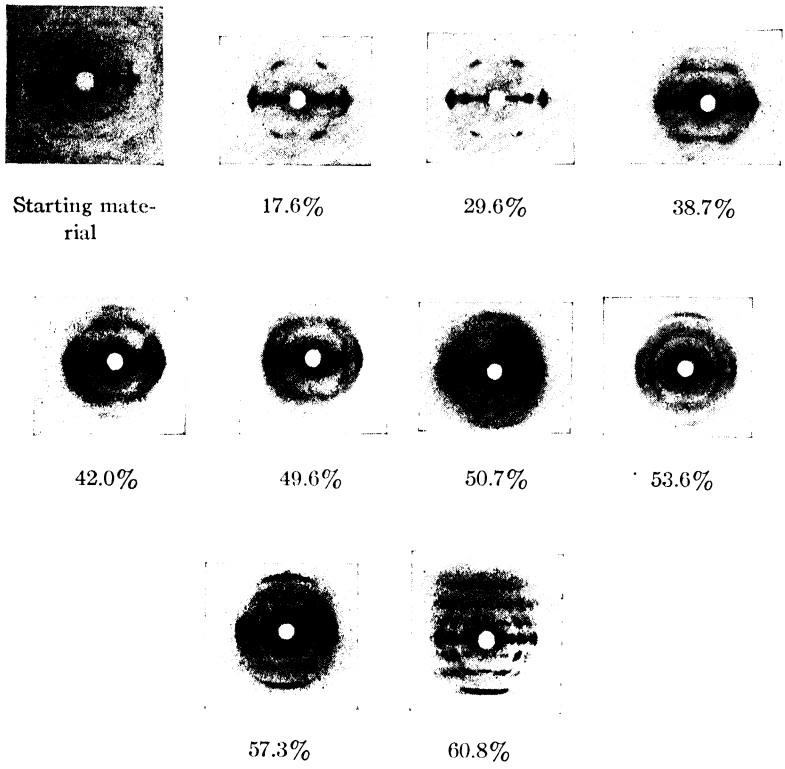


Fig. 17. Course of acetylation of cellulose followed by x-ray diagrams. The percentages denote the amount of acetic acid determined by chemical analysis (Hess and Trogus).

the direction at right angles to *a* remains practically constant. This is evident from the behavior of the reflections on the equator. The separation of the chains occurs at the beginning of the reaction; the dilated system of chains now reacts homogeneously and the quantity of reagent taken

[175] M. Mathieu, *Réactions Topochimiques: Nitration de la Cellulose.* Hermann, Paris, 1936.

up is dependent upon the external concentration. As they penetrate, groups of the reagent distribute themselves statistically along the chains on the hydroxyl groups. Changes entirely analogous to those observed by Mathieu in nitrocellulose can also be observed in the course of the acetylation of cellulose,[176] so that here again (apart from special conditions) the reactions must take place homogeneously. Mathieu interprets in a similar way the formation of the addition compounds of nitrocellulose; the absorption of swelling agents is dependent (according to the law of homogeneous reactions) on the external concentration. This was studied particularly in the case of acetone vapor.[177] The parallel arrangement remains intact until complete saturation with acetone is reached and the addition compound is fully formed; at higher acetone concentrations the lattice is disorganized and the product liquefies.

The homogeneous course of the reaction, almost invariably indicated by the sorption isotherms, is in good agreement with this point of view, as is also the decomposition of the addition compounds. It is frequently observed that the arrangement of the chains in the derivative persists during decomposition, and that it is possible to proceed to a considerable extent with the decomposition of the reaction products before the scaffolding is rearranged (Hess[178] and Trogus[179]). Indeed, one added substance may be replaced by another in a permutoid reaction; thus, for example, if the alkali is washed out of alkali cellulose, water may temporarily enter the lattice (water cellulose), according to Sakurada.[180] Both isotherms and x-ray data agree, therefore, with the assumption that during reaction the lattice is dilated. This dilated lattice forms a relatively rigid framework and reacts throughout its extent according to the laws of homogeneous reactions in a permutoid fashion. The cellulose crystallites do not, therefore, form a "crystalline phase" in the sense of the phase rule. An experiment by Champetier[181] shows how easily the inner regions of micelles are reached by penetrating reagents, as cellulose dipped in heavy water exchanges its hydroxyl hydrogen quantitatively for deuterium.

[176] D. Krüger, M. Lüdtke, F. Oberlies, *Z. angew. Chem.*, **47**, 283 (1934). D. Krüger *unpublished* experiments.

[177] M. Mathieu, *Compt. rend.*, **199**, 55 (1934). J. Desmaroux, *ibid.*, **199**, 148 (1934).

[178] K. Hess, C. Trogus, and O. Schwartzkopf, *Z. physik. Chem.*, **A162**, 187 (1932).

[179] C. Trogus, *ibid.*, **B22**, 134 (1933).

[180] I. Sakurada and K. Hutino, *Kolloid-Z.*, **77**, 346 (1936).

[181] G. Champetier and R. Viallard, *Compt. rend.*, **205**, 1387 (1937); *Bull. soc. chim.*, **5** (5), 1042 (1938).

III. COMPOUNDS OF CELLULOSE

1. Addition Compounds with Bases, Acids, and Salts

(a) *Action of Ammonia and Organic Bases on Cellulose*

Few data respecting the absorption of gaseous ammonia by cellulose are available. According to Grace and Maass,[182] dry cotton takes up 4% of ammonia at room temperature and atmospheric pressure; the system takes 50–100 hours to reach equilibrium. It may be supposed that ammonia is bound in a manner similar to water; a more exact investigation of sorption isotherms of the various preparations would be of considerable interest. The x-ray diagram of cellulose is not changed by gaseous ammonia, so that no intramicellar reaction occurs. In concentrated aqueous ammonia, cellulose is only slightly swollen and the lattice is not affected.

Liquid ammonia penetrates into the crystallites (Hess and Trogus,[183] Barry, Peterson, and King,[184] Hess and Gundermann[185]). At temperatures above −30°C. an addition compound with a fiberperiod 10.3 Å., known as ammonia cellulose I is formed, which has the following unit cell: a = 11.97 Å.; b = 10.3 Å.; c = 11.15 Å.; and β = 47°: the glucose residues lie in the (101) plane. At lower temperatures, a compound of the 15.3 Å. type, ammonia cellulose II is formed. The diagram may be indexed postulating a hexagonal cell in which $a = c$ = 14.5 Å.; β = 60°; and b = 15.2 Å. Ammonia cellulose I probably has the formula $C_6H_{10}O_5NH_3$, while ammonia cellulose II is probably $C_6H_{10}O_5(NH_3)_2$.

Arguing from the volume of the unit cell, 2764 Å.^{3}, and an assumed density of 1.42, Hess and Gundermann suppose that the cell contains $9C_6H_{10}O_5(NH_3)_2$ groups. In our opinion, as many cellulose chains in the initial material must run upward as downward, so that in any case the number of glucose residues per unit cell must be divisible by 2. Hence, it seems more probable that the unit cell contains $12C_6H_{10}O_5(NH_3)_2$ residues which would require a specific gravity of 1.41. If this formula if accepted for ammonia cellulose II, the only possible formula for ammonia cellulose I is $C_6H_{10}O_5NH_3$, since formulas such as $(C_6H_{10}O_5)_2NH_3$ are to be rejected because of the presence of a digonal screw axis.

The decomposition of ammonia celluloses leads to the production of various modifications of cellulose, according to the conditions; with warm water cellulose T is formed; with cold water or with dilute alcohol, cellulose II. Removing the ammonia at low pressure at temperatures above −30°C. leads to the formation of cellulose III; at lower tem-

[182] N. H. Grace and O. Maass, *J. Phys. Chem.*, **36**, 3046 (1932).
[183] K. Hess and C. Trogus, *Ber.*, **68**, 1986 (1935).
[184] A. J. Barry, F. C. Peterson, and A. J. King, *J. Am. Chem. Soc.*, **58**, 333 (1936).
[185] K. Hess and J. Gundermann, *Ber.*, **70**, 1788 (1937).

peratures a cellulose with a much distorted lattice is obtained (Hess and Gundermann).

Liquid methylamine, ethylamine, and propylamine in sealed tubes at room temperature give addition compounds whose structure corresponds to that of ammonia cellulose I. Addition compounds can also be obtained from butyl-, *n*-amyl-, hexyl-, and heptylamines if the cellulose is first allowed to swell in ethylamine and then transferred to the higher amine and the ethylamine removed *in vacuo*. The position of the chains is similar to that in soda cellulose III (page 309); the glucose rings lie in the plane (101) with the amine residues in the same plane. The dimensions of the unit cell of butylamine cellulose are: $a = 20.81$ Å., $b = 10.3$ Å., $c = 19.79$ Å., and $\beta = 28°$. The spacing of the midpoints of the two chains in the direction (101) is about 19.84 Å.; the butylamine molecules lie in between.[186]

Cellulose swells in aqueous solutions of ethylenediamine, hydrazine, etc. At sufficiently high concentrations these substances also penetrate into the lattice. The addition compounds formed belong to the 10.3 Å. type, as was shown by Trogus and Hess[187] in the case of compounds with hydrazine, ethylenediamine, and tetramethylenediamine. Certain alkyl quaternary bases dissolve cellulose, as Lieser[188] showed in an interesting paper. Tetramethylammonium hydroxide does not dissolve cellulose, but tetraethylammonium hydroxide, etc., do. According to Lieser and Ebert[189] the intrinsic viscosity of cellulose in tetraethylammonium hydroxide is three times as great as that in cuprammonium solution but, according to Staudinger and Daumiller,[190] it is about the same; according to Lieser and Ebert the viscosity in alkyl quaternary bases and in copper ethylenediamine is about the same. It might be convenient to use the viscosity in benzyltrimethylammonium hydroxide or in dibenzyldimethylammonium hydroxide (triton F) as an index of the quality of cellulose preparations.

(*b*) *Action of Sodium Hydroxide on Cellulose*

The action of caustic soda on cellulose which leads to the formation of alkali cellulose has been much studied because of its great commercial importance. It would take us too far afield to quote all the important literature here; reference may be made to the comprehensive account by

[186] W. E. Davis, A. J. Barry, F. C. Peterson, and A. J. King, *J. Am. Chem. Soc.*, **65**, 1294 (1943).

[187] C. Trogus and K. Hess, *Z. physik. Chem.*, **B14**, 387 (1931).

[188] T. Lieser, *Ann.*, **528**, 276 (1937); *Kolloid-Z.*, **81**, 234 (1937).

[189] T. Lieser and R. Ebert, *Ann.*, **532**, 94 (1937).

[190] H. Staudinger and G. Daumiller, *Ber.*, **70**, 2508 (1937).

Valkó.[191] For the fundamental process underlying the formation of the alkali compound, observations of Neale[192] show that, in the process of swelling, nondiffusible anions, presumably ionized glucose residues are formed:

$$(C_6H_9O_5)^- \text{ or } (C_6H_9O_5H_2O)^-$$

At higher concentrations, the alkali penetrates into the lattice, as is shown by the x-ray diagram. The fundamental process involved will be the same as in the micellar surface reaction, that is, the formation of the ions:

$$(C_6H_9O_5H_2O)^- \text{ and } Na+$$

The lattices of alkali celluloses are composed, therefore, of multivalent cellulose anions with cations sandwiched between.

The course of the reaction was followed, using x-ray analysis, by Katz and Mark,[193] von Susich and Wolff,[194] Hess, Schwarzkopf, Trogus, and Gundermann,[195–197] and Schramek.[198] In spite of this intensive attack, the subject is still by no means cleared up, so that it is not easy to give a critical review of the present situation.

If fibers of native or hydrated cellulose are treated with sodium hydroxide at a concentration below 8–9%, the x-ray diagram remains unchanged; the reaction must therefore be confined to the surface of the micelles and to the amorphous fraction. The sodium hydroxide actually attached to the cellulose at this stage amounts to about 1 mole of sodium hydroxide to 2 moles of glucose, according to Schwarzkopf.[196] This is a case of pseudo-stoichiometrical proportion. At higher concentrations a new diagram appears along with the cellulose diagram, and in 13–19% sodium hydroxide the former alone appears. Over this range of concentration, the "true" cellulose compound, $NaOH:C_6H_{10}O_5 = 1:1$, is formed. There is no general agreement about the lattice of this soda cellulose I; according to Schramek, the dimensions of the unit cell are:

$$a = 25.6 \text{ Å.} \quad b = 10.3 \text{ Å. (fiber period)} \quad c = 13.2 \text{ Å.} \quad \beta = 40°$$

[191] E. Valkó, *Kolloidchemische Grundlagen der Textilveredlung.* Springer, Vienna, 1937.

[192] S. M. Neale, *J. Textile Inst.*, **20,** 373T (1929); **21,** 225T(1930); **22,** 320, 349T (1931).

[193] J. R. Katz and H. Mark, *Z. physik. Chem.*, **A115,** 385 (1925).

[194] G. von Susich and W. W. Wolff, *ibid.*, **B8,** 221 (1930).

[195] K. Hess and C. Trogus, *ibid.*, **B11,** 381 (1930); **15,** 157 (1931); **21,** 349 (1933); *Z. Elektrochem.*, **42,** 696 (1936).

[196] O. Schwarzkopf, *Z. Elektrochem.*, **38,** 353 (1932).

[197] C. Trogus, K. Hess, and J. Gundermann, *ibid.*, **42,** 704 (1936).

[198] W. Schramek, *Kolloid-Z.*, **40,** 87 (1934). W. Schramek and H. Görg, *ibid.*, **42,** 302 (1935).

while Hess gives a fiber period twice as large. It appears, therefore, that the Na^+ ions penetrate between the chains and thus cause the unit cell to expand. Furthermore, as is shown by the intensities of the equatorial spots, the glucose chains are rotated somewhat out of the *ac* plane.

At concentrations above 20% a new compound is formed, soda cellulose, II, as indicated by the appearance of a new x-ray diagram. Its space lattice belongs to the same class as that of ammonia cellulose II; the fiber period is 15.4 Å. The supposition that this form differs from soda cellulose I only in its lower water content appears to us scarcely probable; in fact that the form of cellulose chains is changed leads rather to the conclusion that larger quantities of reagent have penetrated between the chains. In all probability a further hydroxyl group has been ionized and an extra Na^+ ion has been taken up by each glucose residue. Fiber diagrams of soda celluloses I and II are shown in figures 18 and 19.

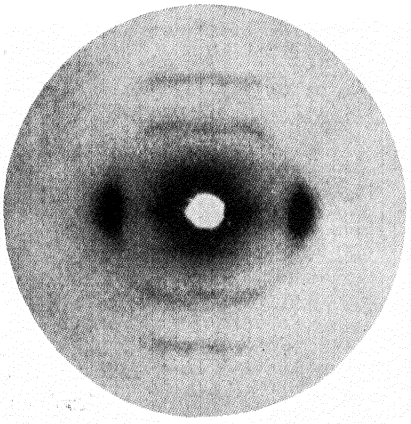


Fig. 18. X-ray diagram of soda cellulose I.

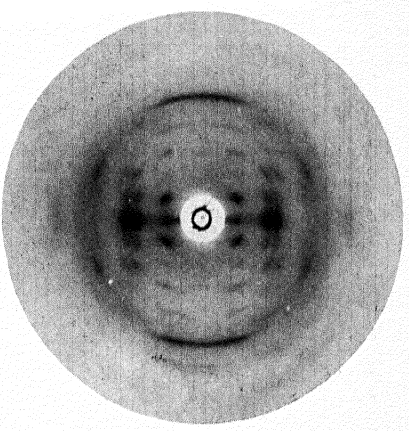


Fig. 19. X-ray diagram of soda cellulose II (Trogus and Hess).

By drying soda cellulose I, a third form, soda cellulose III (perhaps the water-free alcoholate), is formed; according to Gundermann the fiber period is 10.2 Å. In this form, the glucose chains are markedly rotated out of the *ac* plane.

If the soda celluloses are decomposed, various products are obtained, according to the conditions.[199] Soda cellulose I decomposes in hot water to form cellulose T together with cellulose II. Decomposition with moderately cold water leads to the formation of cellulose II. By cautious de-

[199] K. H. Meyer, L. Misch, and N. P. Badenhuizen, Jr., *Helv. Chim. Acta*, **22**, 59 (1939).

composition with water, it is possible to remove the caustic soda by degrees, and the space lattice is then found to be that of a dilated cellulose II. Sakurada[200] therefore supposes that the places formerly occupied by the caustic soda are now taken by H_2O molecules, and speaks accordingly of "water cellulose." A transition from soda cellulose to water cellulose can be shown analytically. On warming in water, the lattice contracts to that of cellulose II.

A form described as soda cellulose IV appears to be, in all probability, a water cellulose still containing a certain amount of alkali.

From soda cellulose II a product is formed (known as IIh) in which the fiber period 15.3 Å. is still retained, while the equatorial reflections have changed, indicating a diminution in the cross-sectional area. This is probably produced by a displacement of the alkali and partial replacement by water.

It follows from all these observations that the chains and parts of chains rearrange themselves slowly in the stable final form when the alkali is washed out, and that the process may be interrupted at unstable intermediate stages.

The formation of alkali celluloses indicated by x-ray analysis is favored by low temperature; 10% caustic soda solution enters the lattice only below 10°C., 12% up to 20°C., and 13% up to 30°C. Above this temperature caustic alkalis are ineffective (von Susich). This indicates that the reaction proceeds exothermally and that equilibrium is displaced in favor of the free products at a higher temperature.

(*c*) *Swelling and Solution of Cellulose in Caustic Soda*

Pure preparations of cotton isolated carefully contain only very small quantities of alkali-soluble materials; the greater part remains undissolved but swells. Technically prepared celluloses (pulps) give various amounts of soluble constituents according to the raw materials and method of preparation. These consist partly of noncellulosic impurities (mannan, xylan) and partly of degraded, alkali-soluble cellulose. In rayon pulps they are undesirable because of their effect upon the yield and properties of the finished product.

In place of the correct expression that "the fiber contains 20% of soluble constituents" it is often said that the "solubility of the cellulose is about 20%." There is, however, a well-understood meaning for the word solubility when used in connection with a number in this way; it is properly

[200] I. Sakurada and K. Hutino, *Kolloid-Z.*, **77**, 346 (1936).

used to indicate the solid content of a saturated solution rather than the content of the solid in soluble constituents. We prefer therefore to use the expression "solvability." The solvability, that is, the percentage of material given up by the cellulose to a solvent expressed as the percentage of the original cellulose, is dependent both on the solvent, in this case the concentration and temperature of the alkali, and on the type of cellulose, in which we can distinguish between the effects of chemical constitution (content of oxidized groups, stage of degradation), and the micellar arrangement.

As far as the solvent is concerned, solvability as well as swelling (increase in volume of the insoluble constituents) passes through a maximum as the concentration of the caustic soda is increased. At room temperature the maximum lies at about 8.5–12% sodium hydroxide,[201] but at lower temperatures it is considerably displaced toward lower concentrations. The maximum swelling of untreated cellulose and the solvability maximum for cellulose which has been chemically altered always follow one another closely.[202] At concentrations below the maximum, sodium hydroxide does not penetrate the lattice; above it, excess alkali reduces the solubility by exceeding the solubility product of soda cellulose. The proportion of a cellulose preparation which can be held in solution by alkali of any given concentration depends upon the direction from which this concentration is approached.

As far as the cellulose itself is concerned the solvability in caustic alkalis increases with increasing hydrolytic, oxidative, or thermal degradation.[203] Jung[204] was able to break up the micellar structure by vigorous grinding so that cellulose dissolved at a temperature below 10° even in 7% alkali to give a reasonably homogeneous mass.

Solution followed by precipitation, as in the preparation of artificial fibers, greatly increases the solvability in caustic soda. This effect depends partly on the chemical degradation which results from conversion to the soluble derivative, and partly on the fact that the micellar structure of regen-

[201] E. Heuser and R. Bartunck, *Cellulosechemie*, **6**, 19 (1925). J. D'ans and A. Jäger, *ibid.*, **6**, 137 (1925). A. Lottermoser and H. Radestock, *Z. angew. Chem.*, **40**, 1506 (1927). W. Weltzien, *Papier-Fabr.*, **32**, 433 (1934). G. Jayme and R. Steinman, *ibid.*, **35**, 337 (1937). H. Rath, W. Kuile, and K. Jäger, *Z. ges. Textil-Ind. Klepzig's*, **39**, 357, 371, 493 (1936).

[202] G. F. Davidson, *J. Textile Ind.*, **25**, 174T (1934).

[203] C. Birtwell, D. A. Clibbens, and A. Geake, *J. Textile Inst.*, **19**, 349T (1928). O. Eisenhut, *Cellulosechemie*, **19**, 45 (1941). A. Marschall, *Jentgen's Kunstseide u. Zellwolle*, **23**, 160 (1941).

[204] K. P. Jung, *Kolloid-Z.*, **98**, 192 (1942). J. Löbering, *ibid.*, **98**, 186 (1942).

erated fibers is much less compact than that of native fibers. In other words, the crystallites of the synthetic fibers are smaller and so more easily soluble than those of the native fibers. The range of swelling and solvability of technical rayons and cottons depends on the raw material (linters or pulp), on the method of preparation (copper or viscose process), on the spinning conditions, and on the concentration, etc.,[205] and may vary between wide limits. Christoph and Schramek[206] found values for the solvability varying between 5 and 4.5% for 9% sodium hydroxide at room temperature. The influence of the mean degree of polymerization (calculated from the viscosity) is small, and in technical fibers it is masked by other factors, for example, the effect of tension as the threads are drawn out of the jets with the consequent reorientation of the crystallites in the thread (Wannow and Schwarz[207]). On the other hand, the change from jet spinning to cup spinning results in a considerable decrease in the solvability in 9.5% sodium hydroxide, although Schramek[208] has shown that this is not due to a particularly good orientation of the structural units or to any restraint of the swelling or solvability by a particularly strong skin on the fibers. It is to be presumed that the effect is due to differences in the size of the crystallites which are difficult to detect by any other method; similar differences occur with amylose.

(*d*) *Mercerization*

The process of mercerization, that is, the production of glistening threads or tissues by the action of concentrated caustic alkalis on the material under tension, is of great commercial importance. If the fibers are not under tension, they shorten; this effect is to be ascribed to the marked, irregular swelling. The solution penetrates into the intermicellar cracks and distends them, so that the micellar strands are thrown into waves. This "waving" also manifests itself in the x-ray diagram of fibers mercerized in the absence of tension; the parallel orientation of the crystallites has disappeared.

The luster of mercerized fibers must be due to a regularizing and smoothing of the cracked surface and of the texture. The surface of native fibers,

[205] W. Weltzien, *Melliand Textilber.*, **6**, 355 (1925). Böhringer and Freund, *Klepzig's Textil-Z.*, **41**, 151 (1938). E. Kühnel, *Jentgen's Kunstseide u. Zellwolle*, **23**, 235 (1941). W. Schieber, *Z. angew. Chem.*, **52**, 561 (1939).

[206] W. Schramek and H. Christoph, *Kolloid-Beihefte*, **48**, 227 (1938).

[207] H. A. Wannow and H. Schwarz, *Kolloid-Z.*, **98**, 180 (1942).

[208] W. Schramek, J. Helm, and A. Stenzel, *Cellulosechemie*, **19**, 5 (1941). H. A. Wannow and H. Schwarz, *Kolloid-Z.*, **98**, 180 (1942).

consisting of relatively broad micellar bundles with cracks between, is rough and the interior of the fiber crevassed. Under the action of the strong caustic alkali, a "liquefaction" of the cohesive linkages at right angles to the direction of the chains occurs at the moment of penetration, and in this "liquid" condition the asperities are smoothed out. Similar effects can also be produced by sulfuric acid.

(*e*) *Action of Other Alkalis on Cellulose*

Lithium and potassium hydroxides react with cellulose in a manner similar to sodium hydroxide; swelling and sorption have been studied by Lottermoser and Radestock[209] and by Heuser and Bartunek.[210] As with sodium hydroxide, the cellulose lattice remains unchanged below certain concentrations but forms addition compounds at higher concentrations, as Katz[211] had already shown by x-ray analysis. According to Susich and Wolff,[212] these limiting concentrations for ramie at room temperature are: LiOH 8.5–9%, NaOH 11–12%, and KOH 15–17%. In terms of molarity, this is equivalent to: 3.4 *M* LiOH, 3 *M* NaOH, and 2.8 *M* KOH. Calculated in mole fractions, this corresponds in each case to a mole fraction of 0.07. The limiting concentrations fall as the temperature is lowered. Potassium hydroxide at concentrations between 17 and 22% forms a potash cellulose yielding a well-developed x-ray diagram with a fiber period of 10.2 Å. (Trogus and Hess[213]). At higher concentrations another modification is formed about which no data are available.

(*f*) *Cellulose and Cuprammonium Oxide Solutions*

As is well known, native cellulose previously treated by bucking and bleaching dissolves in ammoniacal solutions of copper oxide to form a viscous solution, from which it may be regenerated by treatment with acids or alkali, a property of which use is made in the copper silk process. The underlying chemical process is not completely understood. It is certainly not a simple alcoholate formation, as in alkali cellulose; such an

[209] A. Lottermoser and H. Radestock, *Z. angew. Chem.*, **40**, 1506 (1927).

[210] E. Heuser and R. Bartunek, *Cellulosechemie*, **6**, 19 (1925).

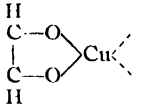
[211] J. R. Katz and H. Mark, *Z. physik. Chem.*, **A115**, 385 (1925); *Cellulosechemie*, **6**, 35 (1925).

[212] G. von Susich and W. Wolff, *Z. physik. Chem.*, **B8**, 221 (1930), as well as *private communications* from the authors.

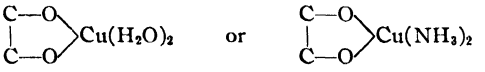
[213] C. Trogus and K. Hess, *Z. Elektrochem.*, **42**, 710 (1936).

assumption, as Hess[214] has emphasized, is not in agreement with the observed increase in optical rotation of cellulose in cuprammonium oxide solution, which indicates intensive interaction with the groups attached to one or two asymmetric carbon atoms.

During the action of cuprammonium solution on cellulose, ammonia is set free from the complex ions; also when cellulose dissolves in solutions of copper oxide in aqueous ethylenediamine, ethylenediamine is set free from its complex with copper. From this it follows that in both cases the original complex $Cu(NH_3)_4^{++}$ or $Cu(ethylenediamine)_2^{++}$ is destroyed and cellulose enters into the copper complex. By analogy with familiar reactions involving glycerol, tartaric acid, and other compounds of low molecular weight, it may be assumed that copper enters into complex formation with two hydroxyls to form a ring:

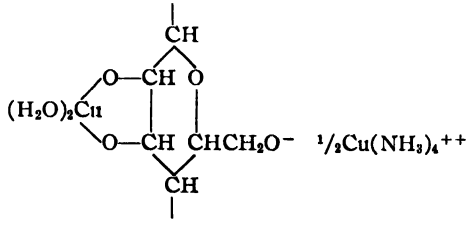


From stereochemical considerations it is suggested that only hydroxyls attached to carbon atoms 2 and 3 are concerned. It is probable that as a result of the entry of copper the electrochemical properties of the molecule are changed and, among other things, the dissociation constant of the cellulosic hydroxyl groups (which according to Neale is 2×10^{-14}) is increased. It may also be that the solubility is influenced directly by the entrance of copper, if the complex:



is particularly hydrophilic. The cellulose must be present in solution as an anion, as it is precipitated in the neighborhood of the anode during electroosmosis (Hess[214]). The formation of the complex is not, therefore, the only reaction involved. One must suppose that cellulose reacts like an acid with the alkali present, by means of the hydroxyls which are not taking part in the formation of the complex. Part of the copper is still present as the strong amine base. The situation may be represented in the following way:

[214] K. Hess, *Chemie der Zellulose*, Akadem. Verlagsgesellschaft, Leipzig, 1928. K. Hess and C. Trogus, *Z. physik. Chem.*, **A145**, 401 (1929).



We have already seen that the viscosity of solutions of cellulose in cuprammonium oxide is dependent on the degree of degradation of the initial material. The viscosity of the solution remains constant if oxygen is completely excluded; in the presence of oxygen the viscosity falls. It is, therefore, necessary to exclude oxygen rigorously in quantitative studies of the viscosity. In addition, it is advisable to add glucose or a little cuprous salt. In the complete absence of oxygen and of light, according to Staudinger and Ritzenthaler,[215] various cellulose materials may be precipitated repeatedly from cuprammonium solutions, using weakly acid or neutral precipitants (Seignette salt), without change in viscosity.

Staudinger regards this behavior as evidence that the solution contains free molecules and no micelles. This conclusion obviously assumes that the micellar equilibrium must be changed by reprecipitation. The assumption is incorrect, however, as examples are known of soap solutions, solutions of methyl cellulose, and of many proteins which, after reprecipitation, show no change in the micellar or association equilibrium prevailing in a given solvent. It is not possible, therefore, to prove in this way the absence of micelles or associated molecules.

With regard to the state of cellulose in solution, the high viscosity suggests an analogy with the dissolved condition of other chain polymers in suitable solvents, for example, nitrocellulose in acetone. At concentrations above 0.1%, the chains touch at many points, so that one can no longer speak of free molecules and the system is more a felt than a solution. From the detailed and careful investigations of Hess and Messmer[216] on the dependence of the optical rotation of a solution upon its composition, it appears that all glucose residues act uniformly, and that equilibrium is established between copper-containing residues and those which contain no copper. This equilibrium observes the mass action law for bimolecular reactions in homogeneous systems.

By the addition of alkali, a compound can be precipitated from solutions

[215] H. Staudinger and B. Ritzenthaler, *Ber.*, **68**, 1226 (1935).

[216] K. Hess, W. Weltzien, and E. Messmer, *Ann.*, **435**, 19 (1923). K. Hess and E. Messmer, *Ber.*, **54**, 834 (1921).

of cellulose in cuprammonium oxide which, according to Norman, has the composition $C_6H_7O_5CuNa$. This compound can also be obtained from native or alkali cellulose by the action of cuprammonium oxide in the presence of alkali. In this condition the cellulose is much swollen and exhibits a changed x-ray diagram; it is evident that an intramicellar reaction has taken place. The x-ray studies of Hess and Trogus[217] show that a series of compounds is formed according to the concentration of the copper solution. The compound first formed has an identity period along the fiber axis of 10.2 Å.; in higher concentrations of copper a compound with an identity period of 15.2 Å. is formed.

(g) *Action of Mineral Acids and Salts on Cellulose*

We shall consider first the reactions which take place when acids or salts are allowed to act upon alcohols of low molecular weight. Strong anhydrous acids, such as sulfuric and hydrochloric acids, dissolve in alcohol and in ether with considerable evolution of heat, from which it is to be concluded that forces of attraction between the hydroxyl groups or the ether oxygens, on the one hand, and the acid or, more correctly, the proton, on the other, are saturated during solution. This addition of protons to ROH or ROR is known as the formation of oxonium salts.

The formation of oxonium salts in aqueous solutions of alcohols and ethers is shown by various features of their physicochemical behavior. The dipoles of ROH or R_2O compete with those of water in the formation of complexes with the hydrogen ions. A quantitative comparison of the affinity of the hydrogen ions for water and for alcoholic hydroxyl supports the value of 0.58 determined electrochemically by Allemann.[218] Taking into consideration the fact that the concentration of hydroxyl groups in liquid water is about four times as great as that in liquid butyl alcohol, we obtain a partition coefficient of 2 for protons between alcoholic hydroxyl and aqueous hydroxyl. This indicates that the alcoholic hydroxyl groups have a greater affinity for protons that those of water. A consequence of this phenomenon is the increased solubility in water of alcohols such as butyl alcohol in the presence of strong acids.

Equilibria such as that reached in the system butyl alcohol–water–HCl, for example, are, of course, affected by the hydrocarbon residues also, as well as by the anions, which (according to their tendency to hydration) monopolize more or less water themselves, and may oppose the increase in solubility due to the hydrogen ions.

[217] K. Hess and C. Trogus, *Z. physik. Chem.*, **A145**, 401 (1929); **B6**, 1 (1929).
[218] E. Allemann, *Z. Elektrochem.*, **34**, 373 (1928).

The salts of certain cations are able, like acids, to form addition compounds with alcohols; of these the salts of Li^+, Ca^{++}, and Zn^{++} with suitable slightly hydrophilic anions such as Cl^-, Br^-, I^-, and CNS^- may be mentioned. Just as in the case of acids, an affinity exists between the cation and the alcoholic hydroxyl, and this may lead to competition between the latter and the OH^- of water in the formation of a complex with the cation. The relative affinity of lithium ions for water and alcohol can be determined, as for protons from the partition coefficient of the lithium ion in the system butyl alcohol–water. According to Allemann,[218] this amounts to 0.08, that is, 0.3 when referred to the number of hydroxyls present. Nothing quantitative is known in regard to other metallic ions, but the existence of numerous oxonium complexes with $ZnCl_2$ and $CaCl_2$, as well as the increased solubility of alcohols in the presence of these salts, etc., indicate the presence of specific forces of attraction between Zn^{++} and Ca^{++} ions and the oxygen atom (Pfeiffer,[219] Meyer and Dunkel[220]).

It is known that certain mineral acids are especially suitable for the separation of oxygen-containing compounds in crystalline form as oxonium salts. This is particularly true of perchloric and nitric acids. Many compounds, otherwise practically insoluble in water, are converted directly by concentrated perchloric or nitric acid into crystalline oxonium perchlorates or nitrates.

Besides these addition reactions in which the primary valences are not affected, acids and salts are also able to react with alcohols in a strictly "chemical" manner; the esterification of alcoholic hydroxyls and the splitting of glucosidal linkages under the influence of hydrogen ions may be mentioned. In this connection it is worthy of note that Li^+, Ca^{++}, and Zn^{++} ions in concentrated solution may have the same catalytic effect as hydrogen ions on the hydrolysis of glucosides.

After this introduction, the behavior of cellulose may now be considered. In concentrated mineral acids cellulose swells; the x-ray picture, however, remains unchanged up to fairly high concentrations of acid, such as 24% hydrochloric acid (Mark). In hydrochloric acid stronger than 40%, cellulose dissolves (Willstätter and Zechmeister[221]). The behavior in other hydrogen halide acids is similar. Forty per cent hydrochloric acid contains about 3 moles of water to 1 mole of HCl. If the constant expressing the affinity between hydroxyl groups and protons is as large for cellulose as it is for butyl alcohol, that is twice as large as for water, almost all the

[219] P. Pfeiffer, *Organische Molekülverbindungen.* 2nd ed., Enke, Stuttgart, 1927.
[220] K. H. Meyer and M. Dunkel, *Z. physik. Chem.*, **Bodenstein-Festband 1931**, 553.
[221] R. Willstätter and L. Zechmeister, *Ber.*, **46**, 2401 (1913); **62**, 722 (1929).

glucose groups will be ionized by the addition of protons. Accordingly an ionized anion chain lattice is formed which passes into solution when a certain concentration is reached.

Both sulfuric and phosphoric acids dissolve cellulose at certain concentrations; according to Ekenstam,[222] the most suitable concentrations are 10.5 moles per liter for sulfuric acid and 14.1 moles per liter for phosphoric acid; perchloric and nitric acids also have some solvent action.

Ekenstam concludes that cellulose forms addition compounds in which acid and water unite with cellulose in the same proportions as those in which they occur in acid of the optimum solvent concentration. This conclusion is completely unjustified, since the solubility of a cellulose oxonium salt may be reduced by the addition of acid. For the formation of the salt a minimal concentration of acid is necessary; if it is to dissolve, a certain maximal concentration of acid must not be exceeded.

Lithium halides, zinc chloride, calcium thiocyanate, and sodium mercuric iodide also produce swelling; these are all salts which form addition compounds with alcohols. It is suggested that the process of swelling and solution produced by these salts is entirely analogous to that produced by acids, although larger complexes are to be expected; the lithium ion, for example, is able to combine with a number of water or alcohol molecules so that complex ions such as $(CH_3OH)_4Li^+$ may be formed. If we compare the action of aqueous lithium bromide solution on cellulose with that on butyl alcohol, we find that, as the concentration of lithium bromide increases, more and more lithium bromide and water are taken up by butyl alcohol as solution takes place: the lattice forces holding together the cellulose phase, or alternatively the intermolecular forces holding together the butyl alcohol phase, are overcome by the energy of hydration of the complex COH—Li^+.

The reversible adsorption of protons and cations leading to swelling and finally to solution is always associated with an irreversible reaction, hydrolytic splitting of the glucosidic linkages which leads to shortening of the chains. The velocity of this reaction (the heat of activation of which is 30,000 cal.) (Freudenberg[223-4]) is much influenced by temperature, while the velocity of swelling is much less influenced. The shortening of the chains is indicated by the diminution in viscosity of the solutions; it can

[222] A. Ekenstam, *Über die Celluloselösungen in Mineralsäuren.* Lindstedt, Lund, 1936.

[223] K. Freudenberg and E. Bruch, *Ber.*, **63**, 535 (1930). K. Freudenberg, W. Kuhn, W. Dürr, F. Bolz, and G. Steinbrunn, *ibid.*, **63**, 1510 (1930).

[224] See also K. Freudenberg, *ibid.*, **62**, 383 (1929). K. Freudenberg, E. Bruch, and H. Rau, *ibid.*, **62**, 3078 (1929). K. Freudenberg and G. Blomquist, *ibid.*, **68**, 2070 (1935).

also be detected chemically, but only within the limits of accuracy of our analytical methods. It would not be possible, for example, to demonstrate the formation of new adehyde groups on the splitting of a chain of 1000 glucose units into two fractions, although the change in physical properties would be immediately apparent. Dissolved cellulose is rapidly hydrolyzed by sulfuric acid and by concentrated hydrochloric acid (as the fall in viscosity shows), whereas 83% phosphoric acid hydrolyzes it relatively slowly.[222,225] Many salts, such as calcium thiocyanate, lead to solution only when the temperature is raised; the process of solution is made possible in this case by a previous breakdown which can be detected viscometrically.

Hydrolytic breakdown and imbibition also play a part in the preparation of parchment paper by treatment with sulfuric acid; products of lower molecular weight are formed which cement together the fibers. Their presence is indicated by the coloration produced with iodine.

(*h*) *Crystalline Addition Products of Acids and Salts with Cellulose*

At certain concentrations acids and salts are able to form crystalline molecular compounds with cellulose as with alcohols. The addition products formed with nitric acid, perchloric acid, and lithium thiocyanate are well known.

The addition of nitric acid was first described by Knecht.[226] Cellulose swells in 70% nitric acid (specific gravity 1.42) retaining its fibrous structure. If the nitric acid is allowed to evaporate in a desiccator, a product remains which, according to Knecht, has the composition $C_6H_{10}O_5HNO_3$. From the investigations of Hess and Katz,[227] it appears that a permutoid reaction leads to the formation of a new lattice. This was subsequently studied in greater detail by Andress.[228]

Andress showed that the new lattice persists if the excess nitric acid is removed in a high vacuum. A product remains containing 18% HNO_3, corresponding approximately to the composition $(C_6H_{10}O_5)_2HNO_3$. This formula for the crystalline phase is favored by Andress. We prefer Knecht's formula and suppose that *in vacuo* the nitric acid bound at the micellar surface is removed and only that within the lattice remains. The value obtained by Andress is thus another example of pseudostoichiometrical proportions. Trogus[229] has adduced further evidence in favor of the proportions represented by the formula $C_6H_{10}O_5HNO_3$.

[225] A. J. Stamm and W. E. Cohen, *J. Phys. Chem.*, **42**, 921 (1938). S. Schmidt-Nielsen and A. Markestad, *Kgl. Norske Videnskab. Selskabs, Forh.*, **11**, 82, 97 (1938).

[226] E. Knecht, *Ber.*, **37**, 549 (1904).

[227] K. Hess and J. R. Katz, *Z. physik. Chem.*, **A122**, 126 (1926).

[228] K. Andress, *ibid.*, **A136**, 279 (1928).

[229] K. Trogus, *Cellulosechemie*, **15**, 104 (1934).

The identity period along the fiber axis can be determined with certainty from the x-ray diagram as 10.28 Å., and it is clear on comparing this diagram with that of native cellulose that the spacing of the cellulose lamellae (assemblages of chains running in the same direction), in the direction at right angles to the plane *ab*, remains constant during the penetration of the nitric acid (the reflection (002) has the same position as in cellulose). On the other hand, the spacing along *a* is increased and, in addition, the monoclinic angle is more acute than in native cellulose. Nitric acid groups have therefore penetrated between the chains running in the same direction, corresponding to the scheme in figure 9 (page 309). Possible axes and angle are given by Andress as:

$$a = 12.20 \text{ Å.}$$
$$b = 10.28 \text{ Å.}$$
$$c = 9.73 \text{ Å.}$$
$$\beta = 53°7'$$

Cellulose II is formed when the compound is decomposed with water.

Perchloric acid at concentrations between 9.5 and 10.5 moles per liter reacts with cellulose, which swells and undergoes changes in lattice structure (Andress and Reinhardt[230]). At higher concentrations cellulose is rapidly dissolved and destroyed. The sorption isotherm for perchloric acid has been determined by the same authors and is shown in figure 15, page 337. The authors conclude that a compound is formed of the composition $(C_6H_{10}O_5)_2HClO_4$. In our opinion it appears to provide a particularly good example of a reaction which begins as a micellar surface reaction, the glucose residues at the surface of the micelles first reacting homogeneously to form the compound $C_6H_{10}O_5HClO_4$, and continues as a heterogeneous permutoid reaction, in which the rest of the glucose residues, those within the micelles, are attacked. The identity period along the fiber axis determined from the x-ray diagram is 10.3 Å. Further indexing cannot be given with certainty.

Cellulose II is formed by decomposition of this compound with water. By a clever technique, the authors succeeded in obtaining the x-ray diagram of a compound formed at a higher concentration of perchloric acid. This they call an ester but it is probably an oxonium diperchlorate since it is rapidly decomposed by water. According to Gundermann,[231] trigonal screw axes are present and the compound belongs to the group with a fiber period of 15.4 Å.; this indicates that more of the reagent has

[230] K. Andress and L. Reinhardt, *Z. physik. Chem.*, **A151**, 425 (1930).
[231] J. Gundermann, *Z. physik. Chem.*, **B37**, 387 (1937).

penetrated into the lattice than in the case of the monoperchlorate belonging to the group 10.3 Å.

Under suitable conditions, crystalline addition compounds are also formed with metallic salts. Katz and Derksen[232] mention a compound with lithium thiocyanate belonging to the group 10.3 Å.

2. Viscose

(*a*) *Formation of Xanthate from Cellulose*

It was shown by Cross, Bevan, and Beadle[233] fifty years ago that carbon disulfide reacts with soda cellulose to form a water-soluble compound, from the aqueous solutions of which cellulose may be precipitated by the addition of acids; it is by means of this reaction, the viscose process, that most artificial silk (rayon) and cellulose foil (for example, cellophane) is prepared. In spite of its great commercial importance, the reaction is by no means clear in all its details although the main features have been explained. A whole series of secondary reactions accompany and mask the primary reaction. The conditions suitable for reaction have almost all been determined empirically and it may well be that a complete understanding of all the processes involved would not lead to any considerable progress in the process of manufacture. Nevertheless, it appears desirable to consider the viscose process in the light of the theory of reaction adopted here.

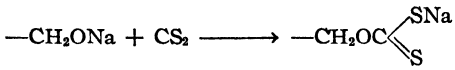
Good quality "rayon pulp" is first treated with 18% sodium hydroxide. In this way soda cellulose I is formed; the micelles react in the permutoid fashion. After being pressed and macerated the alkali cellulose is brought into contact with air. The longer this treatment (known as ripening) lasts, the lower is the viscosity of the solution subsequently obtained. In this process an oxidative breakdown takes place with the formation of carboxyl groups, which can be shown to be present in the finished viscose, and which are perhaps of some importance for the properties of viscose. If cellulose is not sufficiently broken down it yields solutions which are much too viscous and difficult to filter; if the process is carried too far, the tensile strength, both dry and wet, of the finished product is decreased. Oxidative breakdown in a heterogeneous system is certainly not an ideal way of reducing the chain length; the fibers must be disintegrated mechanically so that all the chains may be attacked as far as possible to the same extent.

The alkali cellulose is treated with the vapor of carbon disulfide, and is

[232] J. R. Katz and J. C. Derksen, *Rec. trav. chim.*, **50**, 149, 736 (1931).

[233] C. F. Cross, E. T. Bevan, and C. Beadle, *Ber.*, **26**, 1090, 2520 (1893).

thereby transformed into an orange-colored material which dissolves in water to give viscose, a reddish brown solution, usually containing about 6% alkali and 8–10% cellulose. The compound formed is the alkali xanthate which is known from alcohols of lower molecular weight:



Quantitative experiments on the amount of carbon disulfide adsorbed show that not all the glucose residues are xanthated. Workable products, soluble in dilute alkali, are obtained when the ratio of carbon disulfide to glucose residues is only 1:3. According to Lieser the ratio of 1 carbon disulfide to 2 glucose residues is not much exceeded in the xanthating of solid alkali cellulose even if a considerable excess of carbon disulfide is used. Lieser[234–5] assumes that the reaction is confined to the micellar surface and that the interior of the micelles is not involved. The proportion of 1 carbon disulfide to 2 glucose residues, which has been regarded by many authors as evidence of a stoichiometrical compound between 1 cellobiose unit and 1 molecule of carbon disulfide, is interpreted by him as a pseudo-stoichiometrical proportion.

In the presence of excess carbon disulfide the xanthate in sodium hydroxide solution, on the other hand, undergoes further xanthate[236] formation and by means of solution in organic bases it is possible according to Lieser[237] to take this process as far as the formation of the trixanthate. Lieser's views of the micellar character of the reaction involved in the formation of xanthate from solid alkali cellulose under technical conditions, with a low concentration of carbon disulfide and comparatively short reaction time, have been confirmed by x-ray analysis by Hess and Trogus[238] and by Schramek and Küttner.[239] The commercial product obtained after treatment with carbon disulfide vapor still shows the reflections of alkali cellulose I, showing that no intramicellar transformation has taken place. Only after complete xanthate formation do the reflections disappear.

On the other hand, Centola[240] and Bredée,[241] using both x-rays and analyt-

[234] T. Lieser, *Ann.*, **464**, 43 (1928); **470**, 104 (1929); **483**, 132 (1930); *Kolloid-Z.*, **81**, 234 (1937); *Papierfabrikant*, **36**, 272 (1938).

[235] T. Lieser and E. Leckzysk, *Ann.*, **522**, 56 (1936).

[236] W. Schramek and E. Zehmisch, *Kolloid-Beihefte*, **48**, 93 (1938).

[237] T. Lieser, *Ann.*, **464**, 43 (1928); **470**, 104 (1929); **483**, 132 (1930); *Kolloid-Z.*, **81**, 234 (1937). T. Lieser and E. Leckzysk, *Ann.*, **522**, 56 (1936).

[238] K. Hess and C. Trogus, *Cellulosechemie*, **13**, 84 (1932).

[239] W. Schramek and E. Küttner, *Kolloid-Beihefte*, **62**, 331 (1935).

ical methods, have shown that it is probable that xanthate formation may occur as an intramicellar reaction if the time is long. It is probable that even in commercial practice xanthate formation does, to an extent, invade the crystalline parts of the soda cellulose. In the commercial reaction a vapor is allowed to work on a swollen, but externally dry, crumbly fiber. The reaction takes place under conditions which from the point of view of reaction kinetics are difficult to control. Attempts have therefore been made[242] to grind the cellulose to a fine powder before treatment, after which it can be dispersed to a sludge in 8% sodium hydroxide by intensive stirring. It is then allowed to react to form the xanthate with carbon disulfide which is emulsified in the solution. With this procedure the sludge is slowly converted into a solution of viscose, in which if conditions are suitably controlled, the desired concentration of xanthated cellulose and caustic soda are attained directly.

(*b*) *Solution and Ripening of Viscose*

It is not at present possible to say anything precise about the state of xanthated cellulose in solution. As xanthate formation is incomplete, it is to be presumed that the chains hang together at points which have not reacted, while parts which were converted to xanthate are surrounded by solvents. In this way aggregates arise which have been termed "micelles" without regard to the fact that in this case we are not dealing with crystalline associations. The solution is unstable; it undergoes complex changes known as "ripening," which lead eventually to the formation of a jelly. The viscosity of the solution first decreases and then rises again until a gel is formed; this must be the effect of several processes working together. There is apparently no decomposition during ripening,[243] so that the initial fall in viscosity can scarcely be due to molecular breakdown. It depends rather upon the fact that the solution equilibrium, that is the equilibrium between free chains and those associated into micelles, is only slowly attained. The view that the initial decrease in viscosity results from gradual solution and dissociation of the micelles is confirmed by the analogous behavior of methyl cellulose (Chap. H, sect. IV 3*c*).

Centola[240] would relate the viscosity minimum with a peptizing of the cellulose xanthate by "ester exchange"; but this appears to be improbable

[240] G. Centola, *Atti congr. intern. chim. 10th Congr. Rome*, **4**, 722 (1939).
[241] H. L. Bredée, *Kolloid-Z.*, **94**, 81 (1941).
[242] J. Löbering, *Kolloid-Z.*, **98**, 186 (1942).
[243] H. Schwarz and H. A. Wannow, *Kolloid-Z.*, **97**, 193 (1941).

from the chemical point of view, since, in the presence of water, radicals which have once become dissociated cannot again form esters, equilibrium being in favor of the saponified product.

The principal reaction in ripening, and that which leads to the increase in viscosity and finally to the formation of the jelly, is undoubtedly chemical; it consists in the gradual decomposition of xanthate with the splitting off of sulfur-containing radicals, whereby whole parts of chains may lose their lyophilic groups. It is probable that the chains associate at these points (as the paraffin chains of fatty acid molecules in soap solutions associate) and thus form larger particles (secondary particles). This leads to an increase in viscosity and finally to coagulation. This irreversible coagulation is undoubtedly the result of the formation of numerous points of linkage of this kind which eventually connect together all the chains in the solution, thus preventing the slipping of chains over each other and abolishing viscous flow. Brownian movement, which can be observed in the case of individual submicroscopic particles, gradually ceases (Berl and Bitter[244]). If the course of ripening and the subsequent coagulation are followed by measurements of the intensity of the light scattered from an incident beam, the intensity is found to increase, from which one can conclude that optically discrete regions of supermolecular dimensions are formed.

The decomposition of cellulose xanthate proceeds in the same manner as that of alkyl xanthates of low molecular weight. In alkaline solution (reaction I) the xanthate decomposes to give cellulose and an unstable alkali dithiocarbonate, which saponifies to give carbonate and the disulfide. Alternatively cellulose and trithiocarbonate may be formed (reaction II):

$$\text{Cell—O—CS—SNa} + \text{NaOH} \longrightarrow \text{Cell—OH} + \text{NaO—CS—SNa} \qquad \text{(I)}$$

$$\text{NaO—CS—SNa} + 2\ \text{NaOH} \longrightarrow \text{Na}_2\text{CO}_3 + \text{Na}_2\text{S} + \text{H}_2\text{O}$$

$$\text{Cell—O—CS—SNa} + \text{Na}_2\text{S} \longrightarrow \text{Cell—OH} + \text{Na}_2\text{CS}_3 \qquad \text{(II)}$$

In acid solution, as in an acid spinning bath, the xanthate is mostly decomposed into cellulose and carbon disulfide.

As a secondary reaction, disulfide may be formed in small quantities by the action of atmospheric oxygen:

$$—CH_2O—\underset{\underset{S}{\|}}{C}—S—S—\underset{\underset{S}{\|}}{C}—OCH_2—$$

Staudinger and Daumiller[245] observed that for xanthates of similar structure, the ratio of the η_{sp} for xanthate 8% sodium hydroxide solution to the η_{sp} for cellulose in cupram-

[244] E. Berl and J. Bitter, *Cellulosechemie*, **7**, 137 (1926).

[245] H. Staudinger and G. Daumiller, *Ber.*, **71**, 1995 (1938).

monium solution is approximately the same over a range of samples of different degrees of polymerization. From this they conclude that the colloidal particles in xanthate solutions are of the same character as the colloidal particles in solutions of cellulose in cuprammonium and that in both instances we are concerned with single macromolecules rather than micelles. The assumption that isolated macromolecules are present in alkaline xanthate solution contradicts, however, not only the facts quoted above but also the results of Lieser and Bredée,[246] and more recent observations of Schramek.[247]

In practice, the degree of ripeness is assessed by precipitation with a solution of ammonium chloride; as ripening proceeds, less and less ammonium chloride is required to effect coagulation because, with progressive splitting off of xanthate groups, the dissolved particles grow and form ever larger and more strongly lyophobic structures. In addition, chemical methods are also employed. The conversion of cellulose xanthate to a disulfide by means of iodine (discovered by Cross and Bevan) is used in various ways. A reliable and accurate method for following the saponification of the ester during maturation has been developed by Fink, Stahn, and Matthies[248]; viscose is precipitated with diethylchloracetamide as Cell—O—CS_2—CH_2—$CON(C_2H_5)_2$ and the nitrogen content of the precipitate is obtained by a Kjeldahl determination. Each nitrogen atom corresponds to one cellulose dithiocarbonate group present in the original viscose. The degree of esterification is expressed as the γ value (the number of moles of carbon disulfide united with 100 $C_6H_{10}O_5$ units).

As might be expected the development of secondary particles hinders the parallel orientation of the chains during spinning. Threads spun from overripe viscose are therefore less well oriented and less solid under similar conditions of coagulation than those obtained from immature viscose. In the preparation of foils it is necessary to avoid as far as possible the orientation of the chains in the process of spinning.

(*c*) *Process of Spinning*

Viscose solution is formed into threads by being forced through jets into an acid spinning bath; this has the effect of removing the xanthate groups, so that cellulose is regenerated and coagulated; a similar reaction may be observed in xanthates of low molecular weight. The solution is thus converted into a strongly swollen fiber which is drawn off under tension. We shall now consider the process of spinning in somewhat greater detail.

[246] H. L. Bredée and J. De Booys, *Kolloid-Z.*, **79**, 31, 43 (1937).

[247] W. Schramek, *Papier-Fabr.*, **36**, 226 (1938).

[248] H. Fink, R. Stahn, and A. Matthes, *Z. angew. Chem.*, **47**, 602 (1934).

Viscose solution, like all other solutions of high polymers which are not extremely dilute, is no true solution of independent particles, but consists rather of a plastically swollen solid, a kind of molecular felt, in which the individual chains make many contacts with one another. This mass is forced through a hole some 50 to 90 μ in diameter into a bath consisting of about 12% sulfuric acid and 22% sodium sulfate, usually with some addition of magnesium or zinc sulfate. This composition has been found empirically to be suitable for viscose of a mean degree of ripeness. Lower concentrations of sulfuric acid lead to too slow decomposition; higher concentrations have too strong a swelling action on the fiber and make possible extensive plastic extension with a consequent increase of tensile strength at the expense of extensibility (Lilienfeld silk). The salt content causes a decrease in the swelling; magnesium and zinc salts also seem to have a specific effect of which the mechanism is obscure.

As the viscose enters the bath, hydrogen ions initiate decomposition at the edges of the cylinder; the cellulose chains adhere at several points to form an elastic, tubular network. A number of threads adhere over a short length to form submicelles and these in turn form nuclei for the formation of larger micelles which develop into typical fringed crystallites. The network permits exchange of the sodium ions inside with the hydrogen ions of the spinning solution, but prevents the entry of sulfate ions, which are bulky on account of their strong hydration and high charge. The chemical reaction can thus proceed inside the tube and at the same time water will diffuse out through the semipermeable wall on account of the difference in osmotic pressure. The result of these processes is that the walls of the tube collapse into the folds which are responsible for the typical stellate cross section of the finished fiber. The contents of the tube, originally a viscous fluid, are gradually converted into a highly swollen, elastic but deformable material with a loose three-dimensional structural network. The swollen fibers are stretched as they pass through the precipitation bath—in some processes there may be two baths whose precipitating action is graded. The tension acts selectively on the different regions of the fiber; the external parts which have a well-developed molecular network are oriented and caused to crystallize, while the core undergoes plastic flow. The well-oriented cortical area is much less strongly stained by dyes than the core.[249]

Moist fiber still contains from 100 to 150 parts of water to every 100 parts of dry material, as can be demonstrated by a method suggested by

[249] W. Schramek and J. Helm, *Kolloid-Z.*, **85**, 291 (1938). K. Wuhrmann, *Helv. Chim. Acta*, **28**, 666 (1945).

Hubert, Matthes, and Weisbrod[250] on fibers freed of surface water by vigorous centrifuging. The fiber is washed, treated with sodium sulfide to remove sulfur, bleached, and dried. The continuous rayon fibers may be cut to the desired staple length either before or after decomposition of the xanthate and are then prepared ready for use. When dried after removal of the sulfur, or more particularly after several cycles of wetting and drying, the swelling power of the regenerated cellulose decreases irreversibly. This is undoubtedly due to crystallization, a process which is initiated with the onset of coagulation and proceeds throughout fiber formation. A high proportion of crystalline material and a large mean size of the crystals give a product with high swelling resistance and low solvability in alkali (*cf.* Chap. H, sect. V 2*d*), but they also decrease the extensibility. Slow coagulation such as occurs in the two-bath process, treatment of the finished fibers with steam, or (according to Hubert[250]) treatment with liquid ammonia, all have this effect.

New developments in this field are threefold and consist in: (*1*) attempts to reduce sensitivity of the rayon to water or alkalis (improvement of resistance to laundering) by the use of very pure pulp and carefully selected spinning conditions; (*2*) improvement of tensile strength, without seriously affecting extensibility, by stretching the xanthate thread in suitable baths; and (*3*) production of a surface and crimp like that of wool, by suitable cycles of swelling and shrinking. The "formolizing" of fibers, which consists in after-treatment with formaldehyde in a weak acid bath, improves the water resistance considerably and is of great commercial importance. In the absence of any exact knowledge of the reactions involved in the manufacturing processes, or of any quantitative relation between them and the properties of the finished product, it is in general necessary to make successive empirical determinations of all the factors involved, such as type of pulp, preripening, xanthating time and ripening, composition of precipitation bath, form of the jets, rate of drawing off the threads, tension, and temperature.

3. Esterification of Cellulose

Cellulose cannot be esterified as easily as alcohols of lower molecular weight. The explanation lies in the fact that it is impossible to work in monophasic solution because all available solvents tend to promote saponification. In diphasic systems, in the presence of undissolved cellulose, the hydroxyl groups can only be brought into effective contact with the reagent under certain special conditions.

[250] E. Hubert, A. Matthes, and K. Weisbrod, *Kolloid-Z.*, **98**, 173 (1942).

(a) *Nitration*

Seventy to seventy-five per cent nitric acid or nitric acid above 90% reacts with cellulose to form solid esters; the reaction proceeds ultimately, as Vieille[251] showed as early as 1882, to equilibrium. Nitric acid of about 80% concentration dissolves and nitrates at the same time; acid containing

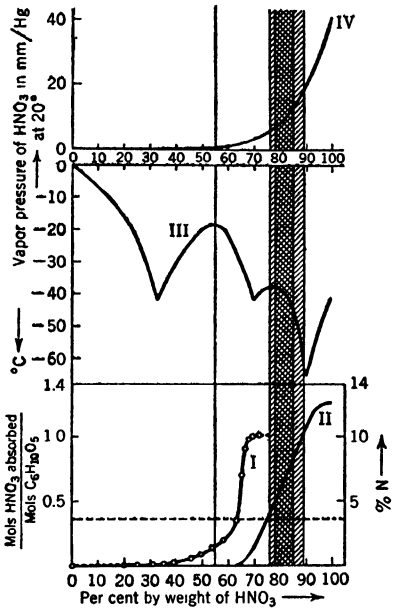


Fig. 20. Formation of Knecht's compound (curve I); nitrogen uptake of cellulose fibers (curve II); freezing point of nitric acid (curve III, from Küster-Kremann); partial pressure of free nitric acid in an aqueous solution at 20°C. as a function of the acid concentration (curve IV, from Taylor); hatched area = marked swelling; cross-hatched area = solution of the fiber in the acid (Trogus).

3 moles of water to 1 mole of acid does not react with cellulose; as Knecht discovered, higher concentrations form oxonium nitrate. The behavior of various concentrations of acid is shown in figure 20 taken from Trogus.[252]

More complete esterification can be obtained in the presence of sulfuric acid, but even so, the system ultimately reaches equilibrium:

$$—COH + HNO_3 + H_2SO_4 \rightleftharpoons —CONO_2 + H_2SO_4 + H_2O$$

[251] A. Vieille, *Compt. rend.*, 95, 132 (1882).

Equilibrium is determined by the final concentrations of the components of the reaction. The diagram in figure 21 (after Trogus[252]) indicates the effect on equilibrium of changes in the concentrations of nitric acid, water, and sulfuric acid. As Berl and Klaye[253] have shown, the isotherms are smooth, suggesting a reaction in a homogeneous system; this means that all the glucose units take part equally in the reaction. The isotherms do not suggest, for example, that the reaction ceases after the nitration of the surface or that distinct intermediate steps, such as pure mononitrate or

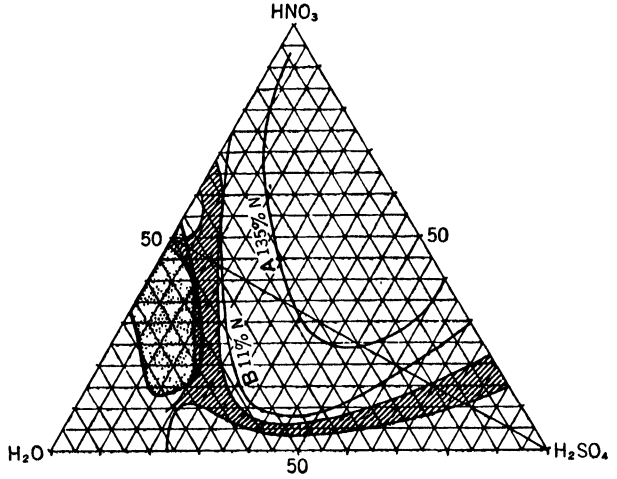


Fig. 21. Action of mixtures of nitric acid, sulfuric acid, and water on cellulose; dotted area is region in which Knecht's compound is formed; hatched area is region in which swelling is marked.

dinitrate, are formed; the final product is the trinitrate. Hydrolytic decomposition cannot be avoided entirely, although its extent at low temperatures may be limited to one of 3000 glucosic bonds.

Nitration can be carried out with comparatively little decomposition in a mixture of nitric and phosphoric acids, and also (according to Trogus[252]) in a mixture made up of 80 volumes of nitric acid (density 1.52), 20 volumes of glacial acetic acid, and 5 parts of acetic anhydride, allowed to act for 20 hours at 0–5°. By this means, products are obtained with a nitrogen content of 13.85%.

The course of the reaction is best followed by means of x-rays. Such studies have been made since the discoveries of the x-ray diffraction pattern of nitrocellulose by Herzog and von Náray-Szabó,[254] von Náray-

[252] C. Trogus, *Cellulosechemie*, **15**, 105 (1930).

[253] E. Berl and R. Klaye, *Z. ges. Schiess- u. Sprengstoffw.*, **2**, 403 (1907).

[254] R. von Herzog and S. von Náray-Szabó, *Z. physik. Chem.*, **A130**, 616 (1927).

Szabó and von Susich,[255] Trillat,[256] Miles, [257] Champetier,[258] Berl and Hefter,[259] and in particular detail by Trogus and Hess,[260] and by Mathieu.[261] Most of these studies have been made on ramie, the crystallites of which are relatively large and well oriented.

In these investigations, observations have not been made at equilibrium as in the case of the isotherms. Primarily the commercial process has been followed, so that the results of the two types of investigation are not necessarily comparable.

Up to a nitrogen content of about 8% the hydrated cellulose fiber shows the lattice of hydrated cellulose after washing out the acid (Miles). Nitration is therefore limited essentially to the micellar surface, while the conversion of cellulose into hydrated cellulose shows that the reagent has also penetrated into the micelles. Presumably the reagent penetrating the micelles has lost nitric acid (by reaction at the surface) and gained water, so that only oxonium nitrate or sulfate can be formed. At a moderate degree of nitration, at and above a nitrogen content of 10.5%, the character of the diagram changes. Up to a nitrogen content of 13%, the reflections are diffuse and indicate that considerable distortion of the lattice has taken place; as Mathieu points out, only the diatropic reflections and the equatorial reflections (002) are at all sharp. From this it may be concluded that the spacing of the chains in the micelles along the *c* axis is to some extent periodic and also that the periodicity along the fiber axis persists; on the other hand, there is no regularity in the arrangement of the substituents, indicating that they are distributed along the chains at random (Mark[264]). As with other derivatives, the chains are separated along *a*, the mean spacing increasing to 13–14 Å. as compared with 8 Å. in the initial material; on the other hand, the spacing of the laminae at right angles to *a* remains unchanged. Such an incompletely oriented arrange-

[255] S. von Náray-Szabó and G. von Susich, *Z. physik. Chem.*, **A134**, 264 (1928).

[256] J. J. Trillat, *J. phys. radium*, **1**, 340 (1930); **2**, 65 (1931).

[257] F. D. Miles, *Trans. Faraday Soc.*, **29**, 110 (1933); *J. Phys. Chem.*, **34**, 2598, 2607 (1934).

[258] G. Champetier, *Bull. soc. chim.*, **1**, 613 (1934).

[259] E. Berl and O. Hefter, *Cellulosechemie*, **14**, 65 (1933).

[260] C. Trogus and K. Hess, *Z. physik. Chem.*, **B12**, 268 (1931); *Z. Elektrochem.*, **40**, 193 (1934).

[261] M. Mathieu, *Réactions Topochimiques; Nitration de la Cellulose.* Hermann, Paris, 1936.

[264] H. Mark, *Physik und Chemie der Cellulose.* Springer, Berlin, 1932, page 268.

ment can hardly be referred to as a crystalline phase; it is understandable that the substance should react homogeneously like a liquid.

At still higher degrees of nitration a new diagram develops: a diffuse equatorial reflection, already present in the diagram at a lower degree of nitration, becomes sharper and new reflections appear. A new crystalline phase is formed: cellulose trinitrate.[265] This diagram is not, however, very definite in the presence of the nitrating mixture. It becomes clear on washing and is still sharper after prolonged boiling ("stabilizing" with water or acetic acid), in which process parts of the chains which were not well oriented come together and recrystallize. Fibers which have been nitrated with gaseous nitric acid yield sharp diagrams.

Taking all these observations into account, the process of nitration may be analyzed as follows: the reagent first penetrates into the fibers, the velocity of penetration depending upon the porosity and the water content; having reached the micellar surfaces, the acid continues to penetrate into the lattice, but with a different velocity. Wherever diffusion takes place, nitration occurs and the acid mixture becomes locally poorer in nitric acid and richer in water; in this way different degrees of nitration may obtain temporarily in different zones of the fiber or of the micelles. Finally, these differences disappear entirely as a result of diffusion, and random nitration occurs. Whether or not the required uniformity of nitration is eventually obtained depends upon the conditions of the reaction, on the previous treatment and humidity of the material, on the composition of the acid mixture, on the duration of the reaction, and on the temperature.

Trogus and Hess regard the process of nitration as a heterogeneous micellar reaction; we consider, however, that although the reaction may begin as such, it soon passes into one of the homogeneous type.

The process of nitration is complicated by two secondary reactions: the formation of sulfuric acid ester (more correctly a mixed ester with nitric and sulfuric acids[266]) and hydrolytic breakdown. Sulfuric acid reacts with cellulose in the following way:

$$> CHOH + H_2SO_4 \longrightarrow > CHOSO_3H$$

It may be the blocking of some hydroxyl groups by sulfuric acid that prevents the preparation of a trinitrate with theoretical nitrogen content. The bound sulfuric acid present in commercial products has to be removed

[265] S. von Náray-Szabó and G. von Susich, *Z. physik. Chem.*, **A134**, 264 (1928). C. Trogus, K. Hess, and J. R. Katz, *ibid.*, **B7**, 17 (1930).

[266] See also T. Tomonari, C. Trogus, and K. Hess, *Z. angew. Chem.*, **45**, 99, 126 (1932)

by treatment with hot water, if necessary with the addition of soda ("stabilization"). This process also has the effect of removing nitrates of lower molecular weight and of increasing the size of the crystallites.

Both sulfuric acid and nitric acid hydrolyze cellulose; the finished nitrate, on the other hand, is much more resistant, since the negative NO_2 groups hinder the attachment of the acid residue to the ether oxygen. This resistance of nitrocellulose to acids is made use of in filter cloth made from nitrated cotton fabric. Hydrolysis cannot be entirely avoided by any process of nitration; it can only be reduced by suitably chosen conditions of reaction. Berl[267] nitrated cellulose, then denitrated the compound, and renitrated. This process was repeated and the hydrolysis at each stage followed qualitatively by measurements of viscosity. It was found by Gralén that 0.03% of glucosidic linkages are hydrolyzed at each nitration.

For many commercial purposes cellulose nitrates of a given degree of hydrolysis are required, since, both for solutions and for molding powders, too great a chain length is unsuitable on account of the resulting high viscosity. (It is true, of course, that a certain minimum chain length is necessary if the tensile strength of the commercial end product is to be of the right order.) In every case hydrolytic breakdown as well as nitration should affect all chains uniformly, and if hydrolysis cannot be performed simultaneously with nitration, it should take place as a separate reaction either before, or better after, nitration. Marked local differences in the progress of the reaction due to local heating and concentration differences may well influence the uniformity of hydrolysis.

It follows from what has been said that preparations of nitrocellulose may differ in the length of the cellulose chains, in nitrogen content, and in the distribution of the nitro groups, which may either be uniformly and statistically distributed, or concentrated at the surface of micelles or fibrils.

(*b*) *Cellulose Trinitrate*

Prepared in the form of stretched fibers and stabilized, cellulose trinitrate yields a beautiful fiber diagram with the strikingly large identity period along the fiber axis of 25.6 Å. (von Náray-Szabó and von Susich; later Hess and Trogus, and Mathieu). The identity period therefore contains 5

[267] E. Berl and O. Hefter, *Cellulosechemie*, **14**, 65 (1933). S. Rogowin and M. Schlachover, *Z. angew. Chem.*, **48**, 647 (1935). See also H. Mark, *Physik und Chemie der Cellulose*, Springer, Berlin, 1932, page 270.

glucose units. According to Trogus and Hess and Mathieu the other axes are:

$$a = 13.0 \text{ Å.}$$
$$c = 8.9 \text{ Å.}$$
$$\beta = \text{approximately } 90°$$

From the size of the unit cell it may be assumed that two chains traverse the unit cell and these should be directed in opposite senses. It appears to us premature to speculate about the form of the chains until the space group and the indexing are definitely established.

Cellulose trinitrate shows negative uniaxial birefringence, although the birefringence of cellulose is positive. The nitrate groups must therefore lie with their most polarizable axis normal to the chain axis, an interpretation which agrees with the three-dimensional model. The dinitrate and the intermediate 2.5-nitrate show positive uniaxial birefringence.

(*c*) *Molecular Compounds of the Trinitrate*

The trinitrate is capable of forming crystalline compounds with a number of organic solvents without losing its fibrous structure. These were discovered by Hess, Trogus, and their collaborators[268]; examples are the compounds with acetone (fiber period 25.6 Å.), with cyclohexanone (fiber period 25 Å.), with fenchone (fiber period 25.5 Å.), and with *n*-xylol. Like cellulose, therefore, trinitrocellulose possesses the power of adsorbing suitable molecules between the chains, the identity period along the fiber axis generally remaining constant at 25.6 Å. Desmaroux and Mathieu[269] have shown that these compounds cease to give an x-ray pattern and begin to melt when a certain quantity of solvent has been taken up, for example, three molecules of cyclopentanone per glucose unit. The particularly well-defined compound with methylcyclohexanone has the composition 1 trinitroglucose:3 ketone. The identity period in this case is 15.4 Å. (Tomonari, Trogus, and Hess.[268] According to Gundermann[270] the lattice of this compound possesses a trigonal screw axis.

Of particular interest in view of its commercial importance is the reaction between nitrocellulose and camphor, which occurs during the formation of pyroxylin. This reaction has been investigated by Derksen, Katz, Hess, and Trogus,[271] using birefringence measurements; they came to the con-

[268] C. Trogus, T. Tomonari, and K. Hess, *Z. physik. Chem.*, **B16**, 351 (1932); **B17**, 241 (1932). See also J. Gundermann, *ibid.*, **B37**, 387 (1937).

[269] J. Desmaroux and M. Mathieu, *private communication*, 1938.

[270] J. Gundermann, *Z. physik. Chem.*, **B37**, 387 (1937).

[271] J. C. Derksen, J. R. Katz, K. Hess, and C. Trogus, *ibid.*, **A149**, 371 (1930).

clusion that a molecular compound of nitrocellulose and camphor is formed; the camphor molecules lie with their most polarizable axis at right angles to the fiber axis.

Further x-ray studies by Hess and his collaborators also indicate the formation of compounds; several compounds of nitrocellulose and camphor displaying different x-ray diagrams may be obtained. The identity period along the fiber axis in camphor nitrocellulose III (following the nomenclature given by Hess[272]) amounts to 25.4 Å. as in trinitrocellulose; camphor trinitrocellulose III is the compound first formed by the action of a solution of camphor in methanol on trinitrocellulose. On increasing the concentration of camphor, compound II is formed with the same fiber period 25.4 Å. Under special conditions the form I is obtained, with the fiber period 38.4 Å., and finally form IV, with the period 25.4 Å. It appears that the nature of the solvent (acetone, alcohol, etc.) influences the formation of particular types of compound. The stoichiometrical relationships of these compounds are uncertain, but it is established that the camphor penetrates into the lattice and it is, therefore, justifiable to speak of the formation of molecular compounds.

(*d*) *Products of Moderate Degree of Nitration*

According to Hess a dinitrate which yields a characteristic x-ray diagram is also obtained under certain conditions. His diagrams, however, only show one of the transition stages from the crystalline to the amorphous condition, such as occur temporarily in the swollen products. We assume with Mark and Mathieu that the nitro groups are distributed statistically over the hydroxyl groups of the chains.

The sorption isotherms of *o*-nitroaniline in aqueous solution on nitrocelluloses (having the composition of the dinitrate) follow Henry's law up to a nitroaniline content of 20% (Meyer, Schuster, and Bülow[273]). This shows that, at this high figure for the nitroaniline content, saturation is still not reached, and the number of units adsorbing the nitroaniline is large in comparison with the number of molecules of nitroaniline which have entered the system. From this it follows that all glucose nitrate units must be capable of reacting in the same way. The marked differences between groups at the surface and in the interior of the micelles observable in the case of cellulose are absent here; nitrocellulose reacts only homo-

[272] K. Hess, J. Tomonari, and C. Trogus, *Z. physik. Chem.*, **B16**, 374 (1932). M. Wadano, K. Hess, and C. Trogus, *ibid.*, **B30**, 159, 183, 232 (1935).

[273] K. H. Meyer, C. Schuster, and W. Bülow, *Melliand Textilber.*, **7**, 29 (1926).

geneously and in the permutoid manner. Substances which penetrate into the material may fairly be said to dissolve in it, as both the forces involved and the ultimate distribution of the dissolved substance are the same as for solution in a liquid. This interpretation is supported by a consideration of the course of adsorption of cyclohexanone vapor[274] (see Chap. H, Fig. 5).

The solubility of nitrocellulose in various solvents is influenced by the degree of nitration; the nitrate groups have affinities for esters and ethers, while the hydroxyl groups are hydrophilic. Hence solubility in organic solvents increases with the degree of nitration. Collodion cotton, that is, nitrocellulose with 2–2.5 nitrate groups per glucose unit, swells to a limited extent in alcohol as in ether and is soluble only in suitable mixtures of both. The solubility in alcohol and ether, plotted as a function of the nitrogen content, passes through a fairly sharp maximum. One may assume that the ether is able to solvate the nitrate groups and separate them from each other, but that it cannot overcome the molecular cohesion of the surviving hydroxyl groups. In the presence of ether alone, therefore, the nitrocellulose chains are still held together by hydroxyl linkages and form a three-dimensional net. The same is true for alcohol or water which solvate only the hydroxyl groups; solvents such as ethylene chlorhydrin or acetone solvate both groups. The effect on solubility of the number of polar groups in the cellulose ester, on the one hand, and in the solvent or mixture of solvents, on the other, has been considered in detail by Highfield.[275]

(*e*) *Denitration*

As a rule, solutions of alkali sulfides are used for thorough saponification. The reaction leads to complete denitration. Tomonari[276] made a study of partially denitrated products obtained after very careful denitration with alcoholic ammonium sulfide, using various solvents and x-ray analysis. Starting with trinitrocellulose, he found that a considerable diminution in solubility in acetone took place, although the diagram of trinitrocellulose had not disappeared. This change in solubility was noticeable as soon as the nitrogen content had changed from 13.8 to 13%. This shows that denitration does not proceed in the permutoid manner, and that it leads to a product other than that obtained in the course of nitration. Chains and micelles are completely denitrated in certain regions, and these areas, which cannot be detected by x-ray analysis and are scarcely detectable by analyti-

[274] C. G. Boissonnas and K. H. Meyer, *Helv. Chim. Acta*, **20**, 783 (1937).

[275] A. Highfield, *Trans. Faraday Soc.*, **22**, 57 (1926); *Z. physik. Chem.*, **A124**, 245 (1926).

[276] T. Tomonari, *Z. Elektrochem.*, **40**, 207 (1934).

cal methods, form points of linkage which can be ruptured only by a solvent for cellulose. The process of denitration is therefore markedly heterogeneous. This can be shown in denitrated ramie fibers by examination under the polarizing microscope (Frey-Wyssling[277]). If denitration is continued, the reflections of the cellulose pattern appear in the x-ray diagram; both native and regenerated forms have been observed (Tomonari), the lattice of both being distorted.

(*f*) *Acetylation of Cellulose*

Complete acetylation of cellulose is not easily attained. In dry cellulose the pores are collapsed as is shown, for example, by its refusal to react with Grignard reagent; the pores only open in polar solvents. But even if one can effect penetration of the reagent into the pores, for example, by using a mixture of glacial acetic, acetic anhydride, and potassium acetate, the reaction is still confined to the surface of the micelles and the amorphous parts of the fiber, as Centola[278] has shown. Native fibers of ramie can be acetylated up to 40% without causing the disappearance of the original cellulose interference points. No cellulose acetate can be dissolved out of the acetylated product, from which Centola deducts that the acetylated parts of the chains must still be connected to one another by cellulose crystallites which happen to include unacetylated parts of the same chain.

Even esterification with alkalis and acid chlorides does not in general yield very good results. Pyridine is usually used as an organic base, but it is not capable of penetrating into the lattice in a permutoid manner and hence cannot be regarded as an ideal reaction medium. For complete acetylation substances must be used which solvate the hydroxyl groups and destroy the lattice. The choice of materials which fulfil these conditions without at the same time preventing esterification or introducing undesirable by-reactions as a consequence of their hydrolytic effect is very small Sulfuric acid or zinc chloride are used as swelling agents and accelerators; perchloric acid is particularly effective, although the presence of this reagent entails a certain amount of molecular decomposition. If some decomposition does not matter, esterification can be made easier by simultaneous decomposition and solution of the products of reaction in the reagent. In commercial acetylation, for example, a solution is used which at once esterifies and decomposes the material and, at the same time, dissolves the ester as it is formed. If it is required, on the other hand, to

[277] A. Frey-Wyssling, *Protoplasma*, **26**, 45 (1936).

[278] G. Centola, *Atti congr. intern. chim. 10th Congr. Rome*, **4**, 129 (1939).

acetylate cellulose without decomposition, this may be effected by first bringing it into a reactive form by precipitation from cuprammonium solution, followed by gradual replacement of the water by pyridine; it can then be easily acetylated to give a soluble product (Staudinger and Daumiller[279]).

It is possible in a relatively short space of time to convert cellulose in the form of fibers almost completely into the triacetate, using acetic anhydride and an acid catalyst in the presence of a liquid in which the ester is insoluble, such as benzene. The product may be converted by partial saponification into substances poorer in acetic acid and soluble in acetone, the fiber structure being retained. Acetylation without hydrolytic breakdown of fibers is obtained by long treatment with pyridine and acetic anhydride at a high temperature. Triacetates obtained from ramie in this way swell only to a limited extent and are insoluble in the usual solvents (Hess and Lubitsch[280]). It seems that insolubility depends upon the existence of small regions, probably in the interior of the crystallites, which have escaped acetylation and are responsible for the coherence of the whole.

Fibers can also be acetylated by a solution of potassium acetate in glacial acetic acid and acetic anhydride. This reaction has been studied in ramie by Hess and Trogus using the x-ray method, figure 17, page 342, reproduces x-ray diagrams of acetylated products. It is apparent that, up to about 30% acetyl, that is, up to half the theoretical acetyl content, the lattice of native cellulose is almost unchanged. The interior of the micelle must therefore be intact: neither acetylation nor mercerization has taken place. It follows that the acetyl groups must all lie at the surface of the micelles, and we have, consequently, a particularly clear case of a micellar surface reaction. At higher acetyl contents, the oblique reflections become more diffuse, while the meridian reflections remain clear; the equatorial reflections disappear and new spots appear. Finally, a new lattice is formed, which is clearly recognizable by the time that the acetyl content reaches 62%. This leads us to suppose that, over the transition region, intramicellar acetylation takes place statistically along the fiber axis in a permutoid manner. Hess, on the other hand, interprets the diffuse diagrams as mixed diagrams, and supposes that the micelles are acetylated from the outside inward, and that the reaction proceeds slowly into the interior of the micelles.

Hess has recorded two crystalline forms of cellulose triacetate, cellulose triacetate I which is formed on acetylation below 50°C., and cellulose triacetate II, formed at higher temperatures. The identity period along

[279] H. Staudinger and G. Daumiller, *Ann.*, **529**, 219 (1937).

[280] K. Hess and N. Lubitsch, *Ann.*, **466**, 1 (1935).

the fiber axis is 10.3 Å. in both; the lattice of the form II is more perfect than that of form I; this can be seen from the sharpness of the diagram and the abundance of reflections. Form I takes up organic solvents into progressive expansion of the lattice; form II does not. On saponification, form I yields cellulose T, form II, cellulose II. At low temperatures, form I is obtained from solvents. In the light of these facts, it appears to us that I is a form with a distorted lattice.

Degraded cellulose triacetate can be precipitated from solution in the form of crystalline bundles. The phenomenon is probably similar to that of the crystallization of polyethylene succinate (Chap. D, sect. IV 1*a*).

In commercial practice acetylation is carried out with sulfuric acid or zinc chloride in a mixture of acetic acid and acetic anhydride. The added reagents presumably act in three ways: (*1*) they form addition compounds with the cellulose and thereby break up the lattice structure; (*2*) they have a catalytic effect on esterification; and (*3*) they tend to hydrolyze the product. This hydrolytic degradation appears to be essential for the production of technical cellulose acetate in soluble form.

In order to obtain a product which is soluble in acetone (secondary acetate or Cellite) the acetylation is reversed in solution by adding water until approximately one sixth of the acetyl groups have decomposed. At the same time any sulfoacetate which may be present is decomposed. Acetate poor in acetic acid and soluble in acetone does not show any spot or fiber diagram even when in the form of drawn threads or films. The diagram for cellulose only appears after saponification if this is carried out in such a way as not to affect the structure and it then shows the prevailing degree of orientation (Mark and von Susich). The mode of reaction in the solid phase is in harmony with this behavior; secondary acetates behave like a liquid in which reagents dissolve as they penetrate; that this is so appears from the old experiments of Knoevenagel[281] and from later work by Meyer and Schuster,[282] and Kartaschoff.[283]

Although the triacetate is only soluble in a few solvents such as glacial acetic acid, chloroform, dichloroethane, or methylene chloride, the partially saponified, acetone-soluble product will dissolve in a large number of organic solvents or mixtures of solvents. According to Dobry and Duclaux,[284] it is soluble in concentrated aqueous solutions of magnesium per-

[281] E. Knoevenagel, *Kolloid-Beihefte*, **13**, 233 (1921).

[282] K. H. Meyer, C. Schuster, and W. Bülow, *Melliand Textilber.*, **6**, 737 (1925).

[283] H. Kartaschoff, *Helv. Chim. Acta*, **9**, 152 (1926).

[284] A. Dobry, *Bull. soc. chim.*, **3**, 312 (1936). M. Amat and J. Duclaux, *J. chim. phys.*, **35**, 147 (1938).

chlorate, from which it can be precipitated by the addition of water, a method which the authors recommend for the preparation of ultrafilters.

(g) *Other Cellulose Esters*[285]

Sulfuric acid esterifies cellulose and at the same time causes considerable breakdown, yielding water-soluble cellodextrin sulfuric acids. Gaseous sulfur trioxide converts dry cellulose into a primary sulfuric acid ester soluble in water, with all the hydroxyl groups esterified. The compound has a copper number of 0.3 and so must be degraded to a considerable extent (Traube[286]). Cellulose precipitated from cuprammonium can be esterified in pyridine with chlorosulfonic acid.

In the manufacture of parchment paper, small quantities of sulfuric acid enter into combination with the cellulose. The mixed esters which are formed during nitration have already been mentioned. A product is obtained from alkali cellulose[287] and toluenesulfonyl chloride, in which the micellar surface is covered with toluenesulfonyl groups. By superficial toluene sulfonation, the affinity of cellulose fibers for substantive dyes can be abolished. Use of this reaction is made in the production of cross dyed effects.

In the presence of ammonia the toluenesulfonyl groups are in part exchanged for amino groups; this exchange is successful, however, only to a limited extent. Such aminocellulose takes up acid dyestuffs.[288]

Esters of cellulose with medium or higher fatty acids have been much investigated; they are obtained in the same way as cellulose acetate, by acid esterification or in pyridine. The propionate is used commercially; the tributyrate has been more exactly described.[289]

Esters of higher organic acids have been obtained by treatment with acyl chlorides and pyridine or quinoline (Grün and Wittka,[290] Karrer and Zega,[291] Gault and Ehrmann,[292] Kita, Sakurada, and Nakashima[293]).

[285] See account in D. Krüger, *Celluloseacetate und die anderen organischen Ester der Cellulose.* Steinkopff, Dresden, 1933.

[286] W. Traube, B. Blaser, and C. Grunert, *Ber.*, **61**, 754 (1928).

[287] Textilwerk Horn, German Pat. 396,926 (1922).

[288] P. Karrer and S. C. Kwong, *Helv. Chim. Acta*, **11**, 525 (1928); German Pats. 459,200 and 438,324.

[289] W. O. Baker, C. S. Fuller, and N. R. Pape, *J. Am. Chem. Soc.*, **64**, 776 (1942).

[290] A. Grün and F. Wittka, *Z. angew. Chem.*, **34**, 645 (1921).

[291] P. Karrer, J. Peyer, and Z. Zega, *Helv. Chim. Acta*, **5**, 853 (1922). P. Karrer and Z. Zega, *ibid.*, **6**, 822 (1923).

[292] H. Gault and P. Ehrmann, *Bull. soc. chim.*, **39**, 873 (1926); *Compt. rend.*, **177**, 124 (1923).

[293] G. Kita, I. Sakurada, and T. Nakashima, *Cellulosechemie*, **9**, 13 (1928).

These are probably considerably degraded products. As the length of the paraffin chain introduced into the molecule increases, the esters become more and more waxlike both in respect to their consistency and to their solubility; cellulose tristearate, for example, softens at 75°C., while the laurate does not begin to melt until 90°. The mechanical properties have been systematically investigated by Hagedorn and Möller[294] and Sheppard and Newsome.[295] The monoesters of higher fatty acids are scarcely soluble, in contrast to the more highly esterified products which are soluble in lipoid solvents. A number of mixed organic esters have been described: for example, acetobutyrate, acetolaurate, acetophthalate, etc. (see Hagedorn[296]). Some of these are manufactured on a commercial scale and are used in the manufacture of varnish; others become rubbery at higher temperatures.

Acid esters of cellulose with dibasic acids such as monocellulose succinate have been proposed as ion exchangers.[296a] By impregnating cotton fabric with a concentrated solution of sodium aminoethyl sulfate and subsequent heating, basic groups can be attached to the cellulose.[296b]

4. Cellulose Ethers

Alkali cellulose can be converted into ethers by treatment with alkyl halides or alkyl sulfates.[297] Incompletely methylated products with 1.5–2 methoxyl groups per glucose residue such as occur in technical methyl cellulose are water-soluble; more completely methylated products are insoluble. Degradation favors solubility; strongly degraded products may be water-soluble even if highly methylated.

Cellulose cannot be completely methylated without some degree of degradation, as has already been observed by Karrer and Escher[298]; Johnston[299] explaining this by supposing that products having a degree of methylation exceeding a certain limit become completely insoluble in the methyla-

[294] M. Hagedorn and P. Möller, *Veröffentl. wiss. Zentral-Labs. phot. Abt. I. G. Farbenind. Akt.-Ges. AGFA*, 1, 150 (1930).

[295] S. E. Sheppard and P. T. Newsome, *J. Phys. Chem.*, 39, 143 (1935).

[296] M. Hagedorn and P. Möller, *Cellulosechemie*, 12, 29 (1930). C. J. Malm and C. R. Fordyce, *Ind. Eng. Chem.*, 32, 405 (1940).

[296a] F. C. McIntire and J. R. Schenck, *J. Am. Chem. Soc.*, 70, 1193 (1948).

[296b] C. L. Hoffpauir and J. D. Guthrie, *J. Biol. Chem.*, 178, 207 (1949).

[297] See, for example, K. Hess, G. Abel, W. Schön, and V. I. Komarewsky, *Cellulosechemie*, 16, 69 (1935).

[298] P. Karrer and E. Escher, *Helv. Chim. Acta*, 19, 1192 (1936).

[299] G. G. Johnston, *J. Am. Chem. Soc.*, 63, 1043 (1941). See also J. F. Mahoney and C. B. Purves, *ibid.*, 64, 15 (1942).

tion mixture and so react no further. According to Freudenberg,[300] methylation may be taken nearly to completion with sodium and methyl iodide in liquid ammonia; but even here there must be some considerable decomposition, as is shown by the fall in viscosity.

The work of Hess, Trogus, and collaborators[301] who followed the course of the reaction by means of x-rays showed that the equatorial interference spots of trimethyl cellulose appear even in preparations of a relatively low degree of methylation. Hess explains this as due to reaction starting at the outside of the fibers, forming complete crystallites of trimethyl cellulose in the cortical regions before the reaction spreads to the interior of the fibers at all. This seems plausible, the more so as the methylation mixture is scarcely if at all soluble in water so that the reaction takes place in a system which is quite grossly heterogeneous. Intermediate stages must be present, however, as hydrolysis yields a mixture of glucose residues in all stages of methylation.

Trimethyl cellulose yields a good fiber diagram if prepared in the fibrous form (Hess and Trogus[302]); von Susich[303] has determined the period along the fiber axis as 10.3 Å.

Degraded trimethyl cellulose melts between 227° and 240°C.; on solidification various forms differing in their lattice spacing are obtained according to the conditions (Hess, Trogus, and Abel[304]). Like cellulose triacetate, degraded trimethyl cellulose can be obtained from organic solvents in crystalline bundles which can be recognized microscopically. No accurate information is available about the chain length of these substances.

Water-soluble methyl cellulose is precipitated on warming the aqueous solution. This interesting phenomenon has been studied repeatedly, most extensively by Heymann.[305] We shall discuss this behavior in Chap. H, sect. IV 3*c*. The phenomenon recalls the corresponding behavior of many ethers and alcohols of low molecular weight; butyl alcohol, for example, shows a decline in solubility with rising temperature over a certain temperature range.

Ethyl and benzyl ethers have also been described; as might be expected from the nature of the substituent groups, they are less hydrophilic than the methyl ethers. Benzyl ether (from soda cellulose and benzyl chloride) is

[300] K. Freudenberg and H. Boppel, *Ber.*, **70**, 1542 (1937).

[301] K. Hess, C. Trogus, W. Eveking, and E. Garthe, *Ann.*, **506**, 260 (1933).

[302] K. Hess, C. Trogus, and H. Friese, *Ann.*, **466**, 80 (1928).

[303] K. H. Meyer and H. Mark, *Aufbau der hochpolymeren organischen Naturstoffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, page 145.

[304] K. Hess, C. Trogus, and G. Abel, *Cellulosechemie*, **16**, 79 (1935).

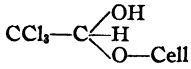
[305] E. Heymann, *Trans. Faraday Soc.*, **31**, 846 (1935).

used for injection molding (see E, III 5*d*). Mahoney and Purves[306] found for ethyl cellulose a mean degree of substitution of 2.48 ethoxyls and 0.52 free hydroxyls per glucose residue; 0.13–0.14 free hydroxyls were in position 2, 0.24–0.28 in position 3, and 0.12, as a primary hydroxyl, in position 6. From this it appears that the primary hydroxyl is the most rapidly etherified. Hydroxyethyl cellulose can be prepared by the action of ethylene oxide on alkali cellulose.[307] It can be obtained in the form of solid, elastic films. Partially hydroxyethylated cellulose yields freely soluble products on nitration or acetylation.

The action of formaldehyde on cellulose in the presence of weak acids gives rise to methylene ethers in which neighboring primary valence chains are connected to one another by methylene bridges, which have a marked effect in decreasing swelling and solubility.[308] This reaction is important in modern techniques for improving the stability of cellulose filaments to water or mechanical abrasion. Other bivalent etherifying agents also have an effect in decreasing the solubility of cellulose derivatives, or in the case of soluble products, of increasing their viscosity; here again their action is presumably due to the formation of cross linkages. By the introduction of ethers incorporating long-chain residues, cellulose fibers can be made water-repellent. For example, cellulose which has been impregnated with octadecyloxymethylpyridinium acetate forms an ether and becomes waterproof on heating (Velan process of Imperial Chemical Industries):

$$C_{18}H_{32}—O—CH_2—NC_5H_5 \\ \quad\quad\quad\

that secondary hydroxyls must be affected. These ethers are soluble in pyridine.[311] A hemiacetal-like compound:



is formed between regenerated cellulose and chloral in pyridine or quino line; this is soluble in pyridine.[312]

5. Commercial Applications of Cellulose Esters and Ethers

(*a*) *Nitrocellulose* (*Cellulose Nitrate*)

Commercial nitrocelluloses may differ in three respects: (*1*) in the mean chain length; (*2*) in the nitrogen content; (*3*) in the distribution of nitrogen. The degree of uniformity is very varied in commercial nitrocelluloses; a nitrocellulose with 11% nitrogen content (2 to 2.5 nitrate groups) contains nitrate groups uniformly and statistically distributed over all the glucose groups if esterification has proceeded to equilibrium; if equilibrium has not been reached, the interior of the fibrils will contain fewer nitrate groups than the exterior, so that the nitrogen content must be taken as a mean value. As a rule, such nitrocelluloses are only partially soluble in typical solvents, since the outer parts dissolve while the inner regions remain intact; the degree of solubility may in fact be used to characterize preparations.[313] It is scarcely to be doubted that such nonuniformity of the initial material lowers the quality of the final product. The solubility in various media and the power of gelatinizing in imbibition agents are determined chiefly by the nitrogen content. This also influences the viscosity; thus, for example, the viscosity in acetone increases with increasing nitrogen content.[314]

For commercial purposes, nitrocelluloses can be divided into three classes according to the nitrogen content: (*1*) Ester-soluble nitrocellulose containing 11.8–12.4% nitrogen and practically insoluble in alcohol. RS nitrocellulose manufactured by the Hercules Powder Co. is an example; its nitrogen content varies between 11.80 and 12.20%. (*2*) Nitrocellulose soluble in a mixture of ether and alcohol, containing 11.3–11.7% nitrogen

[311] W. M. Hearon, G. D. Hiatt, and C. R. Fordyce, *J. Am. Chem. Soc.*, **65**, 2449 (1943).

[312] K. H. Meyer and W. Ruppel, German Pat. 408,821 (1925), Bad. Anil. Soda Fabrik. K. H. Meyer, M. Studer, and A. J. A. van der Wyk, *Monatsh. Chem.*, **81**, 151 (1950).

[313] See F. Ullmann, *Enzyklopädie der technischen Chemie.* 2nd ed., Urban & Schwarzenberg, Berlin, Vol. 4, page 579.

[314] See review by J. Barsha, in J. Alexander, *Colloid Chemistry*, Vol. VI, page 860.

the commercial importance at the present time is slight. This product is called AS nitrocellulose by Hercules. (*3*) Alcohol-soluble collodion cottons containing 10.5–11.2% nitrogen. SS nitrocellulose of Hercules is an example; it contains 10.80–11.10% nitrogen.

In each of these classes products of various degrees of degradation are prepared, which are characterized by their viscosity. Too great a chain length is a hindrance in the preparation of films from solution, or of pyroxylin from the swollen product; too short a chain length influences the quality of the end product unfavorably. The material may be more or less homogeneous with respect to chain length. Since small quantities of very long chains introduce the disadvantage of high viscosity, while, on the other hand, small quantities of very short chains diminish the mechanical strength, the highest possible uniformity is desirable.

Breakdown takes place to a certain extent during nitration, since the aqueous nitration mixtures produce hydrolytic splitting of the chains. Nitrocellulose of very low viscosity may be obtained by heating nitrocellulose in an autoclave with water.

The viscosity is determined for solutions of definite concentration by means of various viscometers of conventional design. In the Cochius viscometer, the time required by an air bubble to rise through a vertical tube, 7 mm. wide and 50 cm. long, filled with the solution, is measured. In the falling ball method,[315] a steel ball, weighing 2.0385 ± 0.007 g. and 7.938 ± 0.01 mm. in diameter, is allowed to fall through a liquid column 25 cm. high and 25.0 ± 0.5 mm. in diameter. The time in seconds taken by the ball to fall through this distance serves to characterize the solution. Since the viscosity depends very largely upon the concentration, it is necessary to record the concentration of the solution with each determination of the time required. The viscosities and uses of various commercial nitrocelluloses are given in Table VIII.

Kraemer[316] has determined the molecular weight of various nitrocelluloses by means of the ultracentrifuge and obtained values from 125,000 to 150,000 for celluloid and of 45,000 for 0.5-second nitrocellulose.

The presence of very short chains, which influence the mechanical properties of the film unfavorably and cannot be detected viscometrically, is tested in the following way. The product is denitrated in the usual way and the alkali- or baryta-resistant part of the regenerated cellulose is determined. A theoretical yield should be obtained from nitrocellulose of the

[315] The numbers refer to the so-called falling ball or Hercules method; this is the approved A. S. T. M. method.

[316] E. O. Kraemer, *Ind. Eng. Chem.*, **30**, 1200 (1938).

TABLE VIII

Viscosity and Uses of Commercial Nitrocelluloses

	Hercules Powder Company			[η]
Nitrogen range	11.8–12.2%	11.4–11.7%	10.5–11.2%	
Type	RS	AS	SS and Herculoid	
Viscosity and uses				
Specialty uses	600–1000 sec.	—	—	~4.0
	250–400 sec.	—	—	
Leather lacquers	125–175 sec.	—	—	~2.0
Silver lacquers	60–80 sec.	—	—	
Bronzing lacquers				
Film, artificial	30–40 sec.	30–40 sec.	20–60 sec. (Plastic)	~1.7
Leather, flexible	15–20 sec.	—	—	
Lacquers				
Lacquers for general	5–6 sec.	—	5–6 sec.	~1.2
use on metal and	0.5 sec.	0.5 sec.	—	~0.6
wood	0.25 sec.	—	0.25 sec.	~0.3
	18–23 cp.	—	—	—

desired long chain length; only strongly degraded nitrocelluloses yield alkali-soluble products.

Highly nitrated cellulose (gun cotton) is used for the preparation of explosives. For this purpose it is gelatinized, that is, kneaded with swelling agents, so that a plastic mass is formed. Complete gelatinization is not necessary for many purposes; in such preparations partially swollen portions are embedded in the swollen mass. Nitroglycerin, acetone, acetic esters, etc., are used as swelling agents. The plastic mass (gelignite) is worked into thin tubes, rods, or plates. In order to avoid electrostatic charging, these are dusted with finely powdered graphite (hence the glossy black appearance of many smokeless powders).

The plastics prepared from swollen nitrocellulose—pyroxylin[317] films and lacquers—are of very great economic importance. In the manufacture of pyroxylin, pyroxylin nitrocotton is kneaded with alcohol and camphor (if necessary with plasticizers such as dibutyl phthalate or tricresyl phosphate) to form a doughlike mass. After evaporating excess alcohol, pressing through filter cloths, and still further kneading and mixing between warmed iron rollers, the product is rolled out into smooth sheets, which are laid one on top of another and pressed together while being heated for a fixed period. In this process internal tensions are relieved. The blocks so obtained are then cut up into sheets of various thicknesses or into rods. Tubes are not drawn from hydraulic extrusion presses.

[317] Pyroxylin is often called "celluloid" in the European literature. Celluloid is a trade name in U. S. A. and designates only products of the Celanese Corporation of U. S.

Pyroxylin may be cut, polished, and stamped, and also worked at higher temperatures. It softens at 70–80°C. at which temperatures it may be bent, pressed, drawn, or blown; separate pieces can be welded together, so that if two sheets are laid between the halves of a hollow mold, and hot air is blown between them, the sheets assume the form of the mold and fuse together where they are in contact.

Pyroxylin films are obtained from concentrated viscous solutions of about 20 parts of film nitrocotton (that is, nitrocellulose of 12% nitrogen and 40 sec. viscosity) with about 3 parts of camphor in about 77 parts of a mixture of ether and alcohol (1 alcohol to 2 ether). The solution is poured out on a smooth surface and the solvent allowed to evaporate. Methanol may be used instead of alcohol; frequently butyl or amyl alcohol is added to the mixture.

Artificial leather is prepared by painting fabrics with solution of collodion nitrocotton and plasticizers (tricresyl phosphate), drying, and stamping.

For spray lacquers, such as are used to a very great extent in the automobile industry at the present time, nitrocellulose degraded by heating with water under pressure is used, together with a great variety of softeners. Pyroxylin is unsuitable for injection molding because of its nonresistance to heat.

(*b*) *Cellulose Acetate*

Owing to the inflammability of nitrocellulose, cellulose acetate is used more and more as a substitute. For example, for photographic films it will probably replace nitrocellulose entirely. The range of application of cellulose acetate is similar to that of nitrocellulose; it may be used for plastic moldings, for films, and for lacquers. As with nitrocellulose, grades may be prepared of different degrees of esterification and different mean chain length. The degree of esterification determines the solubility and the compatibility with softeners; chain length affects the viscosity and mechanical properties of the product. Triacetate which has not been broken down is insoluble; if the chain length is not too great, it is soluble in glacial acetic acid, methylene chloride, and chloroform. Partially saponified cellulose acetate containing about 52–56% acetic acid[318] ("secondary acetate") is soluble in acetone, methyl acetate, and numerous other solvents (see Hercules Powder Co. booklet).

Sheets are prepared in a manner similar to that used for pyroxylin; they are sold as "acetyl celluloid" or under trade names such as Cellon or Ecarit.

[318] The figures indicate the amount of acetic acid obtained on saponification expressed as a percentage of the initial material.

Instead of camphor, triphenyl phosphate, tricresyl phosphate, and dibutyl phthalate are used as plasticizers and, in place of alcohol, a mixture of benzene and alcohol is used; the sheets are worked up as for pyroxylin. In contrast to nitrocellulose, however, cellulose acetate which has been well plasticized is very suitable for injection molding.

Transparency and light resistance are other commercial advantages of cellulose acetate. Cellulose acetate films are cast from acetone solution with added plasticizers.

Cellulose acetate is also used in the manufacture of artificial silk (acetate rayon) and acetate flock, the latter being the staple fiber, prepared by cutting the continuous filament into lengths of a few centimeters. The concentrated solution in acetone or methylene chloride is squirted through nozzles into a warm air shaft where the solvent evaporates and the fiber solidifies and is at the same time drawn out by means of a stretching and winding mechanism. Acetate silk has a characteristic sheen; it cannot be dyed with dyes used for wool, silk, or cotton. It is colored by the addition of pigments, such as aminoanthraquinone and hydroxyazo compounds, which are only slightly soluble in water but dissolve freely in esters. They are emulsified in water in the presence of a protective colloid, and can thus be taken up by the fiber, in which they dissolve.[319] Dyeing is not in this case a salt formation, as with wool, or surface adsorption, as with cellulose, but a process which may be compared with the transfer of an ether-soluble substance from water to the ether phase.

Recently the triacetate has attracted considerable interest on account of its high resistance to water and to heat, and its improved dielectric properties. Thin films between 0.01 and 0.1 mm. in thickness are used in the cable industry (Triafol). Cellulose acetate is also used for lacquers and, in particular, for insulating lacquers, lacquer for cables, electric lamps, and airplane dopes.

Cellulose propionate (Forticel) is used for plastic moldings. It rivets well and has high impact strength and low water absorption.

(*c*) *Mixed Esters*

Cellulose acetate-butyrate is used as a basis for lacquer (trade names Cellit B and Hercose C). It is said to be more compatible with plasticizers

[319] E. Knoevenagel, *Kolloid-Beihefte*, **13**, 192, 233 (1921). A. G. Green and K. H. Saunders, *J. Soc. Dyers Colourists*, **39**, 10 (1923). K. H. Meyer, C. Schuster, and W. Bülow, *Melliand Textilber.*, **6**, 737 (1925); **7**, 29 (1926). V. Kartaschoff, *Helv. Chim. Acta*, **8**, 928 (1925); **9**, 152 (1926).

and resins than the pure acetate and is recommended for the preparation of particularly smooth lacquer[320] (such as is used for airplane wings). On account of its greater water resistance and better dielectric properties, it is also preferable to the pure acetate for the manufacture of insulating lacquers; the Hercules Powder Co. manufactures three brands of different viscosities.

(*d*) *Cellulose Ethers*

Water-soluble methyl cellulose is used in the manufacture of printing paste for printing on fabric (Methocel, Tylose) and in the preparation of adhesives and toilet preparations. It is converted into a three-dimensional network by heating with formaldehyde.

Ethyl cellulose is used as a basis for protective coatings. Thin films are useful as water-proof packing and in sheet form it is used in the manufacture of semirigid containers.

Benzyl cellulose may be used without modification for injection molding. It is resistant to weak acids and alkalis but is attacked by strong acids or alkalis, acetone, benzene, or gasoline It is moderately resistant to alcohol.

Cellulose which has been converted to the sodium salt of celluloseglycolic acid by treatment with alkali and chloroacetic acid is water-soluble and is used as an emulsifying agent and an adhesive.

Appendix: Vulcanized Fiber

Together with the artificial materials prepared from cellulose esters, brie mention may be made of vulcanized fiber, prepared from cellulose which has been rendered plastic. Paper is drawn through a concentrated solution of zinc chloride and the superimposed sheets are pressed together between rollers. In this process the fibers swell and fuse together. The plates obtained in this way are washed in water for weeks or months until all zinc chloride has dialyzed out. They are then dried and smoothed between rollers. The cellulose mass obtained in this way is hard and very tough; it can be bent, cut, bored, and stamped, but it is not thermoplastic. Its range of application is considerable; although scarcely suitable as insulation material, its particularly good mechanical properties render it valuable for articles which are exposed to severe mechanical usage, such as trunks, brake linings, and wheels.

[320] W. E. Gloor, *Ind. Eng. Chem.*, **29**, 690 (1937).

IV. MECHANICAL PROPERTIES OF CELLULOSE AND ITS DERIVATIVES

In view of the great variety of cellulose preparations and their complicated texture, it is not possible to give a quantitative account of the relation between mechanical properties and composition and structure. Many such relationships can, however, be described in a qualitative way and it may be worth while to review them.

1. Tensile Strength

(a) *Breakage as a Result of Rupture of Chains*

The tensile strength of native cellulose fibers is extraordinarily high. From the values for the tensile strength of several other important materials (given in Table IX) it is clear that only the finest steel possesses a much higher tensile strength than the best celluloses.

TABLE IX

Tensile Strength of Various Industrial Materials

Material	Tensile strength, kg./mm.²	Material	Tensile strength, kg./mm.²
Cast iron	10–20	Cotton	20–80
Hardened steel spring	60–300	Irish flax	More than 100
Copper wire	45–50	Viscose (sheet)	4–13
Aluminum	20–30	Well-oriented viscose	40–80
Lead	2–2.5	Acetate (sheet)	4–9
Wood (parallel to fibers)	6–12	Well-oriented acetate rayon	40–100
Silk	35–60		

This strikingly high value may be deduced from the primary valence chain structure of native fibers, as the following considerations show (Mark).[321] In a fiber composed of primary valence chains of indefinite length, each extending over the whole length of the fiber, all the chains must be broken in the same region. From the projection of the unit cell it follows that the cross-sectional area of a chain amounts to about 32 Å., that is, a fiber 1 sq. mm. in cross section will consist of about 3×10^{12} primary valence chains lying parallel to each other. We may suppose that the work necessary to rupture such a fiber is equal to the chemical work required to separate the organic primary valence linkage. This is of the order of 70 kg

[321] H. Mark, *Melliand Textilber.*, 10, 695 (1929).

cal. per mole or 5×10^{-12} erg for an individual primary valence chain, if we assume that this ruptures at the oxygen bridge. Interatomic forces diminish very rapidly with distance, and the potential energy of two atoms displaced by about 2 Å. from their equilibrium position is certainly only of the order of 1 kg. cal.

If now one assumes, as an approximation, that a separation of this amount occurs and that the force remains constant over this distance, that is, if one calculates a "mean" tensile strength, one obtains from the equation, work = force × distance the force necessary for the rupture of a primary valence chain (= work/distance): $15/(2 \times 10^{-8}) \cong 8 \times 10^{8}$ dyne per sq. mm., or about 800 kg. per sq. mm. A more refined calculation by

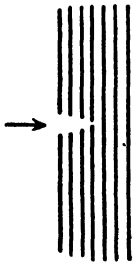


Fig. 22. Fracture resulting from rupture of chains.

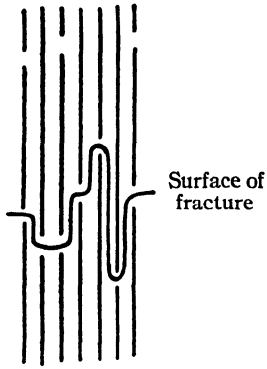


Fig. 23. Fracture resulting from slipping.

de Boer[322] yields a value of 2000 kg. per sq. mm. The theoretical values for the tensile strength considerably exceed the experimentally determined values, and we must assume that in practice the tension is not shared equally between the primary valence chains as we have assumed. If it is not, the most heavily loaded chains will rupture first, the rest will follow (see Fig. 22). The less the orientation, the less the number of chains and micellar threads simultaneously placed under tension, and the less the tensile strength. The highest values have been obtained from slender fibers, as was to be expected; it may be assumed that still higher values will be obtained when it is possible to isolate and test individual fibrils.

With well-oriented bast fibers (ramie), nitration or acetylation of the

[322] J. H. de Boer, *Trans. Faraday Soc.*, **32**, 10 (1935). See also P. H. Hermans, *Rec. trav. chim.*, **58**, 63 (1939).

fibers does not influence the load at which the fiber ruptures (Sakurada).[323] Secondary valences between the chains do not, therefore, play any part in determining the tensile strength. Sakurada concludes from this that the rupture of the fibers takes place by fracture of the chains. Recent observations of Bridgman[323a] with inorganic fibers, however, show that breaking is a very complex phenomenon in which chemical reactions with water or oxygen are involved.

(*b*) *Rupture by Slipping*

The picture of a rope of indefinitely long primary valence chains does not correspond to any artificial fiber. In well-oriented artificial fibers we must suppose that the chains, which are of limited length (of the order of 10^3 Å.), are associated over part of their length to form a true lattice as a result of the interaction forces between the hydroxyl groups. Rupture may occur as a result of fracture of the primary valence chains, or slipping of the chains over one another (see Fig. 23), or both. In artificial silk prepared from material which has been relatively carefully treated, and in which the orientation is good, the internal cohesion of the chains is so considerable that, in practice, chain fracture only is concerned, and conditions are similar to those in the native fiber. The tensile strength of well-oriented artificial fibers therefore approaches that of native fibers.

In slipping, irreversible deformation gradually leads to thinning of the fiber and ultimately to breakage; a slow rise in tension should favor slipping, while a rapid rise in tension should produce rupture of the chains.

(*c*) *Tensile Strength and Humidity Content*

Slipping takes place more readily and becomes more noticeable, the shorter the chains; it is also favored by swelling agents which diminish the cohesion of chains and micellar bundles. A fiber which is built up exclusively from long chains, which fails by fracture of the chains, should not be weakened by water, whereas a fiber consisting of shorter chains, which fails by slipping, will be much more affected. In practice we find that the wet strength of natural fibers is not lower than the dry strength, while the wet strength of artificial fibers is considerably lower than the dry strength (Table X). For practical purposes the dry strength refers to material in an

[323] I. Sakurada, *Papier-Fabr.*, **36**, 252 (1938).

[323a] P. W. Bridgman, *J. Applied Phys.*, **18**, 246 (1947).

atmosphere of 65% relative humidity; the strength of completely dry fibers should be considerably higher. The sensitivity to moisture is greater in poorly oriented fibers containing many amorphous regions than in well-oriented fibers; it is much greater in degraded material than in a product which has been prepared with great care.

TABLE X
Tensile Strength[324]

	Tensile strength, kg./sq. mm.		Relative wet strength $\left(\frac{\text{Wet}}{\text{Dry}}\ \%\right)$	Extension at break, %	
	Dry	Wet		Dry	Wet
Ramie	91–95	108	~117	2.3	~2.4
Cotton	20–80	24–83	~105	16–12	6–13
Flax	84	88	~105	1.8	2.2
Viscose	20–40	10–20	~ 50	8–26	13–43
Highly oriented viscose	62	53	~ 86	9	9
Cellulose acetate fiber	16–21	10–12	~ 65	21–30	29–30

(*d*) *Tensile Strength and Chain Length*

In degraded cellulose as well as in artificial silks, rupture is due not to the breaking of primary valence chains but to slipping between them. In this process, the secondary valence linkages which hold the micelles together must be overcome; slipping will begin when the energy required is less than that for the rupture of a primary valence linkage. If we assume that the energy required to start slipping, by releasing the linkage between hydroxyl group 6 and hydroxyls 2 and 3 of the glucose units in neighboring chains, amounts to 1000 to 2000 cal., then if 70 glucose units of one chain are associated with 70 units of a neighboring chain, 70–140 kg. cal. will be required to effect a separation, which is greater than the energy required to break a chain at a glucosidal linkage. From this it appears reasonable to assume that, in preparations where the chain length is of the order of several hundred glucose units, failure is always by rupture of the chains rather than by slipping. Preparations of high chain length will, therefore, exhibit a tensile strength independent of the number of links in the chain. Accordingly, a certain degree of degradation does not weaken cellulose, and in artificial silks and films, where it is desired to avoid the disadvantages of highly viscous solutions, preparations can be used of a chain length just sufficient to yield a tensile strength approximating that of a natural fiber. These considerations afford an explanation of the fact, which has long been

[324] O. Schmidhäuser, *Melliand Textilber.*, **17**, 905 (1936).

known, that a certain amount of degradation does not diminish the tensile strength; the diminution in viscosity, indicating that breakdown has taken place, is not accompanied by a decrease in tensile strength.

Systematic investigations by Weihe[325] have shown this particularly clearly. Weihe prepared a series of nitrocelluloses of approximately equal nitrogen content and prepared from similar initial material, but covering the whole range of viscosities. From these he cast films by a standard procedure, and found that their mechanical properties were the same over a considerable part of the viscosity range. When the viscosity lay below a certain value (determined by the initial material and the conditions of nitration) a sudden decline in the mechanical properties was observed. The lowest members of the series (from $[\eta] = 0.2$ downward) would not form films at all; in the middle of the series tensile strength and viscosity ran parallel.

In a similar manner Clibbens and Ridge[326] investigated the relationship between the viscosity (in cuprammonium solution) and the tensile strength of damaged natural fibers (flax and cotton), and found that the tensile strength diminishes with falling viscosity over a certain range. According to Staudinger and Sorkin[327] the tensile strength and extensibility of cotton fibers diminish much less rapidly with progressive acid attack than the resistance to flexion. Down to a viscosity of $[\eta] = 3$ cotton shows no appreciable fall in tensile strength. The resistance to flexing is the only property which is already affected by a moderate breakdown.

The strength of pulps depends not only on the chain length, that is, on the degree of hydrolytic breakdown during purification, but also on the quantity of associated substances still present. There is therefore no general correlation[328] between strength and viscosity,[329] although there appears to be a lower limit of viscosity below which the tensile strength is no longer sufficient for commercial purposes. For good rayon pulp $[\eta]$ in cuprammonium should not be less than 3. The presence of a relatively small proportion of components of low molecular weight may reduce the strength of rayons below the commercially required value; it is, therefore,

[325] A. Fausten and A. Weihe, in Berl-Lunge, *Chemisch-technische Untersuchungsmethoden*, Vol. V, 8th ed. Springer, Berlin, 1934, page 940. See also G. G. Jones and F. D. Miles, *J. Soc. Chem. Ind.*, **52**, 251T (1933).

[326] D. A. Clibbens and B. P. Ridge, *J. Textile Inst.*, **19**, 389T (1928).

[327] H. Staudinger and M. Sorkin, *Ber.*, **70**, 1565 (1937). H. Staudinger, M. Sorkin, and E. Franz, *Melliand Textilber.*, **18**, 681 (1937).

[328] E. Hägglund, *Papier-Fabr.*, **34**, 313 (1936). See also H. Staudinger and F. Reinecke, *ibid.*, **36**, 489 (1938).

[329] See also H. Staudinger, *Zellstoff u. Papier*, **18**, 459 (1938).

essential to select raw materials free from components of low molecular weight; excessive breakdown of individual batches must also be avoided. The tensile strength of native fibers and pulps may be very considerably influenced by uneven breakdown, although the viscosity may not fall below the limit.

We have seen that acids and metallic halides have a degrading action, and to these must be added alkali in the presence of air, as well as oxidizing bleaching agents. This oxidative breakdown is markedly accelerated by traces of metallic oxides, such as iron oxide. Traces of strong acids, left by imperfect washing, render the tissue fragile in the course of time, as a result of gradual hydrolytic breakdown, and are particularly dangerous. The cause of abnormal weakening of the fiber is almost invariably to be sought in a previous breakdown, resulting from too ruthless chemical treatment. It is almost always possible to restrict the degrading action considerably without influencing the commercial result required.

(e) *Tensile Strength and Orientation*

The tensile strength of artificial fibers depends to a large extent on the degree of orientation; in poorly oriented fibers the entire load is taken by the fibers lying parallel to the fiber axis, which may make up only a fraction of the total number present. These are the first to rupture, and the rest follow, thus giving a low over-all strength.

By gradually increasing the orientation of suitable material, it is possible to increase the tensile strength about threefold.[330] This would indicate that in moderately oriented fibers the load is taken by about one third of the chains, which happen to lie nearly parallel, an explanation which appears plausible enough. Examples of the difference in tensile strength between oriented and nonoriented materials are provided by the tensile strength of viscose rayon: normal ($\sim$ 25), oriented ($\sim$ 80 kg. per sq. mm.); and acetate rayon: normal (16–20), oriented (80 kg. per sq. mm.).

In well-oriented natural and artificial fibers the anisotropy of the linkages (primary valences along the fiber axis and secondary valences at right angles thereto) is associated with very marked anisotropy in mechanical properties. The stronger the fiber is in the direction of the fiber axis, the weaker it is in the transverse direction, and the more readily it splits on twisting, for example. In nature this is guarded against by the presence of

[330] K. H. Meyer and H. Mark, *Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930, page 202. See also the values for highly oriented and normal viscose in Table IX.

cementing substances and by a thin layer of fibrils wound round the fiber. In artificial fibers, a compromise has to be made; the object is to attain not the maximum tensile strength but the optimal commercially possible tensile strength, bending or knotting strength, elastic and plastic properties.

2. Elastic Properties of Cellulose and Its Derivatives

As we shall see in Chap. I, the free energy of a deformed elastic body (*e.g.*, a fiber under tension) consists of two fractions: (*1*) atoms may be displaced from their potential troughs and, after the removal of the external tension, fall back once more into the troughs (the normal solid body); (*2*) primary valence chains may be straightened by tension (or pressure), returning from this condition of lower entropy (less probability) because of thermal agitation (rubberlike bodies, Chap. C, sect. VI 2*b*). Tests which were made using the methods given in Chap. I, sect. I 2*b*, show that cellulose itself corresponds to case (*1*), but that case (*2*) may occur in the esters.[331] Commercial acetate rayon displays the behavior characteristic of (*2*), which is associated with a low or moderate degree of orientation, and an upper limit to the internal friction as the portions of the chains must be free to glide over each other. Case (*2*) is therefore favored by swelling agents, and is possible only in cellulose derivatives in which the secondary valence forces per chain link are considerably smaller than in cellulose itself. In suitably swollen preparations of cellulose esters, it is even possible to obtain rubberlike extensibility up to several hundred per cent (Hagedorn and Möller[332]). The elasticity of ordinary cellulose ester films and threads compounded with plasticizers has fundamentally the same origin.

Cellulose itself when swollen may show a considerable degree of rubbery elasticity; Meyer, Susich, and Valkó[333] have referred to this in the course of their work on the kinetic theory of rubbery elasticity. Although it cannot yet be thermodynamically proven that we are dealing with another instance of case (*2*), the high reversible deformability suggests that swollen cellulose is essentially an *elastomer*.

Kargin and Michailow,[334] and more particularly Hermans,[335] have stressed the analogy between rubber and cellulose. Freshly coagulated cellulose threads, still strongly swollen, such as may be obtained by allowing a vis-

[331] K. H. Meyer and W. Lotmar, *Helv. Chim. Acta*, **19**, 68 (1936).

[332] M. Hagedorn and P. Möller, *Veroffent. wiss. Zentral-Labs. phot. Abt. I. G. Farbenind, Akt.-Ges. AGFA*, **1**, 144 (1930); *Cellulosechemie*, **12**, 29 (1931).

[333] K. H. Meyer, G. von Susich, and E. Valkó, *Kolloid-Z.*, **59**, 208 (1932).

[334] V. Kargin and N. Michailow, *Acta Physicochim. U.R.S.S.*, **11**, 343 (1939).

[335] P. H. Hermans, *Naturwissenschaften*, **28**, 223 (1940).

cose solution to exude from a capillary into a precipitation bath, may be extended up to 600% and will still, under certain circumstances, return to their original length when treated with swelling agents. Rubbery properties are, however, much less conspicuous in cellulose than in rubber because crystallization is so much more easily initiated.

Native fibers exhibit interesting relationships between elasticity and structure. Their modulus of elasticity is very high, and was determined by Meyer and Lotmar[336] by means of longitudinal acoustic waves. The values are given in Table XI, together with values for artificial fibers. Drying, high tension, low temperature, and, above all, good orientation favor high values. The highest values obtained experimentally may approach the theoretical value for a stretched rope of primary valence chains, which can be estimated from our knowledge of the strength of the C—C or C—O—C linkage (determined from spectroscopic measurements) and from the known deformability of valence angles.

From infrared spectra and Raman lines, the energy of both types of linkage is known, and from this it is possible to calculate the energy required for a given deformation. From this we obtain the force constant of the linkage. By superposing the values for all linkages and angles, the force constant of the chain can be calculated, and hence the force constant of 3×10^{12} chains forming a rope with a cross,section 1 sq. mm. in area. In this way a value of 12,000 kg. per sq. mm. is obtained, which is in good agreement with the highest observed value for the modulus of elasticity.

TABLE XI
Modulus of Elasticity

Material	Modulus, kg./sq. mm.	Material	Modulus, kg./sq. mm.
Native flax	8000–11,000	Unoriented ramie: modulus at higher tension	3000
Native hemp	6000–8000	Viscose hair, damp	135
Native ramie	5000–7000	Viscose hair, dry	1170
Mercerized ramie	8200	Oriented viscose	3400
Unoriented ramie: modulus at low tension	1350	Cellulose acetate film	400

The fact that the values for unoriented fibers are much lower indicates that the molecular process taking place during stretching differs from that occurring in oriented fibers. Unoriented fibers consist of a network of crystallites and chains in contact with each other and clinging together like the threads in a macroscopic tissue. In this, texture determines the elastic properties. Very frequently an irreversible rise in the modulus of elasticity is observed to accompany a lasting deformation; the chains have

[336] K. H. Meyer and W. Lotmar, *Helv. Chim. Acta*, 19, 68 (1936).

been induced by the tension to form new contacts and the structure has become more rigid.

3. Plastic Properties of Cellulose and Its Derivatives

Native cellulose is not susceptible to large plastic deformations because the crystalline regions in which the chains are linked to one another by hydrogen bonds prevent any extensive relative movement. The slight degree of plastic deformability which is observed depends upon irregularities in the texture and rapidly disappears on extension as the structure becomes more compact; if the tension is further increased, the fiber breaks.

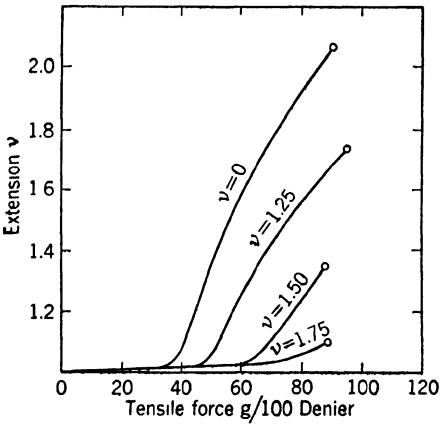


Fig. 24. Stress–strain curves for artificial silk which has been previously stretched (Hermans and de Leeuw).

Extensions of up to 10% at break can be obtained for cotton in the unswollen state but only if it is stretched very slowly; during the slow extension, orientation and consolidation occur. Once so oriented, the fiber has extension at break of less than 3%.

The processes occurring during the deformation of regenerated cellulose fibers have been very thoroughly investigated by Kratky and Hermans,[337] who studied in particular the relation between the stress–strain curve and the state of imbibition and previous orientation. From their results, it is clear that the latter has a very considerable influence, so that there can be no justification for regarding any particular shape of curve as typical of cellulose as such.

[337] P. H. Hermans, *Kolloid-Z.*, **81**, 143 (1937); **83**, 71 (1938). P. H. Hermans and A. J. de Leeuw, *ibid.*, **81**, 300 (1937); **82**, 58 (1938).

Figure 24 reproduces stress–strain curves obtained from air-dried fibers which had been stretched previously in varying amounts. The extensions were 0, 1.25, 1.50, and 1.75; it is clear that the last fiber is scarcely extensible at all. The results are complicated by the influence of the state of imbibition during the preliminary stretching. If stretching takes place in the swollen condition, marked consolidation takes place; if stretched in the dry condition, on the other hand, the fiber is weakened, although in both cases the orientation of the crystalline fraction is found to be equally good. From the stress–strain curve of the previously unstretched, unoriented fiber it is seen that very little extension occurs until the tension has reached a certain value; only after this point (the yield point) has been passed does deformation become considerable. This inflection of the curve[338] undoubtedly indicates that at the yield point a tension is reached which just suffices to overcome the forces of micellar cohesion in the least oriented regions (Hermans). This interpretation agrees with the fact that the addition of plasticizers displaces the yield point into the region of lower tensions.

Further work by Hermans[339] on the relation between orientation and extension has given a more fundamental insight into the mechanism involved in the extension of swollen cellulose threads. The orientation is assessed both by the x-ray diffraction diagram and by measurement of the anisotropy of swelling; for the latter the shrinking of the fiber in the transverse and longitudinal directions is measured. Fibers obtained by extrusion of 8% viscose into 2 *N* ammonium sulfate solution, containing more than 90% fluid, appeared optically isotropic and shrank evenly in length and thickness. They could be stretched up to 2.07 times their original length ("conventional" degree of extension), in which condition they show considerable orientation, and on being dried shrank very much in thickness but little in length. If the stretched and unstretched samples are compared after drying a degree of extension of about 3.9 is found. Although no simple relation between orientation and "conventional" degree of extension could be found in preparations swollen to varying extents, the same relation between orientation and the degree of extension referred to the dry state appeared in all preparations. The significance of this relation is therefore independent of the degree of swelling, and may be called the *characteristic extension.*

[338] See R. Houwink, *Elasticity and Plasticity of Matter*, Oxford Press, London, 1937. S. L. Bass and T. A. Kauppi, *Ind. Eng. Chem.*, **29**, 679 (1937).

[339] P. H. Hermans, *Kolloid-Z.*, **96**, 38, 311 (1941); **97**, 231 (1941). P. H. Hermans and P. Platzek, *ibid.*, **87**, 298 (1939); **97**, 329 (1941). P. H. Hermans and A. J. de Leeuw, *ibid.*, **81**, 321 (1937).

Hermans further showed that under the influence of suitable swelling agents cellulose fibers which had been strongly extended would return to their original length. From these observations it may be concluded that plastic flow does not in fact occur to any important extent during deformation, but that extension is mainly due to a deformation of the net structure. The process involves an increase in the percentage of crystalline material, as Mark[340] and Kargin and Michailow[341] have found; this is to be expected, as the rate of crystallization is always increased by orientation. The phenomena are comparable to the stretching of the swollen fibroin threads as they issue from the silk gland of the silk worm or with the extension of a gutta-percha strip which has been made extensible by warming or the addition of swelling agents. It is interesting in this connection that the maximum degree of extension possible without plastic flow in gutta-percha, cellulose, or silk is of the order of 500–700%.

The attraction between the hydroxyl groups is even more effectively decreased by intramicellar agents than it is by intermicellar agents such as water. In commercial practice, therefore, zinc chloride and concentrated sulfuric acid are used as swelling agents and plasticizers, although they also exercise a hydrolytic effect. Cellulose which has been rendered plastic in this way may be pressed together into sheets in which the cellulose fibers are welded together (vulcanized fiber).

If the hydroxyl groups are masked by acyl or alkyl groups, the cohesion is reduced. A good example of the diminution in the lattice forces is provided by the fusibility of trimethyl cellulose. Reduction of the reciprocal attraction between chains does not suffice, however, to render the material plastic over the temperature range between 80° and 180°C., which would be desirable commercially. Cellulose derivatives which retain the chain length of the initial material, corresponding to a molecular weight of 600,000–3,000,000, are transformed on heating into the rubberlike state, and are scarcely plastic at all. A certain degree of breakdown is therefore necessary as well; not until $[\eta]$ is less than 1 is the material plastic at suitable temperatures, the chains now being short enough to slip over one another. The viscosity at which plastic deformability occurs lies between 10^9 and 10^{10} poises. The transition from the solid state, with a viscosity greater than 10^{10} poises, to the plastic condition takes place over a temperature range of 10–30°C.

[340] H. Mark, *Meeting of the American Chemical Society*, Baltimore, 1939.

[341] V. A. Kargin and N. V. Michailow, *Acta Physicochim. U.R.S.S.*, 11, 343 (1939).

V. CELLULOSE BREAKDOWN

1. Mechanical Breakdown

We have seen above that the forces necessary for the mechanical rupture of cellulose chains are very considerable. While it is true that in many technical processes involving mechanical treatment (such as vigorous wet beating) cellulose is broken down, it appears to us more probable that this is primarily a chemical hydrolysis resulting from oxidation by air or by the action of hydrogen ions, and that mechanical rupture of the chains (which may be produced in fibers under tension) does not occur, or does so only to a very limited extent.

According to Hess,[342] on the other hand, cellulose may very probably be degraded by purely mechanical strains which are induced by rapidly moving balls. In such milling the lattice is destroyed, the solvability and power of adsorption are increased, and the viscosity is considerably decreased. As the rate of degradation is practically independent of the temperature, it may be assumed that none of the usual chemical degradations is involved, as they all have high temperature coefficients.

Solutions of cellulose derivatives of very high chain length may be decomposed by treatment with ultrasonic vibrations but only to a certain degree of polymerization; after a certain stage of degradation the treatment has no further effect. According to Schmid[343] it is the mechanical forces caused by the oscillations which are responsible for rupture of the chains.

2. Chemical Breakdown

(*a*) *Acid Hydrolysis*

Strong acids break up the cellulose chains by hydrolyzing glucosidic linkages. The end product is always glucose, provided that secondary actions (such as reversion, which will be discussed below) are excluded by diluting the solution. The course of the reaction varies with the concentration of acid. Dilute acids have no swelling effect on the fiber; they do not penetrate into the actual micelles or their action is even limited to the surface of the fiber. In this case, therefore, the reaction is markedly heterogeneous. At the surface, linkages are hydrolyzed and the liberated fragments are dissolved and are then transformed by progressive, homogeneous step reac-

[342] K. Hess, E. Steurer, and H. Fromm, *Kolloid-Z.*, 98, 148 (1942).

[343] G. Schmid, E. Beutenmüller, and A. Rief, *Kunststoff-Tech. u. Kunststoff-Anwend.*, 13, 65 (1943).

tions into glucose. The course of reaction in the hydrolysis of wood by dilute acids is apparently of this type. The aldehyde groups liberated in the course of hydrolysis reduce Fehling's solution and react with phenylhydrazine and its sulfonic acid, but not with naphthylhydrazine.[344] As breakdown proceeds, the mechanical strength diminishes and the product becomes partially soluble in sodium hydroxide.

Higher concentrations of acids lead to swelling, and, with the highest concentration, cellulose is dissolved; in this case hydrolytic breakdown proceeds as in a homogeneous phase. According to Ekenstam,[345] hydrolysis proceeds remarkably slowly in a concentrated solution of phosphoric acid. At 0°C. the viscosity of a solution of native cellulose in 83% phosphoric acid diminishes by only about 10% in 20 hours; it is, therefore, possible to use measurements of the viscosity in phosphoric acid for the characterization of cellulose preparations.[346]

Breakdown by hydrochloric and sulfuric acids has been carefully studied. In both processes fragments of moderate chain length (known as cellodextrins) are first produced; as the reaction proceeds, oligosaccharides appear, and the last fragments are cellotetraose[347] cellotriose, cellobiose, and glucose. Breakdown in concentrated salt solutions (for example, zinc chloride, calcium thiocyanate, or lithium iodide) is similar to that in acids. Freudenberg *et al.*[348] have made a detailed study of the velocity and kinetics of hydrolysis in solutions of concentrated acids.

They conclude that all cellulose linkages react in the same way, but that hydrolytic products of lower molecular weight are hydrolyzed rather more rapidly. In other words, the glucosidic linkages in cellulose have somewhat different kinetic constants from those of cellobiose or other sugars of the cellobiose series of low molecular weight. In sulfuric acid (d = 1.420) at 18°C. the decomposition constant of the cellulose linkage amounts to 0.3×10^{-4} sec.$^{-1}$; for cellotriose 0.64×10^{-4} and for cellobiose 1.07×10^{-4} sec.$^{-1}$.

The heat of activation for the splitting of cellobiose is 27,300 cal. per mole; that of a cellulose linkage is 29,800 cal. per mole. The steric factors are: 3.4×10^{16} for cellobiose and 67×10^{16} for cellulose.

[344] F. Müller, *Helv. Chim. Acta*, **22**, 217 (1939).

[345] A. Ekenstam, *Über die Cellulöselösungen in Mineralsäuren.* Lund, 1936.

[346] See also S. Schmidt-Nielsen and A. Markestad, *Kgl. Norske Videnskab. Selskabs, Forh.*, **11**, 82, 97 (1938).

[347] R. Willstätter and L. Zechmeister, *Ber.*, **62**, 722 (1929). See also L. Zechmeister and G. Tôth, *ibid.*, **64**, 854 (1931).

[348] K. Freudenberg, E. Bruch, and H. Rau, *Ber.*, **62**, 3078 (1929). K. Freudenberg and E. Bruch, *ibid.*, **63**, 535 (1930). W. Kuhn, *ibid.*, **63**, 1503 (1930). K. Freudenberg, W. Kuhn, W. Dürr, F. Bolz, and G. Steinbrunn, *ibid.*, **63**, 1510 (1930). K. Freudenberg and G. Blomqvist, *ibid.*, **68**, 2070 (1935).

According to Ekenstam[349] the initial velocity of hydrolysis of native cellulose in concentrated sulfuric acid or, more particularly in concentrated phosphoric acid, is considerably greater than it is in the later stages, from which he concludes that—in addition to the normal glucosidic linkages—natural cellulose contains others which are more easily hydrolyzed. This erroneous conclusion depends upon the fact that the viscosities used for estimation of the molecular weights were measured at too high a concentration, at which η_{sp}/c is still strongly dependent upon the concentration, and they are not therefore comparable.

The temperature coefficient of the velocity of hydrolysis is very high; for a rise of 10°C. the velocity increases five times. There is no reason to suppose that the conditions are any different in heterogeneous hydrolysis by dilute acids. Indeed, at higher temperatures, cellulose is readily destroyed by acid and this fact is made use of in the removal of cellulose from wool (carbonizing). At lower temperatures, cellulose fabrics may be treated to obtain certain effects with sulfuric acid at a considerable concentration without serious damage. The lower the temperature at which such treatment takes place, the less the chains are degraded, and the better the mechanical strength is preserved.

(*b*) *Acetolytic Breakdown*

A special form of breakdown is that known as acetolysis, that is, hydrolysis by acids in the presence of acetic acid and acetic anhydride. In this process, the breakdown products are obtained in the acetylated condition. Cellulose will dissolve slowly in a mixture of 0.9 part of sulfuric acid, 4 parts of glacial acetic acid, and 4 parts of acetic anhydride. If it is precipitated immediately after solution, a degraded cellulose acetate is obtained, which shows a lower viscosity than carefully prepared cellulose acetate when dissolved in the usual solvents; the longer the mixture is allowed to stand, the more degraded the product. The nature of the product varies, therefore, with the duration of treatment: the shorter the period of hydrolysis, the longer the mean chain length of the mixture, the less its solubility, and the higher its viscosity.

The ability of the products of hydrolysis from a certain chain length downward to form microcrystalline spherolites or aggregates is worthy of note. These are not crystals of uniform chemical composition but contain a mixture of polymeric homologues. The phenomenon is similar to the crystallization of mixtures of paraffins or fatty acids; a similar process has been mentioned for polyethylene succinate and for degraded trimethyl cellulose.

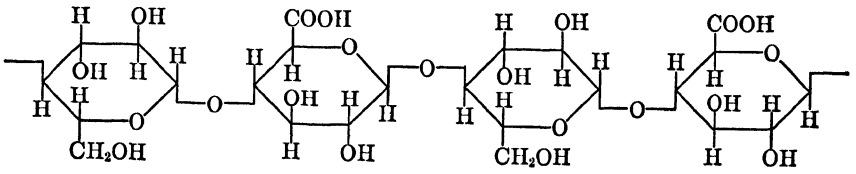
[349] A. Ekenstam, *Ber.*, 69, 549 (1936)

If acetolysis is allowed to continue, well-developed crystals of cellobiose octaacetate separate out. The octaacetate corresponds to the α form of cellobiose and is not, therefore, the form which would be expected from acetylation of the initially formed β-cellobiose. In the presence of sulfuric acid, however, an equilibrium is established between α and β forms, and as the α form is sparingly soluble, it precipitates as the only product; after a still longer period, it too disappears as a result of complete hydrolysis into glucose pentaacetate. According to Bertrand and Benoist[350] the acetate of cellotriose is formed in small quantities together with cellobiose octaacetate.

Trimethyl cellulose may also be subjected to acetolysis; methylated cellobiose and cellotriose as well as larger fragments have been obtained from the mixture (Haworth[351]).

(*c*) *Oxidation and Oxidative Breakdown*

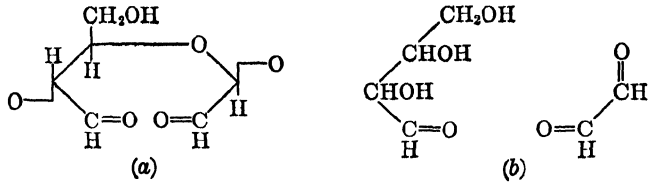
The course of the reaction occurring when cellulose is attacked by oxidizing agents depends upon the nature of the agent used. As Maurer[352] has shown, nitric oxide attacks only the primary alcohol groups (—CH_2OH) of the glucose and their substituents and converts them to COOH, thus giving rise to glucuronic acid derivatives. By treatment of the cellulose fibers with NO_2 at high temperatures the chains which lie at the surface are converted into a polyglucuronic acid called celluronic acid. Since, because of the digonal screw axis, only half the primary hydroxyls lie exposed, the formula of the product must be as follows:



According to Kenyon[353] who has studied the kinetics of this reaction, a nitric ester is formed at carbon atom 6 as an intermediate product, which later decomposes. Preparations of cellulose which have been subjected to strong oxidation at the surface in this way can be used for ion exchange.

[350] G. Bertrand and S. Benoist, *Bull. soc. chim.*, **33**, 1451 (1923).
[351] W. N. Haworth, E. L. Hirst, and H. A. Thomas, *J. Chem. Soc.*, **1931**, 824.
[352] K. Maurer and G. Reiff, *J. makromol. Chem.*, **1**, 27 (1943).
[353] W. O. Kenyon *et al.*, *J. Am. Chem. Soc.*, **69**, 355 (1947).

Periodic acid, first introduced as an organic reagent by Malaprade, only attacks atoms 2 and 3 and splits up the glycol linkage. According to Jackson and Hudson,[354] the first product is the dialdehyde (*a*) which under alkaline conditions decomposes and under acid conditions yields glyoxal and D-erythrose (*b*):



(*a*) (*b*)

The reaction due to the usual chlorine or hypochlorite bleaching agents follows in general the same course as that of periodic acid, as can be seen from the following observation. Cellulose preparations oxidized under acid conditions show a much higher tensile strength[355] and a much higher viscosity as nitrates (Davidson) than one would expect from their cuprammonium viscosity. By boiling with alkali the nitrate viscosity is decreased and the relation between the nitrate and cuprammonium viscosities now becomes the same as that found for cellulose which has not been degraded or which has been bleached under alkaline conditions. The tensile strength of samples showing high nitrate viscosity is considerable and corresponds more nearly to the nitrate than to the cuprammonium viscosity. On treatment with alkalis the tensile strength decreases as the nitrate viscosity. The cuprammonium viscosity of cellulose oxidized under acid conditions decreases on standing.

From this Davidson concludes that oxidation leads to the formation of bonds in the chains which are broken by treatment with alkali; the chain has points of weakness resulting from oxidation at atoms 2 and 3. These cannot be detected in measurements of the nitrate viscosity but should not on that account be overlooked, as textiles containing such flaws may suffer a considerable loss of strength after alkaline washing or bleaching. Davidson therefore recommends determination of the cuprammonium viscosity for the investigation of textile fibers or fabrics, because it allows the recognition of such concealed defects.

Solutions of cellulose in concentrated phosphoric acid are not sensitive to atmospheric oxygen. A very small quantity of oxygen in the form of potas-

[354] E. L. Jackson and C. S. Hudson, *J. Am. Chem. Soc.*, 59, 2049 (1937).

[355] G. F. Davidson, *J. Textile Inst.*, 29, 195T (1938). T. Brownsett and G. F. Davidson, *ibid.*, 32, 25 (1941). T. Brownsett and D. A. Clibbens, *ibid.*, 32, 32, 57 (1941).

sium permanganate is however sufficient to degrade the cellulose, at a temperature of 0°C., to half of its original mean degree of polymerization.

If a solution of ferric chloride in dilute hydrochloric acid is allowed to act on cellulose at a high temperature, carbon dioxide is set free by oxidation of the glucose formed; the sensitivity of cellulose preparations toward this reagent decreases as the degree of orientation is increased, or with increasing content of crystalline components or of α-cellulose. Conrad and Scroggie[356] have made this the basis of a method for assessing the quality of cellulose fibers, in which the volume of carbon dioxide given under standard conditions is determined and taken as a measure of the "accessibility."

Cellulose which has been subjected to superficial oxidation is known as oxycellulose. The acid groups can be titrated (p. 289); if heated with dilute hydrochloric acid they are eliminated as free carbonic acid. The aldehyde groups will reduce Fehling's solution and with hydrazinenaphtholsulfonic acid yield hydrazones which can be coupled with diazobenzenesulfonic acid; this latter reaction has been elaborated by Müller[357] to provide a sensitive test for the presence of oxycellulose.

(*d*) *Distribution of Molecular Weights in Degraded Cellulose*

When a hydrolytic agent acts on cellulose, the course of the reaction depends on the ratio of the local reaction velocity to the velocity of diffusion. Two extreme types are possible; either the outer regions of the fiber may disintegrate into small fragments, or, if penetration is rapid, breakdown may be uniform throughout.

Progressive disintegration from the surface inward, occurs when a native fiber (swollen as uniformly as possible) is exposed to the action of acids or oxidizing agents. This occurs, for example, in oxidative bleaching. Hess[358] in particular has emphasized that the substance (known as oxycellulose) formed in this process is not homogeneous; it is probably a mixture of moderately oxidized fragments with fragments of unchanged cellulose.

In homogeneous reactions decomposition is more even. Decomposition of this kind may be caused by treatment with concentrated acids or acid salts which dissolve or swell cellulose very strongly; oxidation of cellulose dissolved in phosphoric acid[359] or cuprammonium[360] has similar results.

[356] C. C. Conrad and A. G. Scroggie, *Ind. Eng. Chem.*, **37**, 592 (1945).

[357] F. Müller, *Helv. Chim. Acta*, **22**, 208, 217 (1939).

[358] K. Hess, *Chemie der Zellulose.* Akadem. Verlagsgesellschaft, Leipzig, 1928, page 455 *et seq.*

[359] H. Staudinger and J. Jurisch, *Ber.*, **71**, 2283 (1938).

[360] L. Kalb and F. von Falkenhausen, *Ber.*, **60**, 2514 (1927).

Cellulose esters such as cellulose nitrate or acetate may also be hydrolyzed by homogeneous reactions.

According to Eisenhut[361] all the glucose residues react equally during oxidative hydrolysis of alkali cellulose, as they do in acid hydrolysis. In all these types of decomposition the end product is a mixture of polymer homologues, as has been shown experimentally. It has not yet been possible to determine the distribution of molecular weights experimentally, but it may be estimated if certain reasonable assumptions are made. We may define α as the degree of hydrolysis (the fraction of bonds) which are decomposed in a material of very high molecular weight; $1/\alpha$ is therefore equivalent to the mean degree of polymerization, D.P. The weight yield ϕ_i of fragments of degree of polymerization i is given according to Kuhn[362] by the following formula, assuming that all bonds react at the same rate:

$$\phi_i = i\alpha^2(1-\alpha)^{i-1}$$

TABLE XII

i	ϕ_i (0/00)	i	ϕ_i (0/00)
800	9×10^{-11}	20	14.8
500	1.2×10^{-6}	15	13.4
200	1.1×10^{-1}	10	11.1
150	5.6×10^{-1}	8	9.6
125	1.3	5	6.8
100	2.9	4	5.5
75	5.9	3	4.4
50	10.8	2	3.1
25	15.5	1	1.0

In Table XII the yields, ϕ_i, for a degree of hydrolysis $\alpha = 0.04$ (mean degree of polymerization of the hydrolysis product = 25) have been summarized, and in chapter D, figure 1, the distribution of molecular weights for $\alpha = 0.01$ and $\alpha = 0.04$ has been reproduced graphically.

It appears that even when degraded as evenly as possible the product is not homogeneous. By fractional precipitation a rough division into fractions of different viscosity may be obtained; but even the most carefully fractionated preparations are still very inhomogeneous, as Mosimann[363] has been able to show by a careful investigation with the help of ultracentrifuge measurements.

[361] O. Eisenhut, *J. prakt. Chem.*, **157**, 338 (1940).
[362] W. Kuhn, *Ber.*, **63**, 1503 (1930).
[363] H. Mosimann, *Helv. Chim. Acta*, **26**, 369 (1943).

(*e*) *Enzymatic Breakdown*

Numerous bacteria and fungi which attack cellulose are known, but very little is known of the enzymes involved. Grassmann[364] found an enzyme in *Aspergillus oryzae* which could hydrolyze polysaccharides of the type of cellulose to oligosaccharides and, in the same material, another which could attack only oligosaccharides, including cellobiose. Karrer[365] has studied the hydrolysis of cellulose by an enzyme occurring in the gut of snails. Native cellulose was attacked only very slowly; alkali-treated or regenerated cellulose reacted more rapidly, presumably because of the increase in the internal surface. The enzyme is probably produced by the bacterial flora of the intestine.

[364] W. Grassmann, L. Zechmeister, G. Tôth, and R. Stadler, *Ann.*, **503**, 167 (1933). K. Freudenberg and T. Ploetz, *Z. physiol. Chem.*, **259**, 19 (1939).

[365] P. Karrer and P. Schubert, *Helv. Chim. Acta*, **9**, 893 (1926); **11**, 229 (1928).

F. ANIMAL AND VEGETABLE POLYSACCHARIDES AND LIGNIN

I. HEMICELLULOSES: PENTOSANS, HEXOSANS, AND POLYURONIC ACIDS

1. Hemicelluloses in General

We have already seen that natural cellulose fibers contain other substances besides cellulose: hemicelluloses, polyuronic acids, and lignin. The hemicelluloses form a group of substances composed, like cellulose, of chains built up from sugar units linked together by glucosidic linkages; in general, they are more readily hydrolyzed by acids and more readily soluble in alkalis than cellulose. The hemicelluloses are by no means exactly defined, either theoretically or analytically. The polyuronic acids (polyuronides), that is, polysaccharides with acid groups, were often included with the hemicelluloses. Certain hemicelluloses offer considerable resistance to dilute alkalis and to various purifying agents, so that crude cellulose (obtained from wood or other material rich in hemicelluloses) still contains considerable quantities of resistant hemicelluloses (xylan, mannan).

The amount of hemicelluloses present in cotton hairs is small, but it may be quite considerable in wood fibers (from deciduous trees in particular) and may amount to 30% or more of the cellulose present. The hemicelluloses have not as yet been studied sufficiently; the difficulty is that these substances consist for the most part of mixtures, the components of which are difficult to separate, and the chains themselves may be composed of various structural units, including hexose, pentose, and uronic acid residues. Norman,[1] who is one of the most distinguished workers in this field emphasizes in his monograph that when the present methods of investigation are used—fractionation, methylation, and acetolysis—it is often impossible to decide whether sugars and uronic acids obtained by hydrolysis were originally associated as units in the same molecule or in different chains.

The products of hydrolysis include the following sugars and uronic acids:

[1] A. G. Norman, *The Biochemistry of Cellulose, the Polyuronides, Lignin, etc.* Oxford Univ. Press, London, 1937.

glucose, glucuronic acid, xylose, arabinose, galactose, galacturonic acid, mannose, and rhamnose. Aldobionic acids have also been identified, for example, an acid yielding rhamnose and galacturonic acid on hydrolysis. If glucose is present in the hydrolyzed material, it is usually associated with glucuronic acid and xylan, while galactose is associated with galacturonic acid and arabinose. The relationship of these sugars is seen from the following table in which the sugars are expressed in the aldehyde form:

CHO	CHO	CHO	CHO	CHO	CHO
H·C·OH	H·C·OH	H·C·OH	H·C·OH	H·C·OH	H·C·OH
HO·C·H	HO·C·H	HO·C·H	HO·C·H	HO·C·H	HO·C·H
H·C·OH	H·C·OH	H·C·OH	HO·C·H	HO·C·H	HO·C·H
H·C·OH	H·C·OH	H·C·OH	H·C·OH	H·C·OH	H·C·OH
CH_2OH	COOH	H	CH_2OH	COOH	H
D-Glucose	D-Glucuronic acid	L-Xylose	D-Galactose	D-Galacturonic acid	L-Arabinose

For rough fractionation[2] the alkaline solution of crude hemicelluloses, as extracted from the plant material, is first treated with acetic acid which precipitates the so-called A fraction. This is dextrorotatory and contains chiefly glucosans and mannans. The more soluble hemicelluloses are then precipitated with alcohol to give the B fraction, which is levorotatory and contains polyuronic acids and pentosans. Owing to the great diversity of chains present, it is not possible to effect a sharp separation of the two groups. It is found that for wood the proportion of fraction B increases with age.

2. Method for Determination of Constitution of Polysaccharides

In general the structure of polysaccharides is investigated as follows: After fractionating the polysaccharide mixture by a suitable method such as that described above, the individual polysaccharides are submitted to a mild hydrolysis, *e.g.*, with dilute oxalic acid, which often effects a breakdown of the larger complexes (*e.g.*, in the case of pectins, arabans and galactans are separated from the polygalacturonic acids). Then the complete hydrolysis is carried out and the individual sugar units are identified.

The constitution of the larger fragments, and of such polysaccharides as contain only one sugar constituent, is then arrived at by exhaustive methylation and hydrolytic cleavage. The usual procedure is to methylate with dimethyl sulfate and alkali, or with thallous hydroxide and methyl iodide,

[2] M. H. O'Dwyer, *Biochem J.*, **17**, 501 (1923); **20**, 656 (1926).

hydrolyze, and then identify the methylated sugars so obtained; the recently developed methods for chromatographic separation are especially valuable for the identification. In this process originally free hydroxyl groups can be methylated; therefore, any tetramethyl hexoses, trimethyl pentoses, or trimethyl uronic acids isolated can only arise from terminal residues, since only one hydroxyl group, that of the pseudo aldehyde group of carbon atom 1, can have been involved in linkage in the polysaccharide, all others having been free. Trimethyl hexoses and dimethyl pentoses arise from chain residues initially linked at two positions; dimethyl hexoses and monomethyl pentoses arise from residues originally linked at three positions, *i.e.*, those which formed branch points in the chain. The number of "terminal residues" and the number of branch points must be equal and this affords a useful means of controlling completeness of methylation.

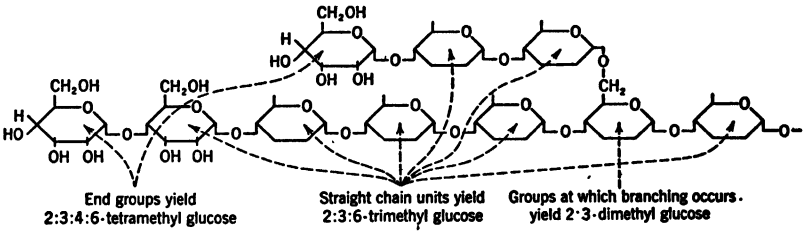
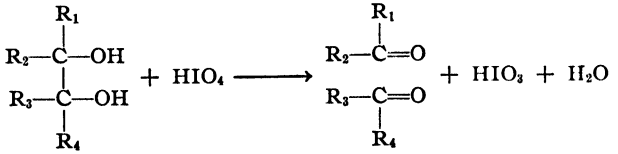


Fig. 1. Determination of constitution of a glucosan by methylation and hydrolysis.

Figure 1 shows schematically the relationship between the constitution of a polysaccharide and the methylated sugars obtainable from it.

A further method available for the elucidation of polysaccharide structure is that involving periodic acid oxidation. This depends on the reaction discovered by Malaprade[3] whereby periodic acid at room temperature effects the oxidative fission of α-glycols, α-ketols, α-diketones, α-hydroxy aldehydes, and α-keto aldehydes (where $\diagup\!\!\diagdown C{=}O$ reacts as $\diagup\!\!\diagdown C(OH)(OH)$) according to the following scheme:



[3] L. Malaprade, *Bull. soc. chim.*, **43**, 683 (1928). B. Criegee, *Z. angew. Chem.*, **53**, 321 (1940). E. L. Jackson, in *Organic Reactions*, Vol. II, Wiley, New York, 1944, p. 341.

where R_1 and R_4 can be either carbon or hydrogen atoms, and R_2 and R_3 carbon atoms or hydroxyl groups. Primary and secondary amino groups behave like hydroxyl groups. When in the above scheme a H—C—OH group has two —C—OH or C=O groups adjacent, it provides a special case where formic acid is produced from the center group. The amount of periodate used can be quantitatively assayed, and in addition, formic acid,[4] formaldehyde,[5] and glyoxal can be separately estimated when they appear among the products of the reaction. Free sugars react in the open-chain aldehydic form.

TABLE I

Analysis of Polysaccharides

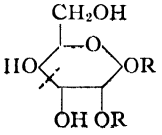
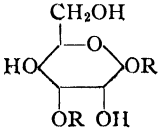
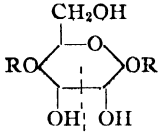
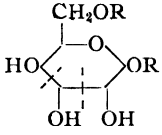
Type of linkage	Point of attack	HIO_4 used per hexose residue, moles	Quantitative estimation of reaction product
1-2		1	—
1-3		0	—
1-4		1	Glyoxal after hydrolysis of polysaccharide
1-6		2	Formic acid and, *after* hydrolysis, glyoxal

Table I illustrates how the method may be used for polysaccharide structural determination. For polysaccharides containing different types of linkage, the relative proportions of the various links present can be esti-

[4] T. G. Halsall, E. L. Hirst, and J. K. N. Jones, *J. Chem. Soc.*, 1947, 1399, 1427. See also 6*a*.

[5] R. Jeanloz, *Helv. Chim. Acta*, 27, 1509 (1944).

mated.[6] This oxidation process can also be applied to the methylated sugar cleavage products discussed above (Table II).

TABLE II

Analyses of Methylated Cleavage Products

	HIO_4 used per hexose residue, moles	Fission product, quantitatively measurable
2,3,4,6-Tetramethylhexopyranose	0	—
2,3,4-Trimethylhexopyranose	1	1-Formaldehyde
2,3,6-Trimethylhexopyranose	1	—
2,4,6-Trimethylhexopyranose	0	—
3,4,6-Trimethylhexopyranose	1	1 Formic acid
2,3-Dimethylhexopyranose	2	1 Formaldehyde
		1 Formic acid[a]
2,4-Dimethylhexopyranose	1	1 Formaldehyde
2,6-Dimethylhexopyranose	2	1 Formic acid[b]
3,4-Dimethylhexopyranose	2	1 Formaldehyde
		1 Formic acid[c]
3,6-Dimethylhexopyranose	2	1 Formic acid[d]
4,6-Dimethylhexopyranose	2	2 Formic acid

[a] The dimethyl mercaptal reacts with 2 moles of HIO_4.
[b] The methyl glycoside reacts with 1 mole of HIO_4.
[c] The dimethyl mercaptal reacts with 1 mole of HIO_4.
[d] The methyl glycoside does not react with HIO_4.

For polysaccharides like cellulose, starch, carubin, and inulin for which the residues within the chain have 1-4, 1-3, or 1-2, but no 1-6, linkages, formic acid can only result from terminal groups with free hydroxyl groups in the 2, 3, and 4 positions. In these cases, the end groups can be quite accurately estimated by oxidation with periodic acid and titration of the formic acid produced, after removal of the excess periodate by reaction with glycol. By means of the optical rotation one may decide between the presence of α- or β-glucosidic linkages. The difference of the optical rotation in water and cuprammonium may give some indications concerning constitution; this method has, however, only been applied to polyglucosans. According to Reeves[6c] the rotation of glucosans with 1-3 linkages is not

[6] K. H. Meyer and P. Gürtler, *Helv. Chim. Acta*, **30**, 751 (1947). O. A. Moe, S. E. Miller, and M. H. Iwen, *J. Am. Chem. Soc.*, **69**, 2621 (1947).

[6a] F. Brown, T. G. Halsall, E. L. Hirst, and J. K. N. Jones, *J. Chem. Soc.*, **1948**, 27. K. H. Meyer and P. Rathgeb, *Helv. Chim. Acta*, **31**, 1545 (1948); **32**, 1102 (1949).

[6b] K. H. Meyer and P. Gürtler, *Helv. Chim. Acta*, **30**, 751 (1947); O. A. Moe, S. E. Miller, and M. H. Iwen, *J. Am. Chim. Soc.*, **69**, 2621 (1947).

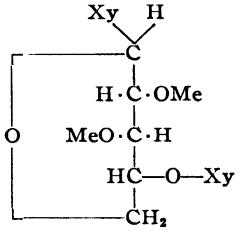
[6c] R. E. Reeves, *J. Biol. Chem.*, **154**, 49 (1944).

altered in cuprammonium, whereas the rotation of 1-6 and still more of 1-2 glucosans shifts to the right and in the case of 1-4 glucosans to the left.

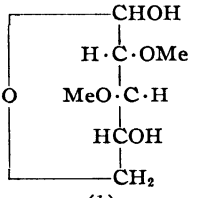
3. Pentosans

(*a*) *Xylan*

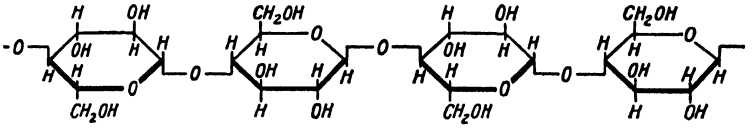
A pentosan can be obtained from almost all lignified cell membranes by treatment with 5% alkali, precipitation of the filtrate with Fehling's solution or alcohol, and decomposition of the precipitate. This may be hydrolyzed to form xylose and is known as xylan. In some cases small amounts of arabinose are also present in the hydrolysate. The structure of this most frequent associate of cellulose has been demonstrated by Hampton, Haworth, and Hirst.[7] They converted xylan into dimethylxylan (*a*)



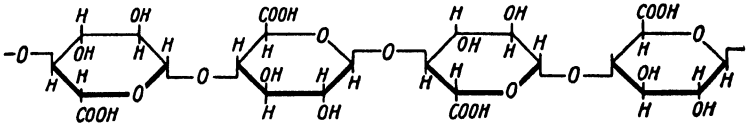
(*a*)
Dimethylxylan



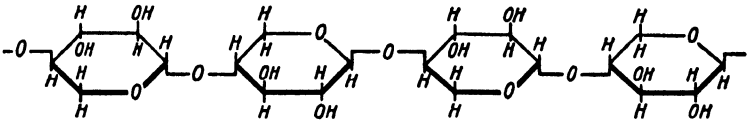
(*b*)
2,3-Dimethylxylose



I Cellulose



II Polyglucuronic acid



III Xylan

Formulas of cellulose, polyglucuronic acid, and xylan

[7] H. A. Hampton, W. N. Haworth, and E. L. Hirst, *J. Chem. Soc.*, 1929, 1739.

and this was hydrolyzed to give a dimethylxylose (*b*), the constitution of which was recognized as 2,3-dimethylxylose. From this it was clear that only the 4 position was available for linkage with the 1 position of the next xylose residue. In this way, the constitution of xylan as 1,4-xylan was demonstrated. Since β-glucosidic linkages are indicated by the optical rotation, we obtain formula (III) which is related to cellulose (I) and polyglucuronic acid (II).

Treatment of xylan with periodic acid brings about fission between carbon atoms 2 and 3 which become aldehydic.[8] Hydrolysis of this oxidation product gives glycerinaldehyde and glyoxal, thus providing confirmation of the Haworth formulation. The spatial arrangement of the atoms of a xylan chain is shown in figure 2.

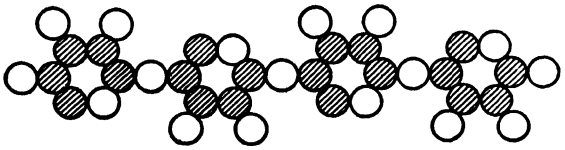


Fig. 2. Three-dimensional model of a xylan chain.

Small amounts of glucuronic acid are found in the xylan of cottonseed husks.[9] According to Haworth,[10] xylan from Esparto grass contains small quantities of L-arabinose units in the furanose form; they occur at the ends of the chains and are split off by 0.2% oxalic acid at 100°. A similar polysaccharide has been isolated from wheat straw,[11] which contains hexuronic acid, arabinose, and xylose in the proportions 1:0.9:23. Wood xylans frequently contain glucuronic acid methyl ester,[12] and this is also true for xylan obtained from alfalfa.[13]

The low viscosity of solutions of xylan in sodium hydroxide indicates a comparatively short chain. On the basis of viscosity measurements, Haworth suggests a chain containing 70–80 sugar units; Staudinger suggests a chain of 100–150 units.[14] The solubility of xylan is due, possibly, to its low molecular weight. According to Schmidt, "skeletal substances" may be obtained from wood, seed husks, and other refuse by careful purification

[8] G. Jayme and M. Sätre, *Ber.*, **75**, 1840 (1942).

[9] P. W. Krznarish, *Cereal Chem.*, **17**, 457 (1940).

[10] W. N. Haworth, E. L. Hirst, and E. Oliver, *J. Chem. Soc.*, **1934**, 1917. R. A. S. Bywater, W. N. Haworth, E. L. Hirst, and S. Peat, *ibid.*, **1937**, 1983.

[11] H. D. Weihe and M. Phillips, *J. Agr. Research*, **60**, 781 (1940).

[12] M. H. O'Dwyer, *Biochem. J.*, **28**, 2116 (1934); **33**, 713 (1939); **34**, 149 (1939). E. Anderson, M. Seeley, W. T. Stewart, J. C. Redd, and D. Westerbeke, *J. Biol. Chem.*, **135**, 189 (1940).

[13] M. Phillips and B. L. Davis, *J. Agr. Research*, **60**, 775 (1940).

[14] E. Husemann, *Naturwissenschaften*, **27**, 595 (1939).

of the lignified material with chlorine dioxide in one or more stages. From these the readily soluble xylans and also polyuronic acids can be extracted with dilute sodium hydroxide. The residual cellulose and xylan remain in definite proportions, for example, 3:1 in red beechwood of any age and from any locality,[15] as well as in the stones of cherries and California plums,[16] and 2:1 in Silesian plum stones at various stages of development.[17]

By treating wood (previously extracted with alcoholic benzene) alternately with chlorine and alcoholic pyridine,[17a] or with alcoholic ethanolamine,[18] it is possible to obtain the total carbohydrate fraction free from lignin; this so-called holocellulose contains, in addition to cellulose and xylan, all the carbon dioxide yielding substances, all the acetyl groups, and part of the methoxyl groups of wood. Holocellulose is a good raw material for obtaining highly viscous α-cellulose for the preparation of cellulose derivatives.[18]

Commercial cellulose materials and even α-celluloses themselves (obtained from holocellulose by treatment with 17.5% sodium hydroxide) may still contain considerable quantities of resistant xylan.[19]

(*b*) *Araban*

In addition to xylan, wood contains small quantities of pentosans which yield arabinose on hydrolysis.[20] See Sect. 4*b* with regard to arabogalactan obtainable from larch wood.

4. Hexosans

(*a*) *Glucosans*

Deciduous and coniferous woods contain polysaccharides (yielding glucose on hydrolysis) which are more soluble in alkalis and more readily attacked by purifying agents than cellulose. Thus, for example, the glucose

[15] E. Schmidt, K. Meinel, K. Nevros, and W. Jandebeur, *Cellulosechemie*, **11**, 49 (1930). E. Schmidt, W. Jandebeur, and K. Meinel, *ibid.*, **11**, 73 (1930). E. Schmidt, Yüan-Chi Tang, and W. Jandebeur, *ibid.*, **12**, 201 (1931). R. Runkel and S. Lange, *ibid.*, **12**, 185 (1931).

[16] J. Pfirschke, *Dissertation*. Breslau, 1936.

[17] R. Bauer, *Dissertation*. Breslau, 1936.

[17a] G. J. Ritter and E. F. Kurth, *Ind. Eng. Chem.*, **25**, 1250 (1933).

[18] W. G. Van Beckum and G. T. Ritter, *Paper Trade J.*, **105**, No. 18, 127 (1937); **107**, No. 22, 109 (1939). L. E. Wise, F. C. Peterson, and W. M. Harlow, *Ind. Eng. Chem., Anal. Ed.*, **11**, 18 (1939).

[19] See F. Lenze, B. Pleus, and J. Müller, *J. prakt. Chem.*, **101**, 213 (1921). M. Steinschneider, R. Schepp, and E. Wultsch, *Papier-Fabr.*, **36**, 36 (1938). G. Jayme and H. Pfretzschner, *ibid.*, **37**, 97, 109 (1939).

[20] E. Hägglund, *Holzchemie*. 2nd ed., Akadem. Verlagsgesellschaft, Leipzig, 1939, page 122.

content of commercial sulfite lye is due to the hydrolysis of the glucosans which occur together with cellulose in pine wood.

The fraction of crude celluloses, commercial cellulose materials, etc., known as β-cellulose, which is soluble under certain conditions in 17.5% sodium hydroxide, contains (along with other hemicelluloses) 1,4-β-glucosans, the chain length of which is much less than the chain length of those present in insoluble α-cellulose. This alkali-soluble cellulose of shorter chain length is certainly already present in part in native wood, but it may also be formed, during the process of purification, by breakdown of cellulose of higher molecular weight. Each type of fiber probably has a characteristic distribution of celluloses of large, moderate, and small chain length; on this depends the yield of more highly polymerized α-cellulose, insoluble in alkali, obtained from any particular plant material. This determines the suitability of the material for the preparation of rayon.[21]

(*b*) *Galactans*

In addition to the polysaccharides related to and associated with cellulose, coniferous and deciduous woods also contain small quantities of galactans. Larch wood contains 8% of what is presumably a galactoaraban of low molecular weight and soluble in water (Schorger and Schmidt[22]); it contains arabinose and galactose residues[23] in the proportion 1:6 and, in view of its relatively low viscosity, probably has a branched chain structure.[24] According to Svedberg[25] this larch polysaccharide is composed of two nearly monodisperse components, with molecular weights of 16,000 and 100,000 in the proportion 1:4. The former is an arabogalactan, the latter a β-galactan containing in equal proportions the following types of galactose residues: residues linked with their neighbors through atoms 1, 3, and 6, residues linked through 1 and 6, and 1 linked end groups.[26] A water-insoluble galactoraban occurs as a reserve in peanuts.

(*c*) *Mannans*

The mannans (yielding mannose on hydrolysis) have been known longer and studied more carefully than the galactans. Mannans are known which

[21] See G. Jayme and P. Schorning, *Papier-Fabr.*, **36**, 235 (1938).

[22] A. W. Schorger and D. F. Schmidt, *Ind. Eng. Chem.*, **8**, 494 (1916).

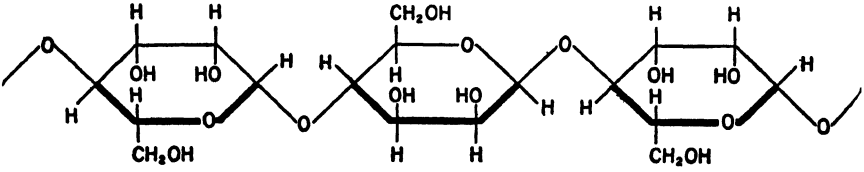
[23] F. C. Peterson, A. J. Barry, H. Unkauf, and L. E. Wise, *J. Am. Chem. Soc.*, **62**, 2361 (1940).

[24] E. Husemann, *Naturwissenschaften*, **27**, 595 (1939); *J. prakt. Chem.*, **155**, 13 (1940). H. S. Owens, *J. Am. Chem. Soc.*, **62**, 930 (1940).

[25] T. Svedberg, *J. Phys. & Colloid Chem.*, **51**, 1 (1947).

[26] W. G. Campbell, E. L. Hirstand J. K. N. Jones, *J. Chem. Soc.*, **1948**, 774.

possess mechanical properties similar to those of cellulose. They are contained in the thickened cell walls of hard seeds, and form the chief constituent of the American ivory nut. The mannan mixture of vegetable ivory contains crystalline micelles, shown by the x-ray diagram (Katz[27]). The high mechanical strength and insolubility in water lead one to suppose that the structure is similar to that of cellulose. Meyer and Mark[28] concluded that this would be possible only with 1,4-β-glucosidic linkages. Ivory nut mannan, soluble in alkali, shows a faint levorotation, which would also suggest a β-glucosidic linkage:



Constitution of ivory nut mannan (mannose residues united by β-1,4-glucosidic linkages) (Meyer and Mark)

The structural similarity of cellulose and ivory nut mannan is shown by the following facts. Grated vegetable ivory can be nitrated like cellulose, as Hopff and Moll[29] showed. Films may be obtained from the solution of nitromannan in acetone. The mechanical strength of such films and the viscosity of the solutions indicate that the structure is similar to that of nitrocellulose.

The formula given by Meyer and Mark has been confirmed by the work of Klages,[30] who methylated and hydrolyzed, and obtained as the chief product of hydrolysis 2,3,6-trimethylmannose, together with small quantities of 2,3,4,6-tetramethylmannose, from which it may be concluded that the latter is formed from the terminal mannose units. The yield of tetramethylmannose gives, therefore, a measure of the mean chain length. Ivory nut mannan A, which can be extracted by dilute alkalis, is found to consist of chains of between 70 and 86 mannose units. Insoluble mannan B has a much greater chain length. Mannans A and B are related to each other as alkali-soluble β-cellulose is to α-cellulose.

Mannans are encountered regularly as constituents of coniferous woods;

[27] J. R. Katz, in K. Hess, *Chemie der Cellulose.* Akadem. Verlagsgesellschaft, Leipzig, 1928.

[28] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, page 168.

[29] H. Hopff and Moll, quoted by Meyer and Mark, *loc. cit.*

[30] F. Klages, *Ann.*, **509**, 159 (1934); **512**, 185 (1934). F. Klages and R. Maurenbrecher, *ibid.*, **535**, 175 (1938).

deciduous woods, on the other hand, contain either no mannans, or, at any rate, considerably smaller quantities. A large part of the mannan present in coniferous woods appears to be closely associated with cellulose; it is somewhat resistant to purifying agents, and is removed from commercial sulfite cellulose only by subsequent treatment with stronger alkali. According to Hess and Lüdtke,[31] the mannan isolated from sulfite cellulose material has the same optical rotation as mannan A from ivory nut: $[\alpha]_D^{18} = -44° 9'$ (in dilute sodium hydroxide). The degree of polymerization is 150–160.[32]

We shall refer later to the mannan of yeast (known as yeast gum), to the mannan of orchid tubers, and to mannocarolose.

5. Polyuronic Acids

Related to these neutral polysaccharides are the acid polyuronides which occur either separately or accompanying cellulose. Schwalbe and Feldmann[33] obtained from straw a polyglucuronic acid which gave glucuronic acid on hydrolysis. Schmidt and Vocke[34] state that polyglucuronic acids occur in the lignified membranes of many if not all phanerogams and Archegoniatae. Galacturonic acid was shown by Hägglund and his collaborators[35] to be present in the sulfite liquors from pine wood; O'Dwyer[36] showed that glucuronic and galacturonic acid occur among the acid products of hydrolysis of beech wood.

It is probable that polyglucuronic acids are not present in wood fibers in the free form but as esters. Schmidt, Meinel, and Zintl[37] point out that the acids are dissolved out of the wood only by fairly strong sodium hydroxide, and not by bicarbonate, although they are readily soluble in a solution of bicarbonate when free. It is conceivable therefore, that they form a cement between the cellulose micelles. Chains of xylan and of other polysaccharides probably occur in between as well. It is also quite possible that mixed chains occur, containing glucose or pentose units alongside units of glucuronic acid, and it is possible that fairly long cellulose chains are united with fairly long uronic acid chains. In a few cases, the existence of such mixed chains has been demonstrated. Renfrew and Cretcher[38] and

[31] K. Hess and M. Lüdtke, *Ann.*, **466**, 18 (1928).

[32] E. Husemann, *J. prakt. Chem.*, **155**, 13 (1940).

[33] G. Schwalbe and S. A. Feldmann, *Ber.*, **58**, 1534 (1925).

[34] E. Schmidt and F. Vocke, *ibid.*, **59**, 1585 (1926).

[35] E. Hägglund, F. W. Klingstedt, T. Rosenqvist, and H. Urban, *Z. physiol. Chem.*, **177**, 248 (1928).

[36] M. H. O'Dwyer, *Biochem. J.*, **17**, 501 (1923); **20**, 656 (1926).

[37] E. Schmidt, K. Meinel, and E. Zintl, *Ber.*, **60**, 503 (1927).

[38] A. S. Renfrew and L. H Cretcher, *J. Biol. Chem.*, **97**, 503 (1932).

Bailey and Norris[39] investigated water-soluble mucilages from the seeds of *Cydonia* and of white mustard. On boiling with 0.5 *N* sulfuric acid these soluble, acid substances give a 33–45% yield of an insoluble substance displaying the properties of cellulose, while about equal quantities of uronic acids and pentosans remain in solution. A more accurate study of the cellulose obtained in this way would be of interest.

Pectin substances, which will be treated separately, consist chiefly of polygalacturonic acids.

II. LIGNIN

Although lignin is not a polysaccharide, we shall consider this most important associate of wood cellulose along with the other associated substances.

1. Methods for Isolation of Lignin and Its Derivatives[40]

Lignin accompanies cellulose in wood, straw, etc.; the cellulose fibers are embedded in lignin, and lignin is also present in the interior of the fiber. If the cellulose material is to be isolated, the lignin must be removed; to this end the material (wood, for example) is heated under pressure with bisulfite or sodium hydroxide, which effects a cleavage of the lignin–polysaccharide linkages. It is now generally assumed that such linkages exist, on the basis of kinetics of the bisulfite reaction (Hägglund); though, to be sure, only the outer chains of the cellulose fibrils can be linked in this way. Furthermore, it has been possible to isolate lignin-polysaccharide complexes from the alkaline sulfite[41] liquors and also from hot-water extracts of wood.[42] Also, Freudenberg's observation[43] that exceedingly finely divided sawdust is soluble in ammoniacal copper solution may be explained by the supposition that the lignin is brought into solution by the cellulose to which it is attached. A familiar and much-used reaction of lignin is the red color developed by lignin-containing material in the presence of phloroglucinol and hydrochloric acid; this reaction is ascribed to the free aldehyde groups of the lignin; it is not specific.

[39] K. Bailey and F. W. Norris, *Biochem. J.*, **26**, 1609 (1932).

[40] Summaries in: K. Freudenberg, *Fortschr. Chem. org. Naturstoffe*, **2**, 1 (1939). A. G. Norman, *The Biochemistry of Cellulose, the Polyuronides, Lignin, etc.*, Oxford Univ. Press, London, 1937. M. Phillips, *Chem. Revs.*, **14**, 103 (1934). E. Hägglund, *Holzchemie*, 2nd ed., Akadem. Verlagsgesellschaft, Leipzig, 1939.

[41] H. Friese, V. Högn, and H. Wille, *Ber.*, **70**, 1072 (1937). H. Friese and E. Clotofski, *ibid.*, **70**, 1986 (1937).

[42] F. Schütz, *Ber.*, **75**, 703 (1942).

[43] K. Freudenberg and T. Ploetz, *unpublished work.*

The yellow or orange color reactions given by wood with aromatic amines is also due to condensation of aromatic aldehydic groups to give Schiff's bases. While many workers agree that aldehydic groups are involved in these color reactions, there is a difference of opinion as to whether they are really due to lignin itself or to substances associated with it. Since, however, all investigations show that any such accompanying substance is not present in wood in the free state, but must first be split off by treatment with zinc chloride, it seems highly probable that the reaction groups concerned are essentially part of the lignin structure.

The molecular weight and constitution have not yet been determined satisfactorily. The great difficulty in the study of lignin lies in the fact that it has not been possible as yet to obtain pure lignin in an unchanged form. Moreover, the larger structural units which appear during breakdown have not been obtained in anything approaching a quantitative yield. Finally, lignin cannot be characterized by any specific properties. Samples of lignin are characterized by the amount of typical lignin groups (methoxyl, hydroxyl, and C—CH_3 groups) which they contain, as well as by their elementary composition. The means employed to remove cellulose and hemicellulose from wood are so drastic that change takes place in the native lignin; on the other hand, the methods by which lignin can be dissolved out probably also lead to changes. This will be clear from the following short summary of the common methods of isolation.

(*a*) *Acid Lignin*

Cellulose and hemicellulose are dissolved out from lignified tissue (with subsequent hydrolysis) by treatment with strong sulfuric acid (Klason[44]) or with 37% hydrochloric acid (Willstätter lignin, Willstätter and Zechmeister[45]). On dilution with water, a brown insoluble substance known as acid lignin is precipitated. This method is also used for the quantitative determination of lignin (72% sulfuric acid is generally used). It has been known for some time that the determination of lignin by this method is fraught with various difficulties and uncertainties; if acid treatment is too short the lignin values are too high; on the other hand, if the acid acts for too long a period and, in particular, at high temperatures, the lignin values are again too high. According to Norman[46] the latter phenomenon de-

[44] P. Klason, *Hauptversammlungsber. des Vereins der Zellstoff- u. Papier-Chemiker und-Ingenieure*, **1908**, page 53; *Cellulosechemie*, **4**, 81 (1923).

[45] R. Willstätter and L. Zechmeister, *Ber.*, **46**, 2401 (1913).

[46] A. G. Norman, *The Biochemistry of Cellulose, the Polyuronides, Lignin, etc.* Oxford Univ. Press, London, 1937, page 347.

pends, not only on the formation of insoluble sugar humic acids, but also on the condensation of lignin with aldehydic or ketonic products of carbohydrate hydrolysis (such as furfurol, formed from pentosan in the presence of acid). In the various methods which have been suggested for the determination of lignin, this source of error is reduced to a minimum by choosing a suitable concentration of sulfuric acid and controlling the duration of hydrolysis.

Freudenberg and Ploetz[47] carried out a series of experiments using sulfuric acid at different concentrations to estimate the amount of insoluble residue and its methoxyl content. They thus selected the conditions which give a minimum residue and a maximum value for the methoxyl content of that residue. For spruce wood, optimal conditions are reached with 72% sulfuric acid, for beech wood 76% acid.

Hilpert and his collaborators[48] have shown for beech wood and straw that it is possible to bring into solution, together with carbohydrates, considerable quantities of "lignin," that is, the fraction which remains as an insoluble residue if the customary methods employing strong hydrochloric or sulfuric acid are used. Hilpert has drawn the conclusion from this that lignin is not present as a normal component of lignified membranes, chemically different from carbohydrates, and sees in the acid lignins, as in other isolated forms of lignin, only reaction products of sensitive carbohydrates. This interpretation is not reconcilable, however, with the results of structural studies of lignin, nor with the observation that samples of lignin prepared by various methods display fundamentally similar properties. The fraction of lignin soluble in strong acids at low temperature is perhaps a lignin-carbohydrate complex.

In the Scholler-Tornesch process for saccharification of wood, considerable quantities of acid lignin are produced for which no satisfactory utilization has yet been found.

(*b*) *Cuproxamlignin*

A less violent method for removing cellulose has been developed by Freudenberg[49,50]: sawdust is extracted first with 5% alkali to remove pento-

[47] K. Freudenberg and T. Ploetz, *Ber.*, **B73**, 754 (1940). T. Ploetz, *Cellulosechemie*, **18**, 49 (1940).

[48] R. S. Hilpert, *Cellulosechemie*, **16**, 92 (1935). R. S. Hilpert and E. Littmann, *Ber.*, **68**, 16 (1935). R. S. Hilpert and H. Hellwage, *ibid.*, **68**, 380 (1935); **70**, 113 (1937). See also E. Hägglund and C. B. Björkman, *Biochem. Z.*, **147**, 74 (1924). K. Storch, *Ber.*, **68**, 2367 (1935).

[49] K. Freudenberg, M. Harder, and L. Markert, *Ber.*, **61**, 1760 (1928). K. Freudenberg, H. Zocher, and W. Dürr, *ibid.*, **62**, 1814 (1929). K. Freudenberg, F. Sohns, and A. Janson, *Ann.*, **518**, 62 (1935).

[50] See also E. Wedekind and O. Müller, *Ber.*, **69**, 1517 (1936).

sans, and then several times alternately with boiling dilute acid and ammoniacal copper oxide solution, by which means the cellulose is gradually removed. According to Freudenberg this "cuproxamlignin" is the most suitable form in which lignin can be isolated for the purpose of investigations of chemical constitution. This form of lignin, too, is probably not unchanged natural lignin, for, even if this method leaves intact the less sensitive parts of the molecule, there is no guarantee that the details of the structure remain unchanged.

(*c*) *Soluble Lignin Derivatives*

By long treatment with hot alkali, lignin may be brought into solution (as in the sodium hydroxide and sulfate process for the isolation of cellulose material) and may be precipitated from solution as alkali lignin by the addition of acids.

During the treatment of wood with bisulfite lye under pressure (sulfite process), lignin passes into solution as lignosulfonic acid. According to investigations by Hägglund,[51] a solid lignosulfonic acid, that is, a sulfonated lignin-carbohydrate complex is formed first, and lignosulfonic acid passes into solution only as the linkage between lignin and carbohydrate is hydrolyzed. The solid lignosulfonic acid formed in the first stage displays the peculiarities of a cross-linked, permutoid-reacting, multivalent acid. Cations are bound in proportions dependent on the concentration of the individual ions in the surrounding liquid. During boiling, Ca^{++} and H^{+} become attached to the lignosulfonic acid in the lye to an extent proportional to their concentration. The nature of the linkage between sulfurous acid and lignin and the mechanism of the process of solution are still obscure.[52]

Lignosulfonic acids present in the sulfite lye may be freed from associated substances by dialysis. They are not homogeneous but are mixtures of compounds of different degrees of sulfonation. The dissolved lignosulfonic acids also differ with respect to their precipitation by salts or organic bases.

No real outlet has yet been found for utilizing the enormous quantities of lignosulfonic acid or alkali lignins which remain in the waste liquor as by-products of the cellulose industry. Apart from the use of the concentrated liquor as fuel, and small amounts of lignosulfonic acid for tanning, cheap soaps, etc., a process has recently been developed in America for their conversion to vanillin by heating calcium lignosulfonate with sodium hydroxide solution under pressure.[53] The yield of vanillin is 4 to 5%; the alkaline

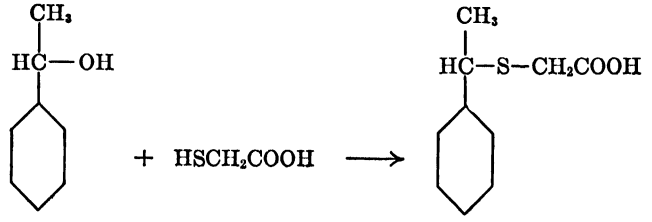
[51] See E. Hägglund, *Holzchemie*. 2nd ed., Akadem. Verlagsgesellschaft, Leipzig, 1939.

[52] See, in this connection, E. Hägglund, *Zellstoff u. Papier*, **16**, 570 (1936). K. Freudenberg, F. Sohns, and A. Janson, *Ann.*, **518**, 62 (1935). B. Holmberg, *Papier-Fabr.*, **36**, 218 (1938). F. E. Brauns and D. S. Brown, *Ind. Eng. Chem.*, **30**, 779 (1938).

[53] A. J. Bailey, *Pacific Pulp & Paper Ind.*, **13**, No. 11, 34 (1939).

liquors containing unchanged lignin are worked up into hard sheets after addition of sawdust. A possible future development for more complete utilization of lignin by-products lies in their hydrogenation (see below) to cyclohexane derivatives which have very considerable application as solvents, preparation of resins, plastics, etc.[54]

Soluble compounds of lignin with thioglycolic acid and other mercapto acids were used by Holmberg[55] for analytical purposes and as a basis for a discussion of chemical constitution. In order to throw light on the mechanism of this compound with thioglycolic acid Holmberg has investigated the behavior of a series of model substances. Phenylethyl alcohol[56] is found to react as follows:



Its ethyl ether is readily split up by thioglycolic acid and gives the same condensation product. It may be assumed, therefore, that lignin carbinol groups or ether groups linking the aromatic residues react with thioglycolic acid similarly.

Lignin is also dissolved by treating sawdust with alcohols, phenols, glycol, dioxan, and similar materials at high temperatures in the presence of hydrochloric acid (or ferric chloride), which acts as a catalyst; it can also be extracted with concentrated formic acid. Alcohol, phenol, dioxan, lignins, etc., are soluble in dilute alkalis and in certain organic solvents.

2. Constitution of Lignin

The mere fact that the various methods for the isolation of lignin[56] frequently involve long treatment at high temperature, shows that a chemical process precedes solution. This has been demonstrated, for example, by Hibbert[57] in the partial cleavage which occurs with alcohol. The whole

[54] E. C. Sherrard and E. E. Harris, U. S. Pat. 2,146,655 (1939).

[55] G. A. Berg and B. Holmberg, *Svensk Kem. Tid.*, **47**, 257 (1935). *Cf.* also B. Holmberg, *J. prakt. Chem.*, **141**, 93 (1934). Also B. Holmberg, *Arkiv Kemi Mineral. Geol.* **A12**, No. 9 (1936). A general review: B. Holmberg, *Oesterr. Chem. Ztg.*, **43**, 152 (1940).

[56] According to L. Lemmel [*Anales soc. españ. fís. y quím.*, **31**, 50 (1933)] lignin is dissolved out at room temperature by acetic ester in the presence of hydrochloric acid. Even in this case, however, the possibility of hydrolysis is not excluded.

[57] W. B. Hewson, J. L. McCarthy, and H. Hibbert, *J. Am. Chem. Soc.*, **63**, 3041, 3045 (1941).

behavior of lignin indicates, as Freudenberg[58] has pointed out, that natural lignin is a three-dimensionally cross-linked substance of very high molecular weight which must first be broken down before it can pass into solution. The molecular weights of soluble lignins[59] (800–4000) can give no indication of the size of the molecules in the natural substance. If we regard lignin as a network of primary valence chains forming a cement for cellulose, and possibly also chemically linked with cellulose chains here and there at the surface of the micelles (Hägglund[60]), the question of the homogeneity of the substance loses all meaning. It is probable that in different plants, at different ages, different groups are linked together to form lignin. The concepts of molecule and molecular weight are therefore as out of place here as, for example, in a discussion of the structure of vulcanized rubber.

Without doubt lignin contains methoxyl groups; the methoxyl content of wood increases with age and a certain proportion of it (10–15%) is bound to carbohydrate (Hägglund). Lignins of different deciduous and coniferous woods can be classified according to their carbon and methoxyl contents, the "spruce type" (gymnosperm type) is characterized by 64–66% carbon and 15–17% methoxyl, the values for the "beech type" (angiosperm) being 61–63% and 20–22%, respectively.

In addition, lignin contains hydroxyl groups which will undergo methylation, the completely methylated substance having 30% methoxyl. The nature of the hydroxyl groups has not yet been established; Freudenberg[61] maintains that for cuproxamlignin the phenolic hydroxyl content is very small, and that secondary and tertiary alcoholic groups are present but no primary groups. On the other hand, the various preparations of alkali-soluble lignin (from which carbon dioxide can effect precipitation), alcohol lignin, thioglycolic acid lignin, and the lignosulfonic acids all appear to contain phenolic hydroxyl groups. Likewise, it is still uncertain whether the O—CH_2—O—grouping is really characteristic of lignin.[62]

Different kinds of lignin all contain 61–66% carbon, 5–6% hydrogen, and about 30% oxygen; the carbon content is thus considerably greater and

[58] K. Freudenberg, *Ber.*, **62**, 383 (1929).

[59] See D. L. Loughborough and A. J. Stamm, *J. Phys. Chem.*, **40**, 1113 (1936).

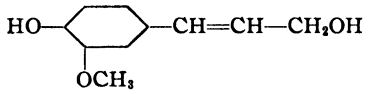
[60] E. Hägglund, *Holzchemie*. 2nd ed., Akadem. Verlagsgesellschaft, Leipzig, 1939, page 208, *et seq.*

[61] K. Freudenberg, F. Sohns, and A. Janson, *Ann.*, **518**, 62 (1935). See also G. F. Wright and H. Hibbert, *J. Am. Chem. Soc.*, **59**, 125 (1937). T. Lieser and V. Schwind, *Ann.*, **532**, 104 (1937).

[62] See K. Freudenberg, *Tannin, Cellulose, Lignin*, Springer, Berlin, 1933; *Papier-Fabr.*, **37**, 28 (1939). K. Freudenberg, F. Klinck, E. Flickinger, and A. Sobeck, *Ber.*, **72**, 217 (1939). M. J. Hunter, G. F. Wright, and H. Hibbert, *ibid.*, **71**, 734 (1938).

the oxygen content considerably less than that of cellulose (44.5% carbon, 6.2% hydrogen, 49.3% oxygen). Most workers infer from this that the structural units of lignin must be aromatic in character.

Quite apart from that the aromatic character of lignin is evident from a number of observations. Many years ago Klason[63] pointed out the similarity between oxidized and polymerized coniferyl alcohol and lignin:

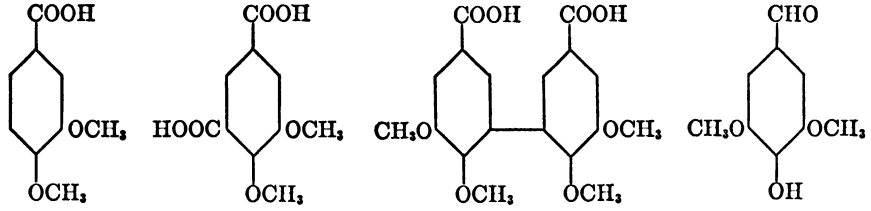


Coniferyl alcohol

Later Herzog and Hillmer[64] drew attention to the similarities in ultraviolet absorption of dissolved lignin and that of isoeugenol and of coniferin, a glucoside of coniferyl alcohol.

Still more important evidence is available from the analyses of products resulting either from oxidation or reductive degradation.

With alkali fusion, one of the oldest procedures, only about 10% of protocatechuic acid was obtainable together with oxalic and acetic acids, etc.; a modification of this due to Freudenberg[65] involves boiling with aqueous alkali, the mixture of phenol alkaline salts then being methylated with dimethyl sulfate and the products oxidized with permanganate. In this way, it was possible to isolate as much as 30–35% of phenolcarboxylic acids, indeed 20% of the lignin could be accounted for as veratric acid (*a*),[66] 6–12% isohemipinic acid (*b*), and a few per cent as dehydrodiveratric acid (*c*):



[63] P. Klason, *Svensk Kem. Tid.*, **9**, 133 (1897); *Arkiv Kemi Mineral Geol.*, **3**, No. 5 (1908); *Ber.*, **B62**, 2523 (1929); **B63**, 1548 (1930).

[64] R. O. Herzog and A. Hillmer, *Ber.*, **60**, 365 (1927); **62**, 1600 (1929); **64**, 1288 (1931). A. Hillmer, *Z. physik. Chem.*, **A167**, 407 (1933); **A168**, 81 (1933).

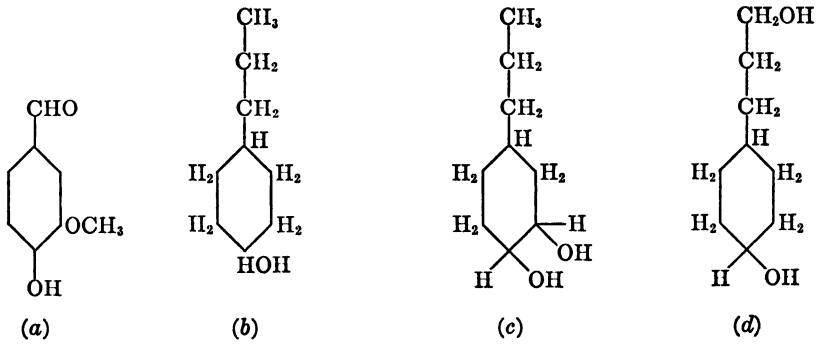
[65] K. Freudenberg, A. Janson, E. Knopf, and A. Haag, *Ber.*, **69**, 1415 (1936). K. Freudenberg, M. Meister, and E. Flickinger, *ibid.*, **70**, 500 (1937). K. Freudenberg, K. Engler, E. Flickinger, A. Sobek, and F. Klink, *ibid.*, **71**, 1810 (1938).

[66] Schimmel & Co., U. S. Patent 2,187,366 (1937).

Up to now the highest yield of aromatic fission products has been obtained by oxidative degradation with nitrobenzene.[66] Applying this method to spruce wood Freudenberg and Lautsch[67] succeeded in isolating 27% vanillin with some 10% of related phenolic acids based on the lignin content of the wood. If the acid products are reckoned as vanillin, which has been destroyed under the conditions of oxidation used, then 40% of the lignin material can be accounted for in the form of a definite decomposition product which contains more than half of the lignin methoxyl.

Maple wood, similarly oxidized, gives 32% 4-hydroxy-3,5-dimethoxybenzaldehyde (*d*, above) and only 3,4% vanillin.[68] Vanillin (*a*, below) results from spruce wood and lignin quite generally after treatment with dehydrogenating agents. On the other hand, from salts of lignosulfonic acids this product is only formed in very small quantities by treatment with alkalis in the absence of oxidizing agents.[69]

Further valuable confirmation of the aromatic structure has been provided by Harris and Adkins.[70] A soluble lignin fraction obtained from aspen wood by treatment with methanolic hydrogen chloride was hydrogenated under pressure in dioxan using copper chromite catalyst. Derivatives of phenylpropane were isolated, *i.e.*, 1-propylcyclohexanol-4 (*b*), 1-propylcyclohexandiol-3,4 (*c*), and 3-(4-hydroxycyclohexyl)propanol-1 (*d*):



(*a*) (*b*) (*c*) (*d*)

Similar products are also obtainable from soluble lignins or sulfuric acid lignin by pressure hydrogenation in aqueous or dilute alkaline solution, in the presence of Raney

[67] K. Freudenberg and W. Lautsch, *Naturwissenschaften*, **27**, 227 (1939). K. Freudenberg, W. Lautsch, and K. Engler, *Ber.*, **73**, 167 (1940).

[68] R. H. I. Creighton, J. L. McCarthy, and H. Hibbert, *J. Am. Chem. Soc.*, **63**, 312 (1941).

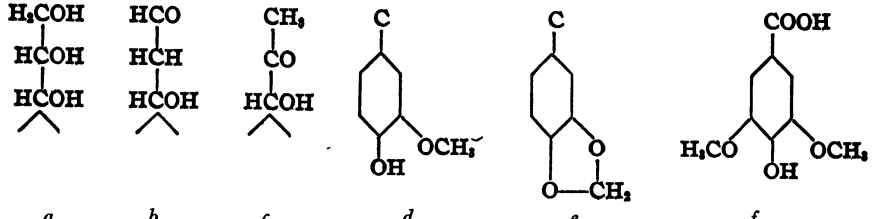
[69] W. Lautsch, E. Plankenhorn, and F. Klink, *Z. angew. Chem.*, **53**, 450 (1940).

[70] E. E. Harris, J. D'Ianni, and H. Adkins, *J. Am. Chem. Soc.*, **60**, 1467 (1938). E. E. Harris and H. Adkins, *Paper Trade J.*, **107**, No. 20, 38 (1938).

nickel.[71] Pressure hydrogenation of wood in dioxan has also given the phenylpropane derivatives (*b*) and (*c*) cited above.[72]

Investigations by Freudenberg and Lautsch[73] have shown that lignosulfonate slags or even sulfite waste liquors can be hydrogenated in weakly alkaline solution, under high pressures at 350°; sulfur is split off and approximately 40% yields of alkyl cyclohexanols and alkyl cyclopentanols are obtained, the latter products arising from cyclohexane derivatives by ring change. By dry distillation of a lignin impregnated with nickel catalyst in a stream of hydrogen, Freudenberg and Adam[74] were able to effect up to 50% conversion of lignin to a phenol mixture, half of which was monocylic, chiefly phenol, guaiacol, and *p*-cresol. The highest yields of hydrogenation products (75% of the lignin) are obtained as cycloparaffin alcohol mixtures by treating lignin with hydrogen donors such as alcohols, *e.g.*, ethyl alcohol in aqueous or sodium hydroxide solution or suspension at 350°.[75]

On the basis of these various degradation processes, giving products which are directly related to the lignin structural units, there can no longer be any doubt about its aromatic constitution. Freudenberg[75a] has expressed the opinion that the structural units in spruce lignin belong to a well-defined group of substances which are closely related biochemically; these are all derivatives of 3,4-dihydroxyphenylpropane. The side chain varies between the biochemically equivalent forms (*a*,*b*,*c*) and the nuclei between the closely related types of vanillyl (*d*), piperonyl (*e*), and perhaps of isovanillyl. According to Freudenberg[76] beech lignin contains along with these, a component derived from pyrogallol in the form of the symmetrical. dimethyl ether, having the same arrangement as syringic acid (*f*):



a *b* *c* *d* *e* *f*

[71] E. E. Harris, J. Saeman, and E. C. Sherrard, *Ind. Eng. Chem.*, **32**, 440 (1940).

[72] H. P. Godard, J. L. McCarthy, and H. Hibbert, *J. Am. Chem. Soc.*, **62**, 988 (1940).

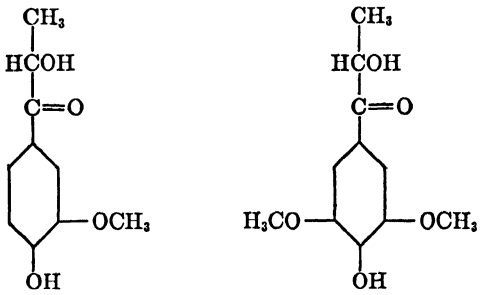
[73] K. Freudenberg, W. Lautsch, G. Piazolo, and A. Scheffer, *Ber.*, **B74**, 171 (1941). W. Lautsch, *Cellulosechimie*, **19**, 69 (1941).

[74] K. Freudenberg and K. Adam, *Ber.*, **74**, 387 (1941).

[75] W. Lautsch, *Brennstoff-Chem.*, **22**, 265 (1941).

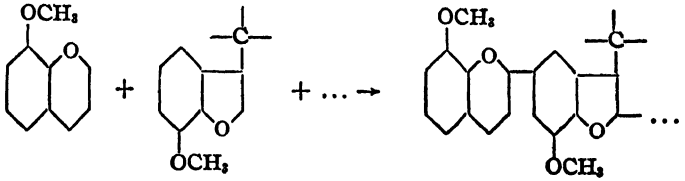
[75a] K. Freudenberg, *Papier-Fabr.*, **36** (Tech. Tl.) 34 (1938).

[76] K. Freudenberg, *Papier-Fabr.*, **36** 4 (1938).

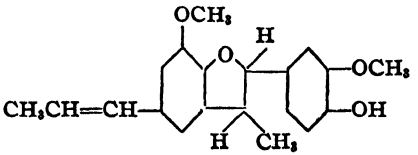


(*g*) (*h*)

These units of low molecular weight are joined together by physiological synthesis in a uniform way, that is, by etherification. A secondary process of condensation follows etherification, perhaps to some extent while the cell is alive, but also later, *post mortem*, in the plant and during chemical treatment for the isolation of lignin; in this latter process benzopyran or benzofuran systems are formed. This may be represented in the following way:



The ease with which benzofuran derivatives can be formed from compounds of the coniferyl type is shown by the dehydrogenation of isoeugenol, which leads to the formation of a substance known as dehydrodiisoeugenol. According to Erdtmann,[77] its constitution is:



Also Hibbert,[78] who has been very much occupied with the problem and

[77] H. Erdtmann, *Ann.*, **503**, 283 (1933).

[78] H. W. Mackinney and H. Hibbert, *Can. J. Research*, **B14**, 58 (1936). See also J. Compton and H. Hibbert, *ibid.*, **B15**, 38 (1937). H. Hibbert, *Paper Trade J.*, **113**, 350. M. Kulka and H. Hibbert, *J. Am. Chem. Soc.*, **65**, 1180 (1943). E. West, A. S. MacInnes, and H. Hibbert, *ibid.*, **65**, 1187 (1943).

for a considerable time had quite other views with regard to the structure of lignin, has made many important contributions in support of its aromatic character. By alcoholysis Hibbert obtained vanilloyl methylcarbinol (*g*) from spruce wood and from beech wood, the syringic derivative (*h*), as well as other compounds, which have the same aromatic nuclei but with different propane residues attached, —$COCOCH_3$ and —CH_2COCH_3. All of these fission products can be satisfactorily included in Freudenberg's scheme. Hibbert suggests that lignin is formed by the condensation of plant respiratory catalysts of the type R—C—C—C—R (where R = guaiacyl or syringyl).

According to Freudenberg,[78a] coniferyl alcohol is transformed in the presence of fungal polyphenol-oxidase by air at room temperature into a dehydrogenated polymer product which has the same composition and the same reactions as genuine lignin.

Remarkably enough, the ultraviolet absorption or reflection spectrum of native lignin (in association with cellulose) does not as yet appear to have been investigated; it should give some indication, at least, whether aromatic groups are present or not, and in what percentage and of what kind. Cellulose does not absorb ultraviolet light, so that its presence would not interfere with the absorption spectrum of lignin; soluble aromatic compounds, such as tannins, could be extracted. Nonetheless, according to Freudenberg, the refractive index of genuine lignin (n_D = 1.6) gives an indication of its aromatic nature.

III. PECTINS

1. Occurrence and Isolation of Pectins

While glucose forms the structural unit of the skeletal and reserve substances of plants, galactose, galacturonic acid, and arabinose form the structural units of a group of carbohydrates characterized by their great swelling power in water, and known collectively as pectins. They appear to be of particular importance in plants, since the young cell walls of meristematic tissues, and, later, the middle lamellae of older cells consist, for the most part, of pectins. Moreover, the flesh of most fruits and roots consist of about half pectin, the pith of many plant stems, *e.g.*, sunflower contains 30–40% pectin.[79] The various pectin substances are apparently able to exert very varied biological functions.

Analogous to animal proteins, which occur in soluble form in the tissue fluids and in insoluble form in the tissues, the pectins of plants are present

[78a] K. Freudenberg, *Sitzber. heidelberg. Akad. Wiss. Math. naturw. Klasse*, **1949**, 151.
[79] H. F. Müller and W. Overbeck, *Ber.*, **75**, 909 (1942).

both in soluble and insoluble forms. Soluble pectin occurs in plant juices; it is particularly abundant, for example, in certain fruit juices, which form jellies; insoluble pectin occurs in the green parts of plants, in roots, and in fruits. To the class of pectins belongs also "pectic acid," the insoluble calcium or calcium and magnesium salt of which occurs in the middle lamella.

On prolonged heating with water hydrolytic breakdown occurs with the formation of smaller molecular fragments which are soluble; pectins can also be brought into solution to a certain extent by conversion of the calcium salts into sodium salts by reaction with sodium oxalate, sodium phosphate, etc. In the course of ripening of fruits or roots the insoluble pectin (protopectin) content diminishes while that of the soluble pectin increases so that presumably the latter is formed by the biological breakdown of the former.

Pectins may be isolated from fruits (citrus, apple) or other sources such as sliced sugar beet, flax waste, etc., which are freed from stones and skins as necessary, finely macerated, and pressed out thoroughly after addition of water. The juices expressed are decolorized with charcoal, clarified with kieselguhr, and the dissolved pectin can then be precipitated with alcohol. The residues, after squeezing out, are usually subjected to prolonged treatment with boiling water, whereby appreciable breakdown occurs and further quantities of pectin pass into solution. To obtain good yields of soluble pectin it is essential to bring about the decomposition of the calcium salt either by treatment with cold acid or by addition of suitable salts as calcium precipitants.

By an alternative procedure, Baier and Wilson[80] have obtained highly viscous pectins from the citrus fruit residues after removal of the dissolved pectins. This involves first treatment with alkali, in which the high molecular pectin is insoluble, so that a mixture of cellulose and calcium pectate remains. After washing this is heated for a short time with dilute trisodium phosphate, whereby sodium pectate is formed and passes into solution, from which it can be precipitated with alcohol. For these products the relative viscosity of a 0.5% solution is between 4.5 and 6.

From flax, and especially from the dust which separates on working up the green flax mechanically, Lüdtke[81] has obtained a large proportion of the pectin by pretreatment with cold 0.5% hydrochloric acid, followed by 0.5% ammonium oxalate at 50–80°, in which the pectin dissolves and is then precipitated by alcohol.

[80] W. E. Baier and C. W. Wilson, *Ind. Eng. Chem.*, **33**, 287 (1941).

[81] M. Lüdtke and H. Felser, *Ann.*, **549**, 1 (1941).

2. Constitution of Pectins

As Ehrlich[82] has shown the complete hydrolysis of pectin yields D-galactose, D-galacturonic acid, and L-arabinose. These three substances are related to each other as are glucose, glucuronic acid, and xylose—they are members of a biological series. In addition methyl alcohol has been found (von Fellenberg[83]) and it is now known that it arises from esterified galacturonic residues.

Pectin, which is insoluble in cold water, is slowly dissolved by heating with water under pressure. Extraction with 70% alcohol then removes a soluble araban and the remaining calcium-magnesium salt gives, on complete hydrolysis, galactose, arabinose, galacturonic acid, and methyl alcohol. On mild hydroylsis a polygalacturonic acid can be obtained which is known as "pectic acid."

Ehrlich has obtained various polygalacturonic acids which he considers to be associates of a tetragalacturonic acid, his so-called pectol acid, to which he ascribes a cyclic tetraglycosidic structure. Furthermore, he suggests that on hydrolysis this product is converted to an open-chain tetragalacturonic anhydride "pectolactonic acid." Ehrlich's molecular weight determinations, however, do not in any way satisfy the requirements for such measurements in the field of high polymers; his theory of "associations" of small units is in contradiction to the physical behavior of the pectins and, likewise, to all that we know of intermolecular forces. The various polygalacturonic acids are rather to be regarded as polymeric homologous polygalacturonic acids of varying chain length. A cyclic formula has also been proposed by Nanji, Paton, and Ling[84] in which four galacturonic residues, galactose, and arabinose are glycosidically linked together, but this is equally irreconcilable with the physical properties of the pectins.

In contrast to the majority of workers in this field, Smolenski[85] and his collaborators have put forward the view that pectins are highly polymerized substances built in the form of chains. For the polygalacturonic acids, easily obtainable from the pectins, they proposed a highly branched structure which today is no longer tenable. In 1930, Meyer and Mark[86] suggested a formula with an unbranched chain. The birefringence of pectin threads, the possibility of drawing out threads from concentrated solutions, the viscosity, and other properties of such solutions appear to them certain evidence of long-chain structure. For polygalacturonic acid, which represents the major part of the pectins, the formula shown below was

[82] F. Ehrlich and R. v. Sommerfeld, *Biochem. Z.*, **168**, 263 (1926). F. Ehrlich and F. Schubert, *ibid.*, **169**, 13 (1926); *Biochem. Z.*, **203**, 343 (1928); *Ber.*, **62**, 1974 (1929).

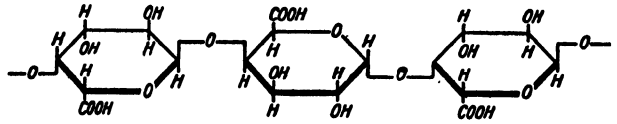
[83] T. von Fellenberg, *Chem. Zentr.*, **1914, II**, 942; *Biochem. Z.*, **85**, 118 (1918).

[84] D. R. Nanji, F. J. Paton, and A. L. Ling, *J. Soc. Chem. Ind.*, **44**, 253T (1925).

[85] K. Smolenski, *Chem. Abstr.*, **19**, 41 (1925).

[86] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, page 219.

advanced. Since then, this formula has been supported by the work of other investigators and its validity can no longer be seriously doubted:



Formula of polygalacturonic acid from pectin (Meyer and Mark)

The high dextrorotation of polygalacturonic acid ($[\alpha]_D^{20} = +280°$ according to Ehrlich) suggests an α configuration for carbon atom 1. Also, the ease of fiber formation, similar to that shown by cellulose derivatives, is only compatible with a highly elongated chain structure, which for a polygalacturonic acid necessitates a repeated α linkage. Here β linkage would give rise to a zigzag chain such as we find in amylose with repeated α-glucosidic linkage.

The behavior of pectins in the ultracentrifuge and likewise the streaming birefringence[87] is further evidence for a chain structure essentially similar to that of cellulose. Observations by van Iterson and Corbeau[88] also suggest that pectins are built up from chain molecules resembling cellulose in structure. They obtained strongly negatively and uniaxially birefringent threads by spinning concentrated aqueous solutions of commercial citrus pectin into a mixture of alcohol and ether; van Iterson also reports that these threads, examined by Burgers, gave an x-ray diagram indicating the presence of oriented crystallites. The fiber period of 13–14 Å. corresponds to three galacturonic acid residues.[89] Films of dried pectins show a higher refractive index at right angles to the plane of the film than in other directions. These facts indicate the presence of long micelles with negative uniaxial intrinsic birefringence. The long axis of the micelles is parallel to the fiber axis in the case of threads, and lies in the plane of the film in the case of films.

Further evidence in support of the chain structure of the pectin molecule has been put forward by Henglein and Schneider.[90] They nitrated pectin and obtained a pectin nitrate (nitropectin) soluble in solvents for nitrocellulose and displaying considerable viscosity in solution; comparatively tough threads and films could be prepared from this compound. From

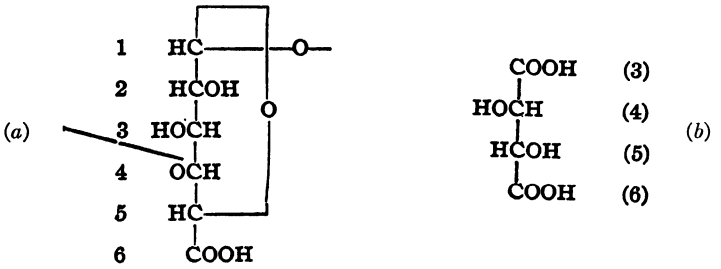
[87] S. Säverborn, *Kolloid-Z.*, **90**, 41 (1940).

[88] G. van Iterson, Jr., L. Corbeau, and W. G. Burgers, *Chem. Weekblad*, **30**, 2 (1933).

[89] K. Wuhrmann and W. Pilnik, *Experientia*, **1**, 330 (1945). K. J. Palmer and H. Lotzkar, *J. Am. Chem. Soc.*, **67**, 883 (1945). K. J. Palmer and M. B. Hartzog, *ibid.*, **67**, 2122 (1945).

[90] F. Henglein and A. Schneider, *Ber.*, **69**, 309 (1936).

determinations of the viscosity, they estimate the molecular weight of carefully prepared pectin to be of the order of several hundred thousand.[91] The physical evidence for the chain structure of pectins has also been supported by purely chemical evidence. Morell and Link[92] demonstrated the presence of end groups in the polygalacturonic acids, which at the same time gives an indication of the chain length. Polygalacturonic acid is esterified with methyl alcohol, in which process both carboxyl groups and terminal aldehyde groups are methylated. The former may be liberated by alkaline saponification, while the aldehyde groups remain resistant to alkali, as does methyl glucoside. The subsequent methoxyl determination gives the content of glucosidically bound methoxyl, and hence the terminal aldehyde groups. In this way, a chemical determination of the mean molecular weight is possible. The authors come to the conclusion that long, slightly soluble, and shorter, more soluble chains are present. A product of hydrolysis containing eight galacturonic acid units was studied in more detail. Morell and Link advocate (as did Meyer and Mark in an earlier study) the 1,4-glucosidal linkage of the units. A direct chemical demonstration of this type of linkage has been brought forward by Levene and Kreider.[93] By the oxidation of polygalacturonic acid with periodic acid and subsequent hydrolysis, they obtained L-tartaric acid, indicating that two hydroxyl groups of the hexosan ring in the configuration of L-tartaric acid were protected from oxidation by glucosidic linkage. The formulas (*a*) and (*b*) make the relationship clear:



Methylation and hydrolysis provide still further support for the formula indicated above; 2,3-dimethyl derivatives[94] are isolated, which means that

[91] G. Schneider and U. Fritschi, *Ber.*, **69**, 2537 (1936). G. G. Schneider and H. Bock, *Z. angew. Chem.*, **51**, 94 (1938). M. Lüdtke and H. Felser, *Ann.*, **549**, 1 (1941). S. Säverborn, *Kolloid-Z.*, **90**, 41 (1940).

[92] S. Morell and K. P. Link, *J. Biol. Chem.*, **100**, 385 (1933). S. Morell, L. Baur, and K. P. Link, *ibid.*, **105**, 1 (1934). L. Baur and K. P. Link, *ibid.*, **109**, 293 (1935).

[93] P. A. Levene and L. C. Kreider, *J. Biol. Chem.*, **120**, 591 (1937).

[94] S. Luckett and F. Smith, *J. Chem. Soc.*, **1940**, 1106, 1506.

only the hydroxyl on carbon atom 4 is available for the glucosidic linkage on the assumption of the highly probably pyranose form. Levene[95] alone prefers the alternative furanose form with 1,5-glucosidic linkages. The polygalacturonic acids isolated from citrus fruits, apples, strawberries, and lupin seeds all have the same constitution.

The relative amounts of the various constituents vary considerably. Citrus pectin is very rich in galacturonic acid, poor in galactose and arabinose; no real decision has yet been reached as to whether, in this case, arabans and galactans are chemically linked to the polygalacturonic acid. According to Lüdtke,[96] flax pectin contains no galactose and only 0.5 to 3% arabinose, this being inseparable as an araban and only isolated after complete hydrolytic breakdown of the pectic acid, from which the author concludes that arabinose residues are directly linked to the uronic residues.

From apple pectin, which is relatively rich in arabinose and galactose, treatment with 70% alcohol, involving no breakdown, effects the removal of a mixture of 54% araban and 46% galactan. This suggests that these polysaccharides are not chemically bound to the polygalacturonic acid, but it is very possible that some decomposition is involved previously in the extraction of the pectin itself. Hirst and Jones[97] have shown that a separation of the galactan can easily be effected since it remains unchanged by the action of 0.05 *N* sulfuric acid which hydrolyzes the araban. To a large extent, Hirst has elucidated the constitution of the araban and of the galactan, which can be split off from the polygalacturonic acid or are originally associated with it. Alkaline methylation of the araban–galactan mixture first brings about the methylation of the former, the methylated araban then being separable. Hydrolysis of this product ($[\alpha]_D^{20} = -86°$) yields 2,3,5-trimethylarabofuranose, 2,3-dimethylarabinose, and a monomethyl derivative which is probably 3-methylarabinose. The structure deduced consists essentially of a straight chain of arabofuranose residues linked in the 1,5 position, with arabofuranosido residues linked here and there through the 2 position, as shown in the formula on page 434.

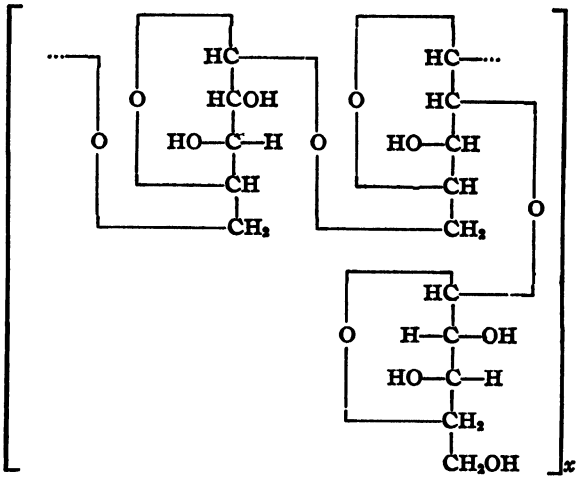
Peanut araban (according to Hirst one of the most readily available arabans) is found to have an essentially similar structure. This is also true for the araban obtainable from citrus fruits and from white lupin seeds.

The galactans consist of chains of galactose residues linked in the 1,4-β positions; lupin seeds are a convenient source for the preparation of pectin galactan. As Hirst pointed out, the structural units arabinose, galactose,

[95] P. A. Levene, G. M. Meyer, and M. Kuna, *Science*, **89**, 370 (1939).

[96] M. Lüdtke and H. Felser, *Ann.*, **549**, 1 (1941).

[97] E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, **1939**, 452, 454. E. L. Hirst, *ibid.*, **1942**, 70. G. H. Beaven, E. L. Hirst, and J. K. N. Jones, *ibid.*, **1939**, 1865.



and galacturonic acid are closely interrelated but the corresponding polymeric substances have essentially different structures and so cannot be formed directly one from another.

In all pectins, the galacturonic acid residues are esterified to some extent; for citrus and apple pectins, the proportion of methyl ester residues is greater than in flax pectin. The capacity for combination with bases depends, of course, on the proportion of free carboxyl groups.

3. Micelle Formation in Solution and Gelation

Information concerning the dissolved state of pectins has been furnished by Kertesz[98] who investigated the change in viscosity and reducing power of aqueous solutions, effected either by heating or by enzyme action. It was found that the higher polymeric pectins are associated in aqueous solution to give micelles which dissociate on heating. Since the lower polymers are less associated a comparatively slight degradation has a very considerable effect on the particle size. Titration curves of the free acids indicated that all the carboxyl groups are accessible to titration.[99]

Säverborn[100] has determined the molecular weight (more correctly, particle size) of various pectins by measuring the velocity of sedimentation in the ultracentrifuge. He investigated "free" pectins, *i.e.*, in the case of

[98] S. Kertesz, *J. Am. Chem. Soc.*, **61**, 2544 (1939).

[99] H. Deuel, *Helv. Chim. Acta*, **26**, 2002 (1943). See also H. Pallman and H. Deuel, *Chimia (Switzerland)*, **1**, 27, 56 (1947). R. Speiser, C. H. Hills, and C. R. Eddy, *J. Phys. Chem.*, **49**, 328 (1945).

[100] S. Säverborn, *Kolloid-Z.*, **90**, 41 (1940).

soluble apple pectin, the product obtained by alcohol precipitation of the apple juices expressed in the cold; in the case of red currant and citrus pectins, the products were obtained by extraction with warm water. He also investigated the nitrate ester derivatives of these substances (Table III). The values should only be taken as provisional; the relatively high value for the free citrus pectin is probably due to micelle formation.

TABLE III

Molecular Weights of Pectins

Pectin source	In water		Nitrate in acetone	
	[η]	Mol. wt. (approx.)	[η]	Mol. wt. (approx.)
Apple (cold extract)...	22	100,000	6–8	40,000 to 60,000
Red currant (cold)....	—	33,000	—	
Lemon (cold).....	40	270,000	—	

The very pronounced increase in viscosity with concentration found for cold-extracted citrus pectin is worth notice (Table IV). This phenomenon can be ascribed to association at high concentrations, and can be regarded as the first stage of gelatinization; similar effects have been observed by Mosimann[101] in the case of gelatin. The quantitative estimation of chain length carried out by Snellman and Säverborn[102] by measurements of streaming birefringence is open to criticism.

TABLE IV

c, g./100 cc.	η_{sp}/c
0.125	204
0.062	110
0.031	86
0.016	76
0.008	45

A warm aqueous solution of pectin at *p*H 2.5–3.5 solidifies on cooling to form a clear, solid jelly resembling a gelatin gel. This property is more marked in the undegraded highly viscous pectins than in those of lower molecular weight. High polymeric pectins can solidify to a solid gel even in 0.5% solution. Gel formation is reversible; on warming, the gel liquefies. Gelation is promoted by sugars and also small amounts of calcium salts, but is retarded by sodium citrate.

[101] H. Mosimann, *Dissertation*. Bern, 1937.

[102] O. Snellman and S. Säverborn, *Kolloid-Beihefte*, **52**, 467 (1941).

As we shall see later, the formation of a reversible, transparent gel is always due to partial crystallization, that is, to the formation of submicroscopic coherent regions. These regions form points of linkage between other parts of the molecules which, owing to their hydrophilic groups, are still surrounded by solvent. This interpretation was developed by Gerngross (fringe micelle theory) in the case of gelatin, and has been generalized by Meyer and van der Wyk.[103] The pectins fulfil the structural conditions necessary for the manifestation of this phenomenon. Polygalacturonic acid chains contain hydrophilic carboxyl groups along with less hydrophilic units bearing methylated carboxyl groups. It may be assumed that the esterified groups unite in micelles, which thus act as points of linkage, while the portions of the chains bearing carboxyl groups remain surrounded by solvent and, to a certain extent, mobile; these are responsible for the elastic properties of the gel.

This interpretation is supported by the fact that neither completely methylated nor completely demethylated polygalacturonic acid forms a jelly. According to Lüers and Lochmüller[104] pectins containing less than 7.3% methoxyl do not gelate; the optimum lies at about 12% methoxyl.

It is obvious that special conditions are necessary if such a structure, consisting of chains which are in part crystallized and in part surrounded by solvent, is to be formed. If the solvation of the carboxyl groups is too great (when the reaction is alkaline, for example), the lattice forces of other groups are overcome and gel formation does not occur; if the carboxyl groups are neutralized by calcium ions, forming desolvated complexes, solvation of the region of the chains between the points of linkage is suppressed and precipitation occurs, accompanied by the contraction of the gel and the separation of liquid (syneresis). The action of sugar is probably similar to salting out.

From what has been said it is understandable that, apart from the chain length, gel formation is determined by the methoxyl content, the calcium content, the acidity, the sugar content, and perhaps by other forces. An elastic structure, such as we may suppose to be present in gels, certainly plays a part in the living plant. A diagram of the gel structure is given in Chapter K, figure 1, page 827.

4. Commercial Pectins

Commercial pectins are used in the preparation of jellies, etc. The most important pectin, citrus pectin, is obtained from citrus fruits by extraction

[103] K. H. Meyer and A. van der Wyk, *Helv. Chim. Acta*, **20**, 1331 (1937).

[104] H. Lüers and K. Lochmüller, *Kolloid-Z.*, **42**, 154 (1927).

with hot dilute sulfuric acid. It consists principally of polygalacturonic acids of different molecular weights and varying methoxyl content. Differences in molecular weight are shown by the different viscosities of pectins of similar analytical composition. Since the method of preparation involves treatment with acid (which necessarily produces glucosidic hydrolysis and hence chain breakdown), it must always be difficult to obtain uniform preparations of constant properties. The problem involved in preparation is, obviously, to hydrolyze just sufficiently to enable the chains to be detached from the network of primitive pectins, but to prevent these chains from undergoing further hydrolysis. For this reason alkaline degradation has recently been considered.

TABLE V

Analyses and Properties of Two Powdered Pectins from the California Fruit Growers' Exchange, Ontario[a]

	Lemon pectin I (1934)	Lemon pectin II (1934)
Water	14.9	14.2
Acidity: 1.0 g. neutralizes so many cc. of 0.1 *N* NaOH (phenolphthalein as indicator)	8.1	12.0
$[\alpha]_D^{20}$	+206.7°	+222.4°
Galacturonic acid, $C_6H_{10}O_7$, %	82.8	79.5
Pectin, %, as calcium pectate	88.2	87.4
Arabinose, %	1.3	1.25
Methyl alcohol	8.5	7.9
η_{rel} at 20°C. in 0.3% solution	2.4	4.1

[a] From F. Ehrlich, in Abderhalden, *Handbuch der biologischen Arbeitsmethoden*. Berlin, 1936, Section I, Part II, page 1568.

In view of the commercial importance of pectins, analytical methods have been studied carefully, in particular those which may provide a measure of the suitability of various pectin preparations for the manufacture of jellies. The chemical methods include: (*1*) the usual method of methoxyl determination; (*2*) the precipitation as "calcium pectate" of the polygalacturonic acid set free from the ester during treatment with sodium hydroxide by addition of calcium chloride in the presence of acetic acid (Carré and Haynes[105]); and (*3*) the determination of galacturonic acid in the mixture of products of total acid hydrolysis by splitting off carbon

[105] M. H. Carré and D. Haynes, *Biochem. J.*, **16**, 60 (1922). M. H. Carré, *ibid.*, **16**, 704 (1922).

dioxide and determining the latter by a gas volumetric method (Tollens and Lefèvre,[106] Nanji, Paton, and Ling[107]).

Of the physical methods of testing, viscometry has been particularly recommended by Myers and Baker[108]; in addition, the power of forming a jelly is determined by allowing an aqueous solution containing a fixed quantity of sugar to gelate and measuring the mechanical strength of the jelly.[109]

5. Pectinases

Enzymes which bring about the hydrolytic breakdown of pectins, *i.e.*, pectinases (formerly known as pectolases), were first discovered by Ehrlich in fruits. They are also present in varieties of *Penicillium* and *Aspergillus*; the common pectinase is present in commercial takadiastase.[110] Pure galacturonic acid[111] can be prepared from pectin by the action of pectinase. Nothing definite is known about the mechanism of the enzyme breakdown; it seems, however, that first larger and then smaller fragments appear, so that it can be assumed that the attack is at random. No mamalian pectinase has been described as yet.

Pectinase preparations are used commercially for the clarification of fruit juices and fruit drinks. The term pectase is used for enzymes which bring about gelatinization of pectin solutions. They are commonly present in plants and are essentially esterases, which hydrolyze the methyl ester groups; they are always present in crude pectinase preparations. Pure pectinase attacks free polygalacturonic acid more rapidly than native pectin. It is, therefore, concluded that the pectinase action is accelerated by the previous saponification brought about by the pectase.

IV. PLANT GUM AND MUCILAGES[111a]

The separation of this group from the hemicelluloses and pectins is to some extent arbitrary. Gums and mucilages occurring in plants are high

[106] K. U. Lefèvre and L. Tollens, *Ber.*, **40**, 4513 (1907).

[107] D. R. Nanji, F. J. Paton, and A. L. Ling, *J. Soc. Chem. Ind.*, **44**, 253T (1925).

[108] P. B. Myers and G. L. Baker, *Bull. Delaware Agr. Exptl. Stat.*, No. 187 (1934).

[109] See G. G. Schneider and H. Bock, *Z. angew. Chem.*, **51**, 94 (1938).

[110] S. Matida and T. Tasiro, *J. Agr. Chem. Soc. Japan*, **15**, 243 (1939).

[111] H. H. Mottern and H. L. Cole, *J. Am. Chem. Soc.*, **61**, 2701 (1939). I. A. Manville, F. J. Reithel, and P. M. Yamada, *ibid.*, **61**, 2973 (1939). E. Rietz and W. D. Maclay, *ibid.*, **65**, 1242 (1943).

[111a] See E. L. Hirst, *J. Chem. Soc.*, **1949**, 522; J. K. N. Jones and F. Smith, *Advances in Carbohydrate Chemistry*, **4**, 243 (1949).

polymeric polysaccharides in which hexose, pentose, and uronic acid units are linked with one another. Their great power of swelling and the absence of pronounced fiber structure indicate that the mode of linkage differs from that with which we are familiar in cellulose. At the moment we know only the units of which these substances are composed, and even here our knowledge is incomplete. Little is known of the mode of linkage. From our present knowledge, however, it seems certain that they have a very irregular structure; moreover, even for very closely related plants considerable differences are found between the structures of the gums and mucilages.

1. Gum Arabic

Gum arabic, the exudate of certain acacias, is a salt of a high polymeric acid, arabic acid, which can be set free by treatment with mineral acids. About 1000 grams of acid neutralize one gram equivalent of alkali.

On hydrolytic breakdown an aldobionic acid, 6-β-D-glucuronosido-D-galactose can be obtained in 28% yield[112-114]; and also a disaccharide, 3-galactosido-galactose. Total hydrolysis gives, besides glucuronic acid, approximately 30% D-galactose, 34% arabinose, and 14% rhamnose. By controlled hydrolysis, Jackson and Smith[115] have been able to isolate a disaccharide, 3-D-galactosido-L-arabinose together with L-arabinose and L-rhamnose, a resistant core remaining which contains galactose and glucuronic residues in the proportion 3:1. Methylation and hydrolysis yield 1 mole of 2,3,4,6-tetramethylgalactose (end group), 5 moles of 2,3,4-trimethylgalactose, 3 moles of 2,4-dimethylgalactose (branch points), and 2,3,4-trimethylglucuronic acid (end group), together with a considerable quantity of 2,3-dimethylglucuronic acid. This indicates a very complicated branched structure, in which residues of L-arabofuranose, L-rhamnopyranose, and also 3-galactopyranosido-L-arabofuranose residues are attached to the central core. Fibers of gum arabic show positive birefringence, but there is no sign of a fiber diagram; the structure of the chain cannot, therefore, be very regular.

[112] L. H. Cretcher and C. L. Butler, *Science*, **68**, 116 (1928).

[113] M. Heidelberger and F. E. Kendall, *J. Biol. Chem.*, **84**, 639 (1929).

[114] S. W. Challinor, W. N. Haworth, and E. L. Hirst, *J. Chem. Soc.*, **1931**, 258.

[115] J. Jackson and F. Smith, *J. Chem. Soc.*, **1940**, 74, 79. F. Smith, *ibid.*, **1939**, 1724; **1940**, 1935.

2. Other Gums

According to Butler and Cretcher[116] hydrolysis of cherry gum yields arabinose, xylose, galactose, mannose, and a uronic acid. Jones[117] obtained 1 mole of D-glucuronic acid, 2 moles of D-galactose 1 mole of D-mannose, and 6 moles of arabinose. In contrast to gum arabic, the fibers of cherry gum are negatively birefringent. Damson gum, obtained from the bark of damson trees, contains, according to Hirst and Jones,[118] an acid polysaccharide from which, by controlled hydrolysis, L-arabinose and D-galactose can be obtained, leaving a much-branched polysaccharide consisting of D-xylose, D-galactose, D-mannose, and D-glucuronic acid residues, the arrangement of which is not yet completely known. Citrus gum also contains similarly complex compounds. Gum tragacanth has been separated into a neutral and an acidic component, similarly complex. The latter has been extensively investigated by Smith[119] and found to have a highly branched structure comparable in its complexity with that of arabic acid. In this case the residues included in the structure are L-fucose, D-xylose, and D-galacturonic acid. Mesquite gum has been found to contain arabinose, galactose, and glucuronic methyl ester.[120]

L-arabinose and D-xylose are readily split off from egg-plum gum by acid hydrolysis leaving a residue which, when methylated and degraded, yields 2,3,4,6-tetra-, 2,4,6- and 2,3,4-tri-, and 2,4-dimethyl-D-galactose, and 2,3-dimethyl-D-glucuronic acid.[120a] In the same way almond-tree gum yields first L-arabinose and D-xylose and later D-galactose and D-glucuronic acid on acid hydrolysis.[120b] Both almond-tree gum and egg-plum gum contain the aldobionic acid found in gum arabic. A ketose previously unknown in nature, D-tagatose, has been found [120c] in the gum of *Sterculia setigera* in company with D-galactose and L-rhamnose.

3. Plant Mucilages

Plant mucilages also yield uronic acids and sugars on hydrolysis. Both flaxseed mucilage[121] and slippery elm bark[122] give the same aldobionic acid,

[116] C. L. Butler and L. H. Cretcher, *J. Am. Chem. Soc.*, **53**, 4160 (1931).
[117] J. K. N. Jones, *J. Chem. Soc.*, **1939**, 558; **1947**, 511.
[118] E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, **1939**, 1482; **1946**, 506.
[119] S. P. James and F. Smith, *J. Chem. Soc.*, **1945**, 749.
[120] E. V. White, *J. Am. Chem. Soc.*, **68**, 272 (1946).
[120a] E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, **1947**, 1064; **1948**, 120.
[120b] F. Brown, E. L. Hirst, and J. K. N. Jones, *J. Chem. Soc.*, **1948**, 1677.
[120c] E. L. Hirst, L. Hough, and J. K. N. Jones, *Nature*, **163**, 177 (1949).
[121] R. S. Tipson, C. C. Christman, and P. A. Levene, *J. Biol. Chem.*, **128**, 609 (1939).
[122] R. E. Gill, E. L. Hirst, and J. K. N. Jones, *J. Chem. Soc.*, **1939**, 1469.

2-D-galacturonopyranosido-L-rhamnose as one of the fission products, the former yielding in addition molecular equivalents of L-galactose and D-xylose.[123] The seed mucilage of *Plantago lanceolata* contains 72% xylose, 11% methylpentose, 15% galactose, and uronic acid residues.[124] There is evidence for 1,2-β linkage of xylopyranose residues and also that one third of the residues are present as end groups (also xylose residues) so that the molecule must be branched to a very considerable extent. The mucilage of the closely related *Plantago psyllium*, on the other hand, gives on hydrolysis arabinose, xylose, and D-galacturonic acid.[125] The seed mucilage of lucerne[126] yields on hydrolysis only 2 parts of D-galactose to 1 part of D-mannose; a possible structure consists of a chain of alternating galactose and mannose residues, the latter carrying side-chain galactose residues.

Some mucilages contain cellulose chains which can be separated (see sect. I 5); certain species of orchid give mucilages which consist entirely of mannose residues (*salep* mannan). In this domain our knowledge is still only of a preliminary character; there is exceedingly little information as yet, either with regard to chain length or mode of linkage of the residues involved.

4. Carbohydrates from Algae[127]

The alginic acids isolated from algae (*Laminaria*) are of interest on account of their considerable powers of imbibition; they have been described by Nelson and Cretcher[128] as polymannuronic acids. Later, Lunde and his collaborators[129] investigated alginic acid viscometrically and by x-ray analysis. By forcing a solution of the acid in dilute alkali through a jet into acid alcohol, they were able to obtain fibers whose x-ray diagram showed signs of fibrous orientation. Together with the mechanical properties, this suggested a structure consisting of primary valence chains. According to their origin and mode of preparation, specimens exhibit different viscosities in solution; the viscosities approach those of cellulose materials,

[123] E. Anderson and H. J. Lowe, *J. Biol. Chem.*, **168**, 289 (1947).

[124] J. Mullan and E. G. V. Percival, *J. Chem. Soc.*, **1940**, 1501.

[125] E. Anderson and M. Fireman, *J. Biol. Chem.*, **109**, 437 (1935).

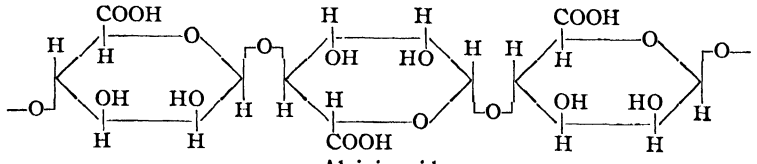
[126] E. L. Hirst, *J. Chem. Soc.*, **1942**, 70. E. L. Hirst, J. K. N. Jones, and W. O. Walder, *J. Chem. Soc.*, **1947**, 1443.

[127] Review: C. K. Tseng, "Phycocolloids," in J. Alexander, *Colloid Chemistry*. Vol. VI, Reinhold, New York, 1946, page 629.

[128] W. L. Nelson and L. H. Cretcher, *J. Am. Chem. Soc.*, **51**, 1914 (1929); **52**, 2130 (1930); **54**, 3409 (1931).

[129] G. Lunde, E. Heen, and E. Öy, *Kolloid-Z.*, **83**, 196 (1938). H. Kringstad and G. Lunde, *ibid.*, **83**, 202 (1938); E. Heen, *ibid.*, **83**, 204 (1938).

and for this reason the authors suggest (with due caution) that the molecular weight may be similar to that of cellulose. By analogy with the formulas of cellulose and pectins, they suggest the following formula with 1,4-β linkage; this has been confirmed by Hirst and also by Lucas and Stewart:[130]



Alginic acid

According to Astbury,[131] the fiber period is 8.7 Å., the rings therefore relate to a zigzag chain, not to one which is fully extended.

Besides alginic acid it is possible to isolate a neutral polysaccharide[132] from *Laminaria*. This substance, laminarin, is found to consist of glucose residues linked only in the 1,3-β positions; its molecular weight is not very high.

Many algae are found to contain sulfate ester groups linked to galactose residues; to this group belongs agar, the polysaccharide obtainable from various tropical species of the *Rhodophyceae*. Hydrolysis of methylated agar yields 2,4,6-trimethyl-D-galactose and, somewhat unexpectedly, a trimethyl-L-galactose from which it is deduced[133] that 1,3 linked D-galactose and also 1,4 linked L-galactose residues are present.

According to Hassid[134] *Iridaea laminaroides* contains the sodium salt of a polygalactan sulfuric acid. The formula on page 443 has been suggested with $n = 6$, but this value for n must certainly be too low for the native substance.

A similar sulfuric ester product, generally known as carragheen, has been obtained from the North Sea alga, *Chondrus crispus*. Percival[135] suggests that the galactose residues are glucosidically linked in the 1,3 position, carbon atom 4 carrying the sulfate ester group.

[130] H. J. Lucas and W. T. Stewart, *J. Am. Chem. Soc.*, **62**, 1792 (1940).

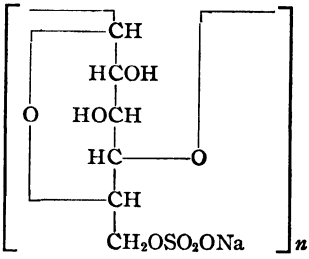
[131] W. T. Astbury, *Nature*, **155**, 667 (1945).

[132] V. C. Barry, *Nature*, **152**, 537 (1943). V. C. Barry and T. Dillon, *Proc. Roy. Irish Acad.*, **B49**, 177 (1943).

[133] E. G. V. Percival, J. Munro, and J. C. Somerville, *Nature*, **139**, 512 (1937). I. A. Forbes and E. G. V. Percival, *J. Chem. Soc.*, **1939**, 1844. T. L. Cottrell and E. G. V. Percival, *ibid.*, **1942**, 749. E. G. V. Percival and T. G. H. Thomson, *ibid.*, **1942**, 750; W. G. M. Jones and S. Peat, *ibid.*, **1942**, 225.

[134] W. Z. Hassid, *J. Am. Chem. Soc.*, **55**, 4163 (1933); **57**, 2046 (1935).

[135] J. Buchanan, E. E. Percival, and E. G. V. Percival, *J. Chem. Soc.*, **1943**, 51.



All these so-called *phycocolloids* have manifold commercial uses, especially in meat canning, pharmaceutical emulsions, cosmetics, etc.

V. CARBOHYDRATES FROM MICROORGANISMS

1. Yeast Polysaccharides

After suitable destruction of the cell walls, hot-water extraction of yeast gives two polysaccharide products: (*1*) yeast gum is precipitated by the addition of cuprammonium solution; (*2*) glycogen (see sect. VII 10) remains in solution. A third carbohydrate substance is present in the residues.

Yeast gum gives only mannose on hydrolysis and is therefore a mannan. According to Haworth, Heath, and Peat,[136] methylation and hydrolytic cleavage yield equal quantities of 2,3,4,6-tetramethylmannose, 3,4 dimethylmannose, and a mixture of trimethylmannoses containing 45% of 3,4,5, 45% of 2,4,6, and 10% of 2,3,4 derivatives. The methylated mannan can be separated into fractions for which the molecular weights vary between 18,000 and 76,000, the intrinsic viscosity of the highest molecular fraction amounting to 0.21 in chloroform. All of the evidence points to a highly complex branched structure which cannot yet be more precisely defined.

The third polysaccharide, normally remaining in the residues, was isolated by Salkowski[137] by repeated extraction with water and hot dilute alkali. Apparently this constitutes the skeletal substance of yeast. On hydrolysis this insoluble polysaccharide yields glucose in the upward mutating form, so that it is essentially a poly-β-glucoside. Zechmeister and Tóth[138] and likewise Hassid[139] have obtained evidence for repeated 1,3-β

[136] W. N. Haworth, R. L. Heath, and S. Peat, *J. Chem. Soc.*, **1941**, 833.

[137] E. Salkowski, *Z. physiol. Chem.*, **92**, 75 (1914).

[138] L. Zechmeister and G. Tóth, *Biochem. Z.*, **270**, 309 (1934).

[139] W. Z. Hassid, M. A. Joslyn, and R. M. McCready, *J. Am. Chem. Soc.*, **63**, 295 (1941).

linkage by the isolation of 2,4,6-trimethylglucose after methylation and hydrolysis. No tetramethylglucose has been isolated as evidence for end groups. The molecular weight is unknown, as estimations made on the basis of viscosity measurements are unreliable.

2. Polysaccharides from Species of *Penicillium*

Penicillium charlesii produces from glucose a galactan known as galactocarolose and a mannan, mannocarolose. According to the particular medium used, one or the other of these carbohydrates predominates. The investigations of Haworth, Raistrick, and Stacey[140] show that galactocarolose contains galactose in the furanose form; the linkage of the units with one another is a 1,5 linkage. Only 9 to 10 galactose units are said be present in the molecule. Mannocarolose is probably composed of mannopyranose units in 1,6-α linkage (Haworth, Raistrick, and Stacey).

A glucosan of higher molecular weight is produced by *Penicillium luteum* Zukal; this is luteinic acid. On mild hydrolysis, malonic acid is split off, leaving luteose, in which more than 80 glucopyranose units are united with one another by 1,6-β linkages (Anderson, Haworth, Raistrick, and Stacey[140a]).

The constitution of varianose, a product of the metabolism of *Penicillium varians* G. Smith, has not yet been cleared up. Varianose is a polysaccharide containing, for the most part, galactopyranose units in 1,4 linkage together with glucose units and an idose (Haworth Stacey[140]).

3. Bacterial Glucosans

A considerable number of bacteria can synthesize glucosans from sugar solutions. *Acetobacter xylinum* produces cellulose. As revealed by the electron microscope,[141] it consists of submicroscopic bacterial threads, in the form of long ribbons, 100 Å. thick and 200 Å. wide. In a culture a thick tangled mass is produced, resembling macroscopically a distended cuticle. This cellulose has the same crystal structure as normal native cellulose; when freshly prepared it is swollen and, like precipitated cellulose, loses the power of imbibition on drying. This bacterial cellulose

[140] W. N. Haworth, H. Raistrick, and M. Stacey, *Biochem. J.*, **29**, 2668 (1935); **31**, 640 (1937).

[140a] C. G. Anderson, W. N. Haworth, H. Raistrick, and M. Stacey, *Biochem. J.*, **33**, 272 (1939).

[141] E. Franz and E. Schiebold, *J. makromol. Chem.*, **1**, 4 (1943). K. Mühlethaler, *Biochim. Biophys. Acta*, **3**, 527 (1949).

gives highly viscous solutions[142] and must therefore have a high molecular weight.

Other bacteria produce insoluble swollen dextrans in which the linkages are α-glucosidic. According to Peat and Stacey,[143] and also Hassid and Barker,[144] the dextran which separates out as a swollen mass from *Betacoccus* (*Leuconostoc*) *arabinosaceus* consists of an unbranched chain of α-glucose residues linked in the 1,6-α positions. The molecular weight lies between 40,000 and 60,000.

The dextran formed by *Betabacterium vermiforme* is considered by Stacey[145] to be of similar structure; on the other hand, in Freudenberg's[146] opinion, the dextran of *Leuconostoc mesenteroides*, which appears as a swollen mass in infected beerwort (so-called frogspawn disease), contains both 1,6-α and 1,4-α linkages and has a branched chain. This dextran has been proposed as a plasma substitute[147] in blood transfusion since it is quite harmless and can give an aqueous solution isotonic with blood. The polysaccharide produced from various sugars by Crowngall bacterium (*Phytomonas tumefaciens*) is a 1,2-β-glucosan[147a] with $[\alpha]_D = -10°$.

In a few cases it has been possible to isolate the effective enzyme from the culture medium, *e.g.*, from that of *Leuconostoc mesenteroides*. Hehre[148] obtained an enzyme which catalyzes the reaction: n sucrose $\rightarrow$ n fructose + glucosan (dextran). Another example has been provided by Hestrin[149] who claims to have isolated from cultures of *Aerobacter levanicum* an enzyme capable of catalyzing the reaction: n sucrose $\rightarrow$ n glucose + fructosan (levan).

4. Other Bacterial Polysaccharides

Among the carbohydrates produced by bacteria, we find galactans, arabogalactans, glucosans, and fructosans. The fructosan formed by *B. mesentericus* from glucose has been investigated by Challinor, Haworth,

[142] H. Fikentscher, *Cellulosechemie*, **13**, 58 (1932).

[143] S. Peat, E. Schlüchterer, and M. Stacey, *J. Chem. Soc.*, **1939**, 581.

[144] W. Z. Hassid and H. A. Barker, *J. Biol. Chem.*, **134**, 163 (1940).

[145] W. D. Daker and M. Stacey, *J. Chem. Soc.*, **1939**, 585.

[146] K. Freudenberg, T. Ploetz, and W. Jakob, *Ber.*, **75**, 1694 (1942).

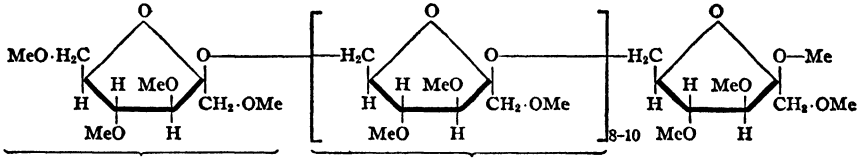
[147] A. Grönwall and B. Ingelman, *Nature*, **155**, 45 (1945).

[147a] F. C. McIntire, W. H. Peterson, and A. J. Riker, *J. Biol. Chem.*, **143**, 491 (1942). R. Hodgson, A. J. Riker, and W. H. Peterson, *ibid.*, **158**, 89 (1945). R. E. Reeves, *J. Biol. Chem.*, **154**, 49 (1944).

[148] E. J. Hehre and J. Y. Sugg, *J. Exptl. Med.*, **75**, 339 (1942).

[149] S. Hestrin and S. Avineri-Shapiro, *Biochem. J.*, **38**, 2 (1944). Further literature: M. Doudoroff and R. O. Neal, *J. Biol. Chem.*, **159**, 585 (1945).

and Hirst[150]; a similar fructosan is formed by certain earth actinomycetes; after methylation and methanolysis 1,3,4-trimethylfructofuranose is obtained along with a little 1,3,4,6-tetramethylfructofuranose. From this result they conclude that the fructosan is composed of fructofuranose units arranged as in the following formula for the methylated fructosan:



Fructosans with the same chemical structure but rather different physical properties have been obtained from *Betabacterium megatherium*, *B. pruni*, and *B. prunicola*[151] (for fructosans of higher plants see sect. VII 14). A polysaccharide of unknown constitution is formed by *Streptococcus salivarius* in a sugar-containing medium.[152]

5. Polysaccharides from Pathogenic Bacteria

Serologists experienced considerable surprise when Heidelberger and Avery[153] discovered polysaccharides of immunological specificity in pathogenic bacteria. Up to that time, such specific properties as the formation of antibodies had been ascribed to proteins and complicated lipoids only. Unfortunately, the constitution and molecular weight of the substances in question are still but little known. The polysaccharides from *Pneumococcus* have been most studied. Different types of *Pneumococcus* (I, II, and III, etc.) are known. The cocci contain capsules consisting for the most part of polysaccharides. The polysaccharides can be separated from proteins since they are resistant to proteolytic enzymes. From 10 liters of a glucose–bouillon culture of *Pneumococcus* III, 2 grams of polysaccharides may be obtained. The substance is a high polymer and strongly acid; according to Heidelberger, Goebel, and Reeves,[154] it consists of a chain of alternating glucose and glucuronic acid residues; each glucuronic residue is

[150] S. W. Challinor, W. N. Haworth, and E. L. Hirst, *J. Chem. Soc.*, **1934**, 676.

[151] R. R. Lyne, S. Peat, and M. Stacey, *J. Chem. Soc.*, **1940**, 237.

[152] C. F. Niven, Jr., K. L. Smiley, and J. M. Sherman, *J. Bact.*, **41**, 479 (1941).

[153] M. Heidelberger and O. T. Avery, *J. Exptl. Med.*, **38**, 73 (1923); **40**, 301 (1924). Review: M. Heidelberger, *Physiol. Rev.*, **7**, 107 (1923). K. Landsteiner, *The Specificity of Serological Reactions*, Thomas, Springfield, 1936.

[154] M. Heidelberger and W. F. Goebel, *J. Biol. Chem.*, **70**, 613 (1926); **74**, 613 (1927). R. E. Reeves and W. F. Goebel, *ibid.*, **139**, 511 (1941).

β-glycosidically linked to carbon atom 4 of a glucose residue and the glucose residues are linked to the 3 position in the glucuronic acid residues. The molecular weight of this polycellobiuronic acid is very high. The polysaccharide from type VIII contains two glucose residues for one cellobiuronic acid; in spite of the difference in constitution it reacts with the antibody produced by type III.

The carbohydrate from *Pneumococcus* II yields glucose on hydrolysis; that from I contains considerable quantities of nitrogen and acetyl groups and is acid; it contains galacturonic acid units and an amino compound. The polysaccharide obtained from cultures of *Pneumococcus* XIV contains acetylglucosamine and galactose[155] in the proportions 1:3; $[\alpha]_D^{20} = 12.5$; it is related to a specific substance isolated from pepsin by Landsteiner[156] which displays reactions similar to those of the specific substance from blood group 1.

Polysaccharides have also been isolated from other pathogenic bacteria, for example, from *B. pneumoniae* Friedländer (Heidelberger, Goebel, and Avery[157]). Three types of this bacillus are known, each of which produces its own carbohydrate. In all three, glucose and an aldobionic acid occur. *Cholera vibrio* contains a carbohydrate yielding, on hydrolysis, galactose, arabinose, and an aldobionic acid, as Linton and Shrivastava[158] showed. A polysaccharide has been isolated from *Bact. typhosum.*[159] It contains D-glucose, D-galactose, and D-mannose; $[\alpha]_D^{16} = +114$; mol. wt. $> 10{,}000$. In this and further investigations, it has been found that the varieties of individual species produce specific carbohydrates which can be distinguished both serologically and chemically. A further example is provided by different strains of tubercle bacilli; the polysaccharide from strain H37 contains D-mannose and D-arabinose, whereas that from A10 contains D-galactose and inositol.[160]

Two polysaccharides have been obtained from strains of human tubercle bacilli: a branched one containing mannose, arabinose, rhamnose, and an aminosugar, M.W. 12,000, and, bound to a lipoid, an unbranched one containing galactose, mannose, arabinose, and glucosamine.[160a] Other specific

[155] W. F. Goebel, P. B. Beeson, and C. L. Hoagland, *J. Biol. Chem.*, **129**, 455 (1939).

[156] K. Landsteiner and M. W. Chase, *J. Exptl. Med.*, **63**, 813 (1936).

[157] M. Heidelberger, W. F. Goebel, and O. T. Avery, *J. Exptl. Med.*, **42**, 701 (1925); **46**, 601 (1927).

[158] R. W. Linton and D. L. Shrivastava, *Indian J. Med. Research*, **1932–35**.

[159] G. G. Freeman, *Biochem. J.*, **36**, 340 (1942).

[160] M. Heidelberger and A. E. O. Menzel, *J. Biol. Chem.*, **118**, 79 (1936). R. J. Anderson, R. E. Reeves, and F. H. Stodola, *ibid.*, **121**, 649 (1937).

[160a] W. N. Haworth, P. W. Kent, and M. Stacey, *J. Chem. Soc.*, **1948**, 1211, 1220.

carbohydrates have been isolated from tuberculin present in culture media of tubercle bacilli (D-arabinose, D-mannose, D-galactose, traces of glucuronic acid[160b]) and from *Corynebacterium diphtheriae* (D-galactose, pentoses, amino sugars[160c]).

The serological importance of these substances lies in the fact that they form antigens when combined with protein. Antigens are substances or mixtures of substances which, injected into a mammal, stimulate the organism to form a new substance, known as an antibody, in its blood. It is characteristic of the antibody, or, more correctly, of the blood serum containing the antibody, that it reacts with the antigen which has induced its formation and with that antigen only (in a test tube, for example) to produce precipitation or another reaction. When precipitation occurs, the antibody is known as a precipitin.

Bacterial polysaccharides, as they occur in nature mixed with bacterial protein or in chemical combination with other suitable proteins, are antigens; the antibody formed in response to their injection reacts not only with the antigen but also with the protein-free polysaccharide. Substances which lead to the formation of antibodies only when in combination with protein, but which have the power of reacting with the antibody in the absence of protein, are known as haptenes. According to Heidelberger, very high polymeric specific polysaccharides act as full antigens even without addition of proteins.

Serological specificity is associated with the various polysaccharides. Their number must be very great, since practically every kind of bacteria produces its own polysaccharide, characteristic for the species.

Substances which in extremely small quantities (0.002γ/kg. man or animal) provoke fever have been isolated from different bacteria, *e.g.*, *Bacterium typhosum*, *Escherichia coli*. Elementary analysis indicates that they must be considered as polysaccharides containing small amounts of amino sugars.[161]

VI. CHITIN AND OTHER POLYSACCHARIDES CONTAINING AMINO SUGARS[161a]

1. Chitin

The organic skeletal substance of arthropods, annelids, mollusks, and fungi is chitin, a nitrogen-containing carbohydrate.

[160b] F. B. Seibert, *Chem. Revs.*, **34**, 107 (1944).

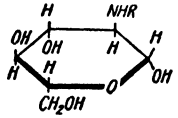
[160c] E. M. Gubarew, *Chem. Abstracts*, **38**, 142 (1944).

[161] P. Weger, *Naturwiss.*, **34**, 59 (1947).

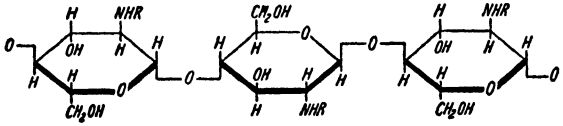
[161a] Summary by L. Zechmeister and G. F. Tóth, in *Fortschritte der Chemie der organischen Naturstoffe*, **2**, 212 (1939).

(*a*) *Constitution*

On hydrolysis with acids, glucosamine and acetic acid are obtained in equivalent quantities and in theoretical yields[162]; under certain conditions, acetylglucosamine may also be obtained, so that it must be assumed that acetylglucosamine is the structural unit of chitin, just as glucose is the structural unit of cellulose. In view of the physical and chemical properties of chitin there is no doubt that many molecules of glucosamine are united to form long primary valence chains. The constitution of glucosamine is shown below:[163,164]



The assumption that chitin consists of primary valence chains of glucosamine units, the value of 10.4 Å. for the identity period along the fiber axis given by Gonell,[165] and the biological analogy between chitin and cellulose, led Meyer and Mark[166] to the conclusion that the units of glucosamine are united in the same manner as the glucose units in cellulose, that is, in 1,4-β linkage, each chain having a digonal screw axis. They ascribed to chitin the constitution shown below and assumed that chitin had a micellar structure of parallel oriented chains similar to that of cellulose. Soon after this formula was put forward it was confirmed by the isolation of chitobiose, a product of hydrolysis, by Bergmann, Zervas, and Silberweit,[167] and by the demonstration of the presence of β-glucosidic linkages by Zechmeister, Grassmann, Tóth, and Bender.[168] The results of more detailed x-ray examination, and of the study of the mode of reaction, are also in agreement with this formula:



Formula of chitin (Meyer and Mark)

[162] G. Ledderhose, *Ber.*, **9**, 1200 (1876). H. Brach, *Biol. Zentr.*, **38**, 468 (1912).

[163] P. Karrer and J. Mayer, *Helv. Chim. Acta*, **20**, 407 (1937).

[164] W. N. Haworth, W. H. G. Lake, and S. Peat, *J. Chem. Soc.*, **1939**, 271.

[165] H. W. Gonell, *Z. Physik*, **25**, 118 (1924); *Z. physiol. Chem.*, **152**, 18 (1926).

[166] K. H. Meyer and H. Mark, *Ber.*, **61**, 1936 (1928).

[167] M. Bergmann, L. Zervas, and E. Silberweit, *Ber.*, **64**, 2436 (1931).

[168] L. Zechmeister, W. Grassmann, G. Tóth, and R. Bender, *ibid.*, **65**, 1706 (1932). L. Zechmeister and G. Tóth, *ibid.*, **66**, 522 (1933).

(*b*) *Crystalline Structure of Chitin*

The fiber diagram of a tendon (apodeme) of the rock lobster (*Palinurus vulgaris*) (Chap. A, Fig 35), obtained by Meyer and Pankow,[169] made possible a thorough evaluation. The unit cell is rhombic with the axes:

a = 9.40 Å.
b = 10.46 Å. (fiber axis)
c = 19.25 Å.

The cell contains 8 acetylglucosamine units (7.9 calculated from the density = 1.415, measured by Meyer and Pankow). These results have been confirmed by Clark and Smith.[170] From the consideration of symmetry elements, space group, and chain structure, the scheme shown in figure 3 was

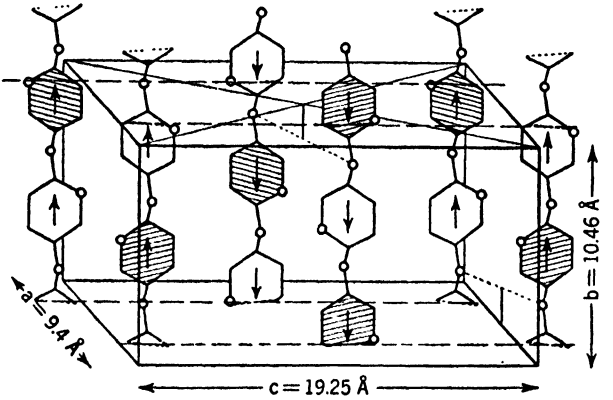


Fig. 3. Diagram of the unit cell of chitin (Meyer and Pankow).

obtained. The analogy with cellulose is apparent in the main features of the diagram: equal numbers of chains are directed in opposite senses, the rings follow one another in a digonal screw sequence (to a first approximation) and all rings lie flat in one plane.

The following difference is apparent: while in cellulose the chains lying flat in one plane have the same direction, here the direction alternates. The structure is similar to that of metastable cellulose III.

The crystalline regions are of approximately the same size and shape as those of cellulose, that is, 100 to 200 Å. thick and very long. This is the case, at least, in the specimen from which the diagram was obtained. Here also the chitin chains are probably as long as the cells. Freshly pre-

[169] K. H. Meyer and G. W. Pankow, *Helv. Chim. Acta*, **18**, 589 (1935).
[170] G. L. Clark and A. F. Smith, *J. Phys. Chem.*, **40**, 863 (1936).

pared solutions of chitin in acids have about the same viscosity as those of wood cellulose. Native chitin may, therefore, have a molecular weight so large as to be unmeasurable. In structures which are obviously fibrous, the chitin chains are oriented, for example, in tendons, in the laminae of beetle elytrae (Kratky[171]) and in the sporangiophores of *Phycomyces*. Most specimens of chitin or of organs consisting largely of chitin, such as butterfly wings, crustacean chitin, or fungal chitin, however, yield powder diagrams only. This may be due to the fact that such structures consist of superimposed laminae in which the orientation of the chains varies from lamina to lamina.[172] Chitin is often associated with other substances of organic or inorganic nature, for example, with calcium carbonate in the carapace of crabs and lobsters. Further x-ray studies of the submicroscopic structure and texture of chitin, in particular lepidopteran scales, have been carried out by Picken.[172a]

(*c*) *Molluscan Chitin*[173]

The jaws of mollusks as well as the internal skeleton (the "bone") of the cuttlefish consist of, or contain, chitin. The internal shell of the squid, *Loligo*, a transparent elongated shield, is pure chitin; in the natural condition it contains 46% of water. This water readily evaporates in air; once the substance has been dried, water is taken up only very slowly. In certain regions, the chitin micelles run parallel; such regions give a fiber diagram which differs somewhat from that of arthropodan chitin. This form is therefore called β-chitin by Lotmar and Picken. The micellar threads are probably much thinner here than in arthropodan chitin. The absence of certain reflections may be due perhaps to the fact that the micelles are very thin in one direction.

(*d*) *Fungal Chitin*

Although it has long been known that the chitin of fungi has the same composition as that of arthropods, the final demonstration of this is due to a detailed study by Diehl and van Iterson.[174] According to these workers, the composition of fungal chitin obtained in pure form from the sporangio-

[171] O. Kratky, *unpublished work.*

[172] W. J. Schmidt, *Die Bausteine des Tierkörpers in polarisiertem Lichte.* Cohen, Bonn, 1924, page 238.

[172a] L. E. R. Picken, *Phil. Trans. Roy Soc. London*, **B234**, 1 (1949).

[173] E. H. Wiebenga, *unpublished work.* W. Lotmar and L. E. R. Picken, *Experientia*, **6**, 58 (1950).

[174] F. M. Diehl and G. van Iterson, Jr., *Kolloid-Z.*, **73**, 142 (1935).

phores of *Phycomyces blakesleeanus* is $C_8H_{13}O_5N$, that is, the same as animal chitin. The physical properties are also analogous and, as van Iterson, Meyer, and Lotmar[175] showed, the x-ray diagram is essentially the same as that of crustacean chitin. The diagram was evaluated simultaneously by Heyn,[176] whose results are essentially the same as those of the first-named authors. Heyn made the interesting observation that a higher orientation occurs in the wall of the sporangiophore. In the cylindrical wall, the long axes of the chitin chains lie inclined at a small angle to the fiber axis, the *c*-axis is radial and the *a*-axis tangential. The plane of the glucose rings is thus approximately radial. The x-ray diagram of fungal chitin is somewhat poorer in reflections than that of arthropodan chitin and, in this respect, may be compared with molluscan chitin. Solutions of fungal chitin in acids have the same viscosity as those of animal chitin (Meyer and Wehrli[177]).

(*e*) *Reactions of Chitin*

Chitin may be nitrated by fuming nitric acid to form mononitrochitin, which yields a well-defined fiber diagram with a fiber period of 10.4 Å. (Clark and Smith, Meyer and Wehrli); the fiber structure is retained. In certain acids chitin dissolves and is gradually hydrolyzed to give viscous solutions; this occurs in 45% phosphoric acid, 65% sulfuric acid, and 45% nitric acid. Strong alkali has at first no effect on the fiber diagram; after prolonged boiling, the acetyl groups are split off and a polyglucosamine consisting of 20–30 glucosamine units is formed. Breakdown of the chains cannot be excluded.

Under the name "chitosan" various more or less deacetylated breakdown products of chitin have been described, which form crystalline salts with acids. Similar phenomena of crystallization have been already noted several times in the course of this account, for example, in mixtures of oligosaccharides and in degraded acetyl celluloses.

It may be mentioned that the heat of activation of acid hydrolysis of the glucosidic linkage in chitin amounts to $\sim$ 30,000 cal. and is the same as that of the linkage in cellulose (Meyer and Wehrli). In the presence of acetic anhydride, acetic acid, and zinc chloride, chitin may be acetylated, thereby undergoing profound degradation (Schorigin,[178] Meyer and Wehrli).

[175] G. van Iterson, Jr., K. H. Meyer, and W. Lotmar, *Rec. trav. chim.*, **55**, 61 (1936).
[176] A. N. J. Heyn, *Protoplasma*, **25**, 372 (1936).
[177] K. H. Meyer and H. Wehrli, *Helv. Chim. Acta*, **20**, 353 (1937).
[178] P. Schorigin and E. Hait, *Ber.*, **68**, 971 (1935).

2. Mixed Polysaccharides Containing Hexosamine Residues

In recent years a number of biologically highly reactive substances have been shown to be polysaccharides, containing not only sugar or uronic acid residues, but also amino sugars. The immunologically specific substances of certain pneumococci have already been mentioned. The specific blood group substances also belong to this type, likewise heparin. Many of these aminocarbohydrates are chemically bound to proteins as glycoproteids; a primary valency linkage may be involved or the linkage may be only of the secondary type as, for example, in the glucoproteid of egg albumin.

(*a*) *Chondroitinsulfuric Acid*

Calcium chondroitin sulfate occurs with collagen in vertebrate cartilage, in amounts corresponding to 40–44% of the dry cartilage weight. The acid can be extracted from finely divided boiled cartilage using 2% sodium hydroxide, and, after picric acid precipitation of the dissolved collagen, the water-soluble calcium salt is precipitated with alcohol. Acid hydrolysis yields galactosamine,[179] glucuronic acid, acetic acid, and sulfuric acid in equivalent quantities, the acetic acid arising from an acetyl amino group.

According to Neuberg[180] glucosamine also appears among the fission products when hydrolysis is effected by means of an enzyme present in *Bacillus fluorescens non liquefaciens*.

In opposition to the views of Fürth and Bruno,[181] the molecular weight is certainly high. Reducing power indicates a chain length of 120–125 sugar residues[182] corresponding to a molecular weight of 20,000–35,000. By extracting the chondroitinsulfuric acid using only calcium chloride, Blix and Snellman[183] claim to have isolated the native form with a molecular weight of 260,000, treatment with dilute alkali causing, in their opinion, a rapid breakdown to a stable product (mol. wt. 30,000–40,000). Exactly what this alkali breakdown involves is not clear; perhaps it is not really a chemical breakdown but the dissociation of micelles or aggregates.

By the hydrolysis of a degraded methylated product, Stacey has succeeded[184] in isolating 2,3,4-trimethylglucuronic acid (as the amide of the

[179] P. A. Levene and F. B. La Forge, *J. Biol. Chem.*, **15**, 69, 153 (1913); **18**, 123 (1914); **20**, 433 (1915).

[180] C. Neuberg and W. Cahill, *Atti accad. nazl. Lincei*, **22**, 149 (1935).

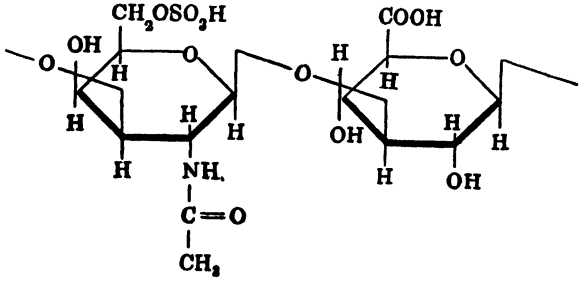
[181] O. Fürth, T. Bruno, and K. Peschek, *Biochem. Z.*, **294**, 153 (1937).

[182] See footnote 185.

[183] G. Blix and J. O. Snellman, *Arkiv Kemi Mineral Geol.*, **A19**, No. 32 (1945).

[184] H. G. Bray, J. E. Gregory, and M. Stacey, *Biochem. J.*, **38**, 142 (1944).

methyl glycoside); the acid, therefore, must occur as an end group. From this a highly branched structure was deduced, but this is not in agreement with the physical properties of the native compound, for which both the viscosity and streaming birefringence, as observed by Blix and Snellman, require an elongated unbranched chain structure. It seems probable that the methylated product was very considerably degraded. Periodate attacks the terminal groups only, all others being resistant. From this and other evidence the following formula has been deduced by Meyer, Odier, and Siegrist:[185]



The optical rotation in water amounts to $[\alpha]_D^{20} = -31°$. It follows that the residues are bound by β linkages.

(*b*) *Mucoitinsulfuric Acid and Hyaluronic Acid*

By suitable hydrolysis, animal mucilages—the mucins—give protein and polysaccharide components, a characteristic of the latter being their glucosamine content.

From the stomach mucosa of young pigs[186] Meyer has isolated two polysaccharides; one is neutral and consists of equimolar quantities of acetylglucosamine and galactose, the second is acidic containing hexuronic acid, acetylglucosamine, and sulfuric acid combined as ester. The neutral polysaccharide gives the reactions of blood group A in quantities as small as 10^{-9} g. Its solutions are highly viscous. The acidic polysaccharides obtainable from mucins are termed in general mucoitinsulfuric acids.

A mixture of polysaccharides has been obtained from hide by Meyer and Chaffee[187]; these give hexosamine, and uronic and sulfuric acids on hydrolysis. The ovomucoid polysaccharide[188] is thought to be a hendecasaccharide

[185] K. H. Meyer, M. Odier, and A. E. Siegrist, *Helv. Chim. Acta*, **31**, 1400 (1948).
[186] K. Meyer, E. M. Smyth, and J. W. Palmer, *J. Biol. Chem.*, **119**, 73 (1937).
[187] K. Meyer and E. Chaffee, *J. Biol. Chem.*, **138**, 491 (1941).
[188] M. Stacey and J. M. Woolley, *J. Chem. Soc.*, **1942**, 550.

consisting of a nucleus of three mannose residues to which seven acetylglucosamine residues and one galactose are attached.

The polysaccharide obtainable from serum protein is thought to contain galactose, mannose, and acetylhexosamine residues.[189]

On aqueous or alkaline extraction of umbilical cord, vitreous humor of the eye or synovial fluids, high polymeric polysaccharides of acidic nature can be isolated after separation from protein material. These have been investigated by Meyer and co-workers[190] and are termed hyaluronic acids. They contain acetylglucosamine and glucuronic acid residues. The former are linked by β-glucosidic bonds to atom 3 of the glucuronic acid residues and these in turn are linked by β bonds to atom 4 of the glucosamine units.[190a]

A similar substance has been isolated from pleural exudates. Hydrolytic fissure gives an acetylhexosamine (probably acetylglucosamine), a hexuronic acid (probably glucuronic acid), possibly a pentose, and in small quantities sulfuric acid originally bound as ester. From measurements of viscosity and streaming birefringence, Blix and Snellman[191] are of the opinion that the hyaluronic acids are very high polymers (mol. wt. 200,000–500,000). Enzymes able to effect their breakdown, known therefore as hyaluronidases, are commonly found in animal tissue[192]; for instance, they are abundantly present in sperm and may serve to break open the outer covering of the ova and so make fertilization possible.[193]

(*c*) *Heparin*[194]

Special mention must be made of the anti blood coagulant heparin, the effect of which was discovered by Howell and McLean. Also a polysaccharide containing glucuronic and glucosamine residues, it is especially rich in sulfuric ester residues, as many as three OSO_3H for each glucuronylglucosamine residue being present. The amino group is not acetylated,[195] but bears sulfuric acid residues ($-NHSO_3H$).[195a] The mode of linkage of the

[189] L. F. Hewitt, *Biochem. J.*, **33**, 1496 (1939).

[190] K. Meyer and J. W. Palmer, *J. Biol. Chem.*, **107**, 629 (1934); **114**, 689 (1936).

[190a] K. H. Meyer and J. Fellig, *Experientia*, **6**, 186 (1950).

[191] G. Blix and J. O. Snellman, *Arkiv Kemi Mineral. Geol.*, **A19**, No. 32 (1945).

[192] K. Meyer and E. Chaffee, *J. Biol. Chem.*, **133**, 83 (1940).

[193] K. Meyer and E. Chaffee, *J. Biol. Chem.*, **140**, Proc. XCI (1941).

[194] J. E. Jorpes, *Heparin in the Treatment of Thrombosis.* Oxford Univ. Press, 1947.

[195] M. L. Wolfrom, D. I. Weisblat, J. V. Karabinos, W. H. McNeely, and J. McLean, *J. A. C. S.*, **65**, 2077 (1943). A. F. Charles and A. R. Todd, *Biochem. J.*, **34**, 112 (1940).

[195a] J. E. Jorpes, H. Boström and V. Mutt, *J. Biol. Chem.*, **183**, 607 (1950). K. H. Meyer and D. Schwartz, *Experientia*, in the press.

residues is unknown. The molecular weight is of the order of 15,000.[196] Heparin is commonly obtained from animal liver or lungs by digestion of the proteins, subsequent precipitation with acid, and fractional reprecipitation with alcohol.

Heparin intervenes in the process of coagulation by blocking the reaction of thrombokinase. In the presence of calcium, this enzyme arising from the thrombocytes brings about conversion of the existing prothrombin (a proenzyme) into thrombin (an enzyme), which in its turn converts the soluble fibrinogen to fibrin. Heparin is of very considerable therapeutical value, especially as a prophylactic against thrombosis.

VII. STARCH AND OTHER RESERVE CARBOHYDRATES[197]

The widespread monosaccharide glucose is often stored by living organisms in the form of readily hydrolyzable polysaccharides. Plants, for example, store glucose in the form of starch grains, insoluble in water but rapidly convertible into soluble sugar when required; such starch may be formed as transitory starch in the chloroplasts during assimilation, or as reserve material in seeds, fruits, tubers, and rhizomes. A similar polysaccharide, glycogen, serves the same purpose in animals, but this is dispersed in the cells, not deposited in granules. Other carbohydrate reserves occurring in plants are water-soluble and are deposited in bulbs or fruits such as the polyfructosans inulin and phlein and also mixed polysaccharides. Lichenin which is chemically related to cellulose is also regarded as reserve material.

The treatment of this group separately from those already discussed, which serve as supporting tissue or have particular physiological importance, is to a certain extent arbitrary; it seemed desirable, as division from a chemical standpoint is not expedient with regard to mixed polysaccharides.

1. Occurrence and Composition of Starch Grains

Starch occurs in plants in the form of grains which can readily be separated from the plastids, the cell components responsible for their formation.

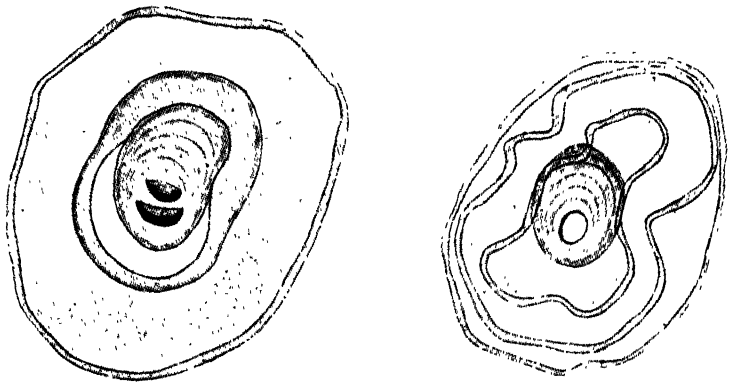
[196] M. Odier, *unpublished work.*

[197] Monographs: A. Meyer, *Untersuchungen über die Stärkekörner*, Fischer, Jena, 1896. E. T. Reichert, *Differentiation and Specificity of Starches*, Carnegie Publication, Washington, 1913. M. Samec, *Kolloidchemie der Stärke*, Steinkopff, Leipzig, 1927. M. Samec and M. Blinc, *Kolloid-Beihefte*, **47**, 371 (1938); **49**, 75 (1939); **51**, 359 (1940); **52**, 57 (1940); **53**, 453 (1941). K. H. Meyer, in *Advances in Colloid Science*, Vol. I, Interscience, New York, 1942, p. 143. R. W. E. Kerr, *Chemistry and Industry of Starch*, 2nd ed., Academic, New York, 1950.

The starch formed as a result of assimilation in the green parts of the plant is deposited temporarily in the form of small granules as assimilation starch or autochthonous starch in the chloroplasts. Larger granules are found in rhizomes, tubers, seeds, and fruits, and in the pith of many plants; they are formed there by the activity of the plastids, from soluble sugar or sugar derivatives transported in the sap to those parts of the plant.

(*a*) *Shape and Internal Structure of Starch Grains*

The grains may be lens-shaped, spherical, oval, or irregular in shape, and the length varies from 0.002 mm. to 0.17 mm.; grains from the rhizome of *Canna* are particularly large. Starch grains are composed of layers which



(*a*) (*b*)

Fig. 4. Starch grains strongly heated and swollen in water (Badenhuizen): (*a*) first and (*b*) later stage.

may be concentric—if the growth nucleus or hilum, about which the layers are deposited, is central (as in cereal starches)—or excentric, as in potato starch. These layers are clearly visible in heated starch; if such starch is treated with water, the layers separate from each other. Figure 4 shows starch grains[198] which have been treated in this way. It is possible that this stratification is due to the alternation of day and night (Meyer). The layers are composed of elongated, crystalline micelles, radially arranged. This structure will be considered in detail when we discuss the chemistry of the starch grain.

[198] N. P. Badenhuizen, Jr., and J. R. Katz, *Z. physik. Chem.*, **A182**, 73 (1938).

(*b*) *Chemical Composition of Starch Grains*

The chief constituent of the starch grain is a carbohydrate, or, more correctly, a mixture of carbohydrates often referred to for convenience as starch, which forms glucose on hydrolysis. Starch grains contain 14–19% of water, 0.01–0.05% of nitrogen, fatty acids, and phosphoric acid. Water is found in the lattice, since, when strongly dried, the grains no longer yield the typical interference pattern, and become amorphous. The composition of the crystalline fraction is presumably $(C_6H_{10}O_5H_2O)_n$, corresponding to a water content of 10%. The remaining water is probably bound at the surface of the crystallites as in cellulose.

Cereal starch contains small quantities of an ether-soluble substance, containing fatty acids and phosphoric acid, which is undoubtedly lecithin-like in character. The nitrogen-containing substances can be removed by treatment with alcoholic hydrochloric acid. Starch purified in this way still contains fatty acids (in maize starch, up to 0.6%), among which Taylor and Lehrmann[199] have identified palmitic, oleic, and linoleic acids. It is probable that the fatty acids of native starch are present in part at least as phosphatides. According to Posternak,[200] cereal starches contain no phosphoric acid bound to the carbohydrate, whereas in potato, arrowroot, and sago starches small quantities of phosphoric acid are linked as ester to carbon atom 6.

"Starch," *i.e.*, the carbohydrate constituent of the grains, is by no means as homogeneous as cellulose; it consists of two differently constituted polysaccharides, amylose and amylopectin. Although this view has been repeatedly expressed both by botanists and chemists and lately especially by Samec,[201] it has nevertheless been disputed by other workers until quite recently.[202] It has been definitely established by Meyer's proof[203] of the structural difference between the two constituents.

Most starches contain about 20% of amylose, which consists of long, straight chains of α-glucose residues linked in the 1,4 positions (*i.e.*, the same structural principle as for maltose). Amylose itself is not a homogeneous compound but a mixture of polymeric homologues of the same structure but different molecular weights (10,000–400,000). It is com-

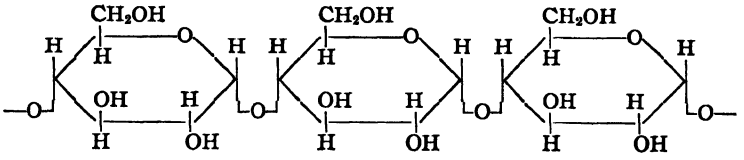
[199] T. C. Taylor and L. Lehrmann, *J. Am. Chem. Soc.*, **48**, 1739 (1926). L. Lehrmann, *ibid.*, **54**, 2527 (1932); **61**, 212 (1939).

[200] T. Posternak, *Helv. Chim. Acta*, **18**, 1351 (1935).

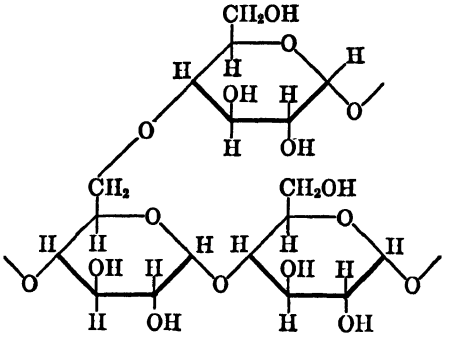
[201] M. Samec, *Ber.*, **A73**, 85 (1940).

[202] N. P. Badenhuizen, Jr., *Rec. trav. botan. néerland.*, **35**, 559 (1938); *Protoplasma*, **33**, 440 (1939).

pletely hydrolyzed to maltose by β-amylase, a process which will be considered in detail later. The formula of amylose is:



On the other hand, the chief component of starchy material, amylopectin, consists of a mixture of branched-chain molecules, wherein not only 1,4-α linkages but also 1,6-α linkages are present. The formula of a branch point is illustrated below:



On the basis of investigations of its enzymatic[204] breakdown which yields, in addition to maltose, residual material of high molecular weight, the so-called limit dextrin, a branched structure has been assigned, as represented in fig. 6 (p. 468). The molecular weight is thought to be 40,000–100,000.

2. Constitution and Properties of Amylose

(*a*) *Earlier Work on Starch Structure*

Although there had been considerable discussion with regard to the two possible constituents, the crude starch, *i.e.*, actually a mixture, was generally used in the earlier investigations on constitution.

As shown by Irvine and Macdonald,[205] starch may be methylated to a trimethyl derivative which gives a good yield of 2,3,6-trimethylglucose on

[203] K. H. Meyer, M. Wertheim, and P. Bernfeld, *Helv. Chim. Acta*, **23**, 865 (1940).
[204] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **23**, 875 (1940)
[205] J. C. Irvine and J. Macdonald, *J. Chem. Soc.*, **1926**, 1502.

hydrolysis. In the majority of the glucose residues, therefore, the 2, 3, and 6 hydroxyl groups are free; hence linkage of the residues one to another can only be 1,4 or 1,5. On decomposition with acetyl bromide, a 30% yield of acetobromomaltose may be obtained (Karrer[206]). Furthermore, maltose is obtained, admittedly in small quantities, by hydrolysis with oxalic acid (Ost[207]). These facts, together with the observation that maltose results from enzyme breakdown, imply that the majority of the glucose units are united by 1,4 linkages, as in maltose. This conclusion is also supported, as Meyer, Hopff, and Mark[208] showed, by the value obtained for the rotation of starch, following Hudson's rule. Finally Freudenberg[209] and co-workers have succeeded in separating both a tri- and tetrasaccharide of essentially maltose structure, by methylating and distilling the mixture resulting from starch acetolysis.

From the cleavage products of methylated starch Haworth[210] was able to isolate 4% of tetramethylglucose which could only arise from terminal groups. From this he deduced originally that the starch molecule consisted of 25 glucose residues which were grouped together in large secondary aggregates.

With the assumption of an open-chain formulation, however, a close analogy between the behavior of starch and cellulose solutions would be expected. But since starch solutions display different properties from those of an unbranched chain polymer, Meyer and Mark[211] concluded that the starch molecule must be branched in some way. Staudinger[212] reached the same conclusion later on the basis of viscometric investigations. Further support for this theory was furnished by the isolation and identification of 2,3-dimethylglucose (Freudenberg[212a]) in the hydrolysate of fully methylated starch, since this gave evidence of branch points in the molecule. But all these results concerning the nature of starch require correction; it is only amylopectin which has a branched structure.

[206] P. Karrer and C. Nägeli, *Helv. Chim. Acta*, **4**, 263 (1921).

[207] H. Ost, *Angew. Chem.*, **17**, 1663 (1904).

[208] K. H. Meyer, H. Hopff, and H. Mark, *Ber.*, **62**, 1103 (1929).

[209] K. Freudenberg and K. Friedrich, *Naturwissenschaften*, **18**, 1114 (1930).

[210] W. N. Haworth, *Nature*, **129**, 365 (1932). E. L. Hirst, M. T. Plant, and M. D. Wilkinson, *J. Chem. Soc.*, **1932**, 2375. See also K. Freudenberg and H. Boppel, *Ber.*, **71**, 2505 (1938); **73**, 609 (1940). E. L. Hirst and G. T. Young, *J. Chem. Soc.*, **1939**, 951. K. Hess and K. H. Lung, *Ber.*, **71**, 815 (1938).

[211] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, page 212.

[212] H. Staudinger and H. Eilers, *Ber.*, **69**, 819 (1936). H. Staudinger and E. Husemann, *Ann.*, **527**, 195 (1937); *Ber.*, **71**, 1057 (1938).

[212a] K. Freudenberg and H. Boppel, *Ber.*, **73**, 609 (1940).

(*b*) *Preparation and Estimation of Amylose*

The earliest method applied to the separation of the two starch components simply involved treatment with hot water. "Crude amylose" passed into solution while the greater part of the starch remained behind in the form of highly swollen mucilaginous granules, the "amylopectin" fraction. From the aqueous solution of crude amylose a pure amylose separated out in a submicroscopic crystalline form in the course of several days or weeks. Actually it is not possible to effect complete separation of amylose and amylopectin by such extraction. Either by mild treatment only a part of the amylose (fraction A_1) passes into solution and the higher molecular weight fractions (A_2) remain in the swollen grains, or by working at higher temperature complete dissolution of the amylose is insured, but there is amylopectin admixed.

The best method for separation has been developed by Schoch.[213] After defatting the starch by careful extraction with methanol, it is heated with water and butanol at 109° under pressure. On cooling an amylose butanol compound separates out; the amylopectin which remains in solution can be precipitated with alcohol. On reprecipitation the amylose butanol compound can be obtained in the form of small crystalline plates.[214] The method can be applied to the estimation of the amylose content of a starch.

An alternative procedure for this analysis makes use of the iodine color reaction. In the presence of iodine, amylose solutions give a pure blue coloration, whereas for amylopectin violet to red-violet colors result; moreover, the color intensity of the amylopectin–iodine complex is relatively much weaker than that of the amylose compound. The method of Hassid and McCready[215] is based upon this and permits the rapid assay of amylose in an amylose–amylopectin mixture. The blue value, *i.e.*, the light absorption of the dissolved starch in the presence of potassium triiodide is estimated colorimetrically and compared with a standard curve.

In addition, amylose has a greater affinity for iodine than has amylopectin. This can readily be seen by adding potassium triiodide dropwise to an amylose–amylopectin mixture containing only small amounts of the former. At first the color is a pure blue characteristic of amylose, all the iodine being absorbed by that component until saturation is reached; then after the further addition of iodine it is absorbed by the amylopectin and the violet amylopectin–iodine coloration appears. Bates, French, and

[213] T. J. Schoch, *Cereal Chem.*, **18**, 121 (1941).
[214] R. W. Kerr and G. M. Severson, *J. Am. Chem. Soc.*, **65**, 193 (1943).
[215] R. M. McCready and W. Z. Hassid, *J. Am. Chem. Soc.*, **65**, 1154 (1943).

Rundle[216] have devised a method for amylose estimation based on this preferential iodine affinity. They measure the concentration of "the free iodine" potentiometrically. On addition of potassium triiodide to pure water, iodine activity increases (Fig. 5, curve *1*). In the presence of amylose, over a certain free iodine concentration, additional iodine forms an amylose complex; the activity therefore remains constant until the amylose is completely saturated (curve *4*). On the other hand, amylopectin does not bind iodine as strongly as shown by the slow increase in activity on addition

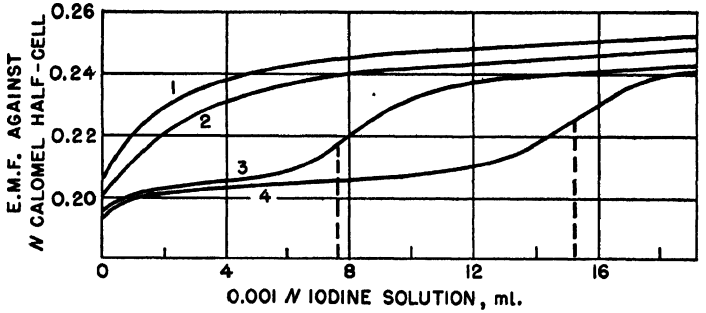


Fig. 5. Titration of amylose in presence of amylopectin: (1) water for comparison; (2) 0.01 g. potato amylopectin; (3) 0.005 g. potato amylose and 0.005 g. potato amylopectin; (4) 0.01 g. amylose.

of iodine (curve *2*). In a mixture the amylose is first saturated, the activity remaining constant; then the amylopectin can take up iodine and the activity rises slowly (curve *3*). The position of the break in the curve is a

TABLE VI

Amylose Content of Starches

Starch	Amylose, %	Starch	Amylose, %
Waxy rice	0	Maize	21
Waxy maize	0	Potato	22
Tapioca	17	Wheat	24
Rice	17	Sago	27
Banana	20.5	Lily bulb	34

measure of the amylose present. Measurement in this way indicates that autoclaved starch contains some 20% of amylose. Table VI shows the amylose content of various starches determined in this way.

By a somewhat less exact procedure, Meyer and Heinrich[217] have estab-

[216] F. L. Bates, D. French, and R. E. Rundle, *J. Am. Chem. Soc.*, **65**, 142 (1943).

[217] K. H. Meyer and P. Heinrich, *Helv. Chim. Acta*, **25**, 1639 (1942).

lished that the starch from potato shoots is much richer, and that the assimilation starch of the leaves is much poorer in amylose than the tuber starch. The molecular weight distribution for amyloses of different starches certainly varies.

Amylose can be synthesized from glucose 1-phosphoric acid by means of an enzyme found in both plants and animals.

(c) *Constitution and Molecular Weight of Amylose*

By comparing the number of end groups with that of the aldehydic residues and also with the molecular weight, it can be concluded that amylose has an unbranched structure, since there is evidence for only one end group per molecule.[218] Moreover, amylose is completely hydrolyzed to maltose by β-amylase, whereas amylopectin is only degraded to a certain extent giving 36–40% of high molecular "limit dextrin" together with 64–60% maltose.

Amylose and other starch constituents form associates in water which gradually attain the size of submicrons and finally separate out in visible flakes. On this account aqueous solutions are not suitable for osmotic measurement of chain length. On the other hand, amylose acetate is molecularly dispersed in tetrachloroethane solution. Since, however, even at small concentrations, the osmotic pressure of high polymeric substances

TABLE VII

Molecular Weights of Amylose Fractions

Amylose	Fraction	Molecular weight	Degree of polymerization
Corn amylose	I	13,000	80
Corn amylose	III	24,000	150
Corn amylose	IV	33,000	200
Corn amylose, extracted with water at 70°	A_1	40,000	250
Corn amylose, remaining in grain at 70°, passing into solution only at 109°	A_2	340,000	2100
Corn amylose, total	$A_1 + A_2$ = Schoch's A	72,000	450
Potato amylose	A_1	32,000	200
Potato amylose	A_2	110,000	700
Potato amylose, total	$A_1 + A_2$	50,000	300

[218] K. H. Meyer, M. Wertheim, and P. Bernfeld, *Helv. Chim. Acta*, **23**, 865 (1940); **24**, 378 (1941).

deviates from the van't Hoff relationship $p = cKT$, it is necessary to measure the pressure p at various concentrations c and extrapolate the reduced osmotic pressure p/c to zero. From this value the molecular weight can be calculated though still with considerable uncertainty.

A simple procedure for molecular weight determination depends on a colorimetric estimation of the free aldehyde groups after their reaction with dinitrosalicylic acid[219]; a brown reduction product is formed on heating. Maltose is used for standardization. Table VII gives the molecular weight values of certain lower fractions—obtained by aqueous extraction of crude amylose (fractions I, III, IV)—and the average molecular weights of complete amylose fractions.

(*d*) *Properties of Amylose*

As already mentioned, amylose obtained by aqueous extraction of starch granules slowly separates out in the cold. This phenomenon has been called *retrogradation*, a term which may be considered as superfluous since the process is simply a slow crystallization, as can be demonstrated by the crystal interferences of the precipitate. The x-ray diagram is sharper than that for native starch and in all cases corresponds to the so-called B spectrum of Katz, which he found for potato starch grains but not for the cereal starches. From the breadth of the interference bands the crystallites must be small and scarcely more than 100 Å. thick. According to Signer[220] aqueous amylose solutions exhibit streaming birefringence only to a very small extent. In contrast to this, amylose solutions in ethylene diamine display this phenomenon; from a detailed investigation it appeared that the chain molecules of amylose are flexible.[220a] For the amylose–iodine complex a definite orientation of the dissolved particles is apparent. Rundle's investigations[221] show that amylose solutions colored with iodine exhibit streaming dichroism; light with its electric vector parallel to the direction of streaming is more strongly absorbed than polarized light perpendicular to it.

Amylose deposited by slow crystallization from water does not give a completely clear solution in hot water and on prolonged standing it crystallizes out again. If the solution is kept at 60° for a considerable time, a less soluble fraction separates out, and that remaining in solution is deposited more slowly in the cold and remains more readily soluble.

[219] K. H. Meyer, G. Noelting, and P. Bernfeld, *Helv. Chim. Acta*, **31**, 103 (1948).
[220] R. Signer, *private communication.*
[220a] J. F. Foster and J. H. Lepow, *J. Am. Chem. Soc.*, **70**, 4169 (1948).
[221] R. E. Rundle and D. French, *J. Am. Chem. Soc.*, **65**, 558 (1943).

In the course of fractionation, the solubility of the individual fractions gradually becomes smaller.[222] It sinks to values less than 0.02% in warm water; the least soluble amylose is hardly soluble in hot water and, after cooling the hot saturated solution, no iodine reaction can be observed. Such amylose dissolves in concentrated aqueous solution of chloral hydrate and can then be precipitated by acetone in a water-soluble form which gives the iodine blue reaction; on standing this amylose again becomes gradually insoluble. On purification, therefore, the solubility decreases; furthermore, slow enlargement of the crystallites occurs, and through that a definite diminution in solubility, just as happens in the aging of inorganic precipitates. Fractionated amylose also crystallizes much more quickly than the crude substance, which means that the more polydisperse they are the greater is the tendency for solutions to remain supersaturated.

The amylose in starch exists in a much more soluble form than purified crystallized amylose. This is due to the smaller size of the crystallites in the granule; it is possible, too, that in the granule mixed crystals of smaller and larger molecules are present, and similarly for the amylopectin. Such mixed crystals are more soluble than the fractionated, more homogenous products.

Starch grains which have been kept in a moist atmosphere at 100° are found to swell much less readily than normally. X-ray examination reveals that after such treatment the crystallites have become much larger.[223] Similar effects are observed when starch is kept at ordinary temperatures for much longer periods.

Aqueous solutions of amylose exhibit peculiar properties due to the supersaturation. This "aging" is shown first in a rapidly increasing resistance to β-amylase (the chief constituent of malt diastase); then ultramicrons appear, next the solution becomes cloudy, and finally a precipitate settles out. This aging, therefore, consists in a process of association, which leads first to invisible associates which are enzyme-resistant and ends by the separation of a solid phase. The purer the preparation is, the more rapid are this aging and precipitation. Whereas crude amylose only begins to crystallize out after several weeks, a fractionated pure amylose may begin to be deposited from solution in the course of a few hours, or even minutes. By treatment of amylose solutions or suspensions with dilute alkali and subsequent neutralization, they can be rejuvenated, a process which is of great importance in connection with enzyme reaction; it seems as if the

[222] K. H. Meyer, P. Bernfeld, and E. Wolff, *Helv. Chim. Acta*, **23**, 854 (1940).

[223] L. Sair and W. R. Fetzer, *Ind. Eng. Chem.*, **36**, 205 (1944). See also T. J. Schoch and D. French, *Cereal Chem.*, **24**, 231 (1947).

amylose is first liberated in a molecular, soluble form from the alkaline solution in which it is molecularly dispersed.

Since aqueous solutions of amylose are supersaturated and consist of a mixture of aggregates which gradually grow larger, ultracentrifugal analysis reveals that they are polydisperse to an exceptional degree. Investigating this, Lamm[224] has obtained evidence for particles with molecular weight varying between 300,000 and 1,800,000 and even still larger ones.

Hydrazine hydrate and ethylenediamine are good solvents for amylose, suitable for viscometric investigation. Amylose dissolves in dilute aqueous alkali and slowly separates out in the form of an alkali–amylose gel. Also from formamide solution a coherent gel separates out on cooling.

(*e*) *Organic Addition Compounds of Amylose*

Amorphous amylose not only absorbs water vapor but also other vapors, *e.g.*, those of alcohols. The fiber diagrams (V spectrum) of the several alcoholates (methyl–heptyl) are almost identical.[225] The powders precipitated from aqueous solution by methanol or butanol, *e.g.*, Kerr's[226] crystalline "amylose" show the alcoholate diagram, so that alcohols are more firmly bound than water.

Addition compounds of amylose with aliphatic amines can be prepared similarly. Ammonia, as well as aliphatic and aromatic amines, also forms a crystalline addition compound. Nothing is known of the composition or structure of these compounds.

(*f*) *Acetylation of Amylose*

Amylose can be readily esterified to the triacetate by pyridine–acetic anhydride, if it is first obtained in a finely divided state by acetone precipitation from aqueous chloral hydrate solution.

The intrinsic viscosity of amylose acetate is roughly two thirds that of a cellulose acetate with the same molecular weight; but since the starch chain has a different structure (*cf.* sect. VII 4) and, for equal molecular weight, the chain length is probably less, this difference is not remarkable and is compatible with the assumption of an unbranched chain. On the other hand, the triacetates of degraded starches show much smaller viscosities, a fact which is explained by the branched structure of the main constituent of starch, *i.e.*, amylopectin.

[224] O. Lamm, *Nova Acta Regiae Soc. Sci. Upsaliensis*, **10**, No. 6 (1937).
[225] P. Zuppinger, *Thesis*, Geneva 1948.
[226] R. W. Kerr and G. M. Severson, *J. Am. Chem. Soc.*, **65**, 193 (1943).

Signer has observed that the streaming birefringence[227] of amylose triacetate is ten times smaller than that of a cellulose triacetate of equal molecular weight; the angle of extinction is much nearer to 45°. Both facts suggest that the particles must be considerably closer to spherical in form than is the case for cellulose.

Amylose acetate can be obtained from chloroform solution in the form of films which are as strong and extensible as cellullose acetate films. The stretched triacetate film gives a fiber diagram with identity period 18.3 Å.[228]

(g) *Methyl Amylose*[229]

Amylose is readily methylated in dilute alkaline solution to the dimethyl derivative using dimethyl sulfate. The product is soluble in cold but insoluble in hot water, and gives a brown coloration with iodine; it is soluble in alcohol, acetone, and chloroform. On continued methylation the trimethyl derivative is obtained, a sticky product which in the slightly swollen state can be drawn out into fine silky threads which are tough and strongly birefringent. Trimethyl amylose is also soluble in cold water, but separates out on warming, and dissolves in acetone and chloroform.

(h) *Inorganic Esters of Amylose*

Nitroamylose can be obtained by treatment with nitric–phosphoric acid but some degradation cannot be avoided. The intrinsic viscosity of the nitro derivative is roughly one third that of a nitrocellulose of the same molecular weight.

By treating amylose with phosphorous oxychloride in pyridine, Karrer[230] has prepared a phosphoric ester in the form of a sticky mass, water-soluble, and precipitated by alcohol; the composition approximates that of a triphosphate $C_6H_7(PO_3H_2)_3$. Amylose sulfuric ester has been obtained by the action of chlorosulfonic acid in pyridine.

3. Constitution and Properties of Amylopectin

(a) *Structure and Molecular Weight*

Rice and corn amylopectins contain about 3 to 4% of end groups[231]; potato amylopectin appears to be slightly more branched (some 5% of end

[227] R. Signer, *unpublished work.*

[228] R. L. Whistler and N. C. Schieltz, *J. Am. Chem. Soc.*, **65**, 1436 (1943).

[229] K. H. Meyer, M. Wertheim, and P. Bernfeld, *Helv. Chim. Acta*, **23**, 865 (1940).

[230] P. Karrer, H. Koenig, and S. Usteri, *Helv. Chim. Acta*, **26**, 1296 (1943).

[231] K. Freudenberg and H. Boppel, *Ber.*, **71**, 2505 (1938). K. H. Meyer, M. Wertheim, and P. Bernfeld, *Helv. Chim. Acta*, **23**, 865 (1940); **24**, 378 (1941).

groups corresponding to the 5% of glucose residues which form the branch points). It is, therefore, possible to characterize an amylopectin by its average molecular weight together with its degree of branching conveniently expressed as a percentage.

The dimethylglucose isolated from the hydrolytic products of methylated amylopectin was identified by Freudenberg[232] as the 2,3 derivative, identi-

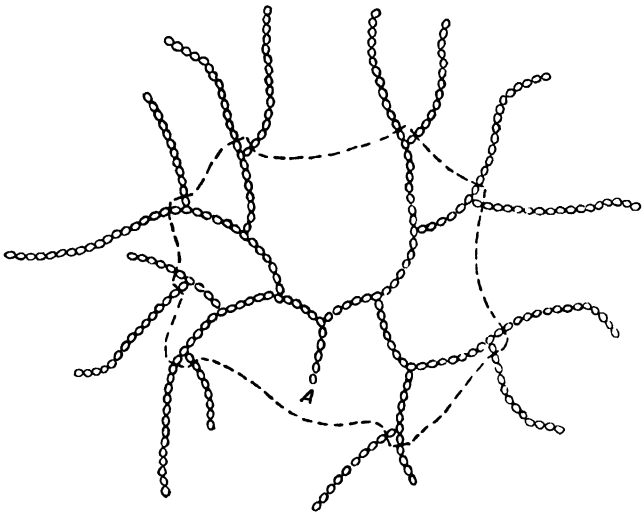


Fig. 6. Structure of amylopectin (according to Meyer): *A* is reducing end group; limit dextrin inside the dotted line.

cal with that previously obtained from methylated glycogen by Haworth, Hirst, and Isherwood.[233] Furthermore, Montgomery[234] has obtained evidence for the occurrence of 1,6-α-glucosidoglucose (isomaltose) in the residues (limit dextrins) after treatment with malt amylase; finally, Meyer and Boissonnas,[234a] have proved the absence of residues containing 1,3 or 1,2 bonds. These results provide evidence of branching with 1,6 linkages.

Further information is available from a study of the enzymatic break-

[232] K. Freudenberg and H. Boppel, *Naturwissenschaften*, **28**, 264 (1940); *Ber.*, **B73**, 609 (1940).

[233] W. N. Haworth, E. L. Hirst, and F. A. Isherwood, *J. Chem. Soc.*, **1937**, 577.

[234] E. M. Montgomery, F. Weakley, and G. E. Hilbert, *J. Am. Chem. Soc.*, **69**, 2249 (1947).

[234a] K. H. Meyer *et al.*, *J. Phys. & Colloid Chem.*, **53**, 319 (1949).

down which will be considered in more detail later. It can be deduced that the amylopectin structure is multibranched and that the outer branches contain some 13 to 15 glucose residues, while the innermost have only 8 to 9 residues[235] (Fig. 6).

The "laminated structure" proposed by Haworth[236] cannot be reconciled with the following facts. On degradation with β-amylose (which attacks amylose at the nonaldehydic end of the molecule) a limit dextrin is formed, the properties of which undoubtedly indicate a compact structure, such as that in figure 6, whereas according to Haworth's formulation it should have an elongated molecular structure (Fig. 7).

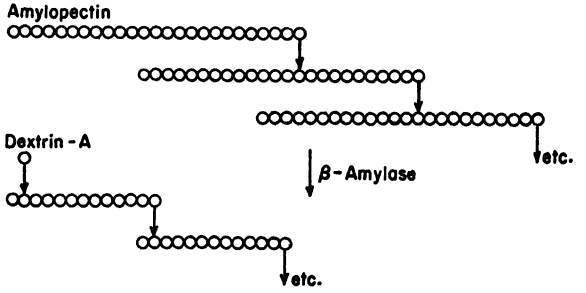


Fig. 7. Structure of amylopectin and limit dextrin (according to Haworth).

Amylopectin from corn has an average molecular weight of 45,000; potato amylopectin is a polymer of much higher D.P., molecular weight approximately 180,000.

(*b*) *Network*

Up to a temperature of 80° starch granules undergo limited swelling in water. It can be concluded, therefore, that a three-dimensional network exists, built of chains for the greater part surrounded by solvent, but which cling together to a certain extent. The small regions of interlocking are readily opened up by mild reagents such as hydrazine hydrate or ethylenediamine at room temperature, chloral hydrate solution at 90°, or water alone at 110°. All reagents which normally have a swelling effect exert a similar cleavage effect at higher temperatures. The forces which bind the chains together in the network are hydrogen bonds just like those which operate in the starch lattice. The large amylopectin molecules with

[235] K. H. Meyer, *Naturwissenschaften*, **28**, 564 (1940).
[236] W. N. Haworth, *Proc. Roy. Soc. London*, **A186**, 1 (1946).

their highly branched structure are particularly well adapted for the formation of a network of crystallites linked together by molecular threads.

(*c*) *Phosphorus Content of Amylopectin*

While amylopectins of cereal starches are phosphorus-free, potato, sago, arrowroot contain about 0.08% of phosphorus which is quite definitely chemically bound. Posternak[237] has succeeded in isolating glucose 6-phosphoric ester (Robison ester) both by acid and by enzyme hydrolysis; in amylopectin, therefore, phosphorus is linked to the primary hydroxyl group.

(*d*) *Properties of Amylopectin*

Amylopectin dissolves in water, concentrated solutions slowly thickening to an opaque jelly. From aqueous solution amylopectin is precipitated by prolonged electrodialysis. The osmotic pressure of solutions of amylopectin and its derivatives indicates a very great particle size of the order of several millions; this statement is confirmed by measurements of the turbidity. In contrast to this the molecular weight determined by reducing group measurements is many times smaller (M 100,000). Since it seems very improbable that one chain, branched or unbranched, contains more than one reducing group, the physically determined particles must contain many (about 100) "chemical" molecules (primary valence chains). Since the larger particles persist also in alkaline solution, in which aggregations such as ordinary micelles due to attractive forces are broken down, it is to be concluded that the molecules are held together by mechanical entanglement.

Solutions of amylopectin exhibit neither streaming birefringence nor streaming dichroism after iodine addition.

After precipitation by acetone from 30% chloral hydrate solution, so that the amylopectin is in a very finely divided state, it may be readily esterified to the triacetate, using pyridine–acetic anhydride. The product gives very brittle films which cannot be drawn even when swollen.

Several varieties of starch, *e.g.*, the so-called glutinous rice (*Oryza sativa* var. *glutinosa*, *Iris germanica*, *Gentiana lutea*, and a variety of *Sorghum*) contain no amylose at all but only lower and higher molecular amylopectins. Despite this they give an x-ray diagram indistinguishable from that of other cereal starch grains. It follows, therefore, that amylopectin can crystallize in the same lattice as amylose. All these varieties give a red

[237] T. Posternak, *Helv. Chim. Acta*, **18**, 1351 (1935).

iodine color reaction. Investigation of one of these[238] varieties (*Oryza sativa* var. *glutinosa*) has shown that in place of amylose a readily water-soluble glucosan is present which has a highly branched structure and a molecular weight of 3000. After extraction of this glucosan, the remaining amylopectin is found to contain 6% of end groups and so must be more highly branched than that of the common rice starch. From the structural point of view this product is more closely related to glycogen but it is more convenient to reserve that name for substances found dispersed in plasma and not to apply it to glucosans which exist in a granular form.

The starch found in waxy corn (a particular variety of corn) also gives a red-violet color with iodine. Here too amylose is completely lacking but there is a considerable proportion of low molecular amylopectin along with more highly polymerized material, both with 4 to 5% degree of branching.

The chief points of difference between amylose and amylopectin may be summarized as follows. The unbranched amylose, molecular weight 10,000 to 400,000, does not form pastes, is not coagulated by electrodialytic removal of salts, and crystallizes from solution. The dissolved particles are oriented by streaming; films of derivatives can be drawn. Amylose gives crystalline addition compounds with alcohols and a fairly stable blue compound with iodine. Amylose is completely degraded by β-amylase. The branched amylopectin forms pastes, coagulates on removal of electrolytes, and crystallizes as a gel. The particles are not oriented by streaming; films of derivatives are brittle. Violet to red-violet iodine complexes are formed, less stable than amylose iodine. With β-amylase, amylopectin is only partly degraded to the so-called limit dextrin.

4. Fine Structure of Starch Grains

The starch grain is composed, as we have seen, of concentrically disposed layers. These consist, as shown by A. Meyer[238a] in his classical work on starch grains, of radially arranged, crystalline, elongated particles. A subdivision of the layers into blocks was at one time described, but the work of Badenhuizen[239] shows that this appearance is probably an artifact. The former view is supported by the birefringence of the grain which behaves as a spherolite, that is, a crystal aggregate of radially arranged, needlelike crystals. Between crossed Nicols a black cross is visible, the arms of which are parallel to the planes of vibration of the Nicols. Detailed investiga-

[238] K. H. Meyer and M. Fuld, *Helv. Chim. Acta*, **24**, 1404 (1941).

[238a] A. Meyer, *Untersuchungen über die Starkekörner*. Jena, 1895.

[239] N. P. Badenhuizen, Jr., *Protoplasma*, **29**, 246 (1937).

tion of the optical properties indicates that the periphery of each layer is more densely packed than the interior.

Many years after Meyer's investigation, the crystalline structure of starch grains was demonstrated by the discovery of the x-ray diagram of starch. The interferences in the x-ray diagram of native starch are rather broad, and indicate that the crystallites responsible for the phenomenon are very small and cannot be more than 100 Å. wide. The diagrams obtained differ according to the variety of starch; despite many years of work Katz[240,241] has only succeeded in establishing the actual fact of crystallinity and that different modifications exist. It is possible to distinguish between A spectrum (cereal starches), B spectrum (potato, chestnut, canna bulb, and starch recrystallized from water), C spectrum (*Maranta* bulb, sago, *Musa* fruits) and the so-called V spectrum given by a starch–butanol (or ethanol) addition compound. On complete dehydration, the diagram becomes amorphous; this means that the single water molecule associated with each glucose unit is indispensable for the crystal structure. From this it may be concluded that the starch molecules are not as closely packed as in cellulose. It is of interest to note that an analogous difference exists between cellobiose, which crystallizes without water, and maltose, which crystallizes with one mole of water of crystallization. If starch is subjected to considerable mechanical deformation, the diagram also becomes amorphous.

By means of a special microtechnique Kreger[242] succeeded in obtaining fiber diagrams of a single elongated starch grain of the bulb of the orchid *Phajus*. It is a B spectrum with fiber period 10.5 ± 10.6 Å. There are probably three glucose residues on each chain in the unit cell. Unfortunately, interpretation of fiber diagrams does not permit clear-cut conclusions concerning the whole unit cell, much less the arrangement within the cell. Despite claims to the contrary[243] x-ray evidence up to the present time has neither revealed the size of the unit cell nor given any information about the form of the molecules.

Nevertheless, the double refraction of the grain gives definite indications. It is found to be uniaxially positive in a radial direction; according

[240] J. R. Katz and J. C. Derksen, *Z. physik. Chem.*, **A150**, 100 (1930).

[241] J. R. Katz and T. B. von Itallie, *ibid.*, **A155**, 199 (1931); **A166**, 27 (1933).

[242] D. Kreger, *Nature*, **158**, 199 (1946); **160**, 369 (1947).

[243] R. E. Rundle and L. W. Daasch, *J. Am. Chem. Soc.*, **65**, 2261 (1943). R. E. Rundle, L. Daasch, and D. French, *ibid.*, **66**, 130 (1944).

to Frey-Wyssling[244] it amounts to 0.013, that is about one fifth of the value given by a highly oriented cellulose fiber. Now, the specific refraction depends upon the position of the glucose rings with respect to the crystal axis (fiber axis). If, as in cellulose, the rings were to lie with their planes along the fiber axis, then one would expect a value for the refraction

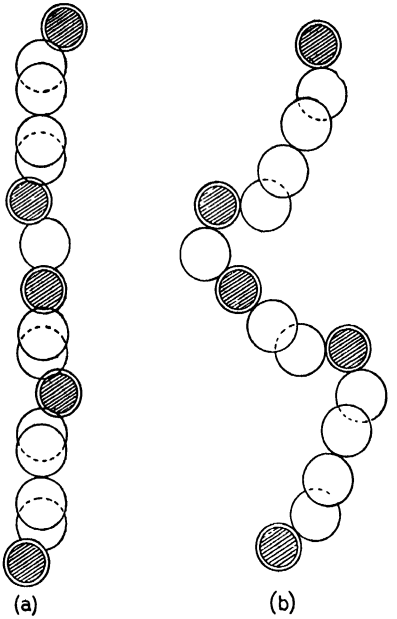


Fig. 8. (a) Portion of the primary valence chain of cellulose. (b) Portion of the primary valence chain of starch. The figures show the glucose rings as seen from the side. Open circles = carbon atoms; hatched circles = oxygen atoms.

equal to that of cellulose; if the rings were perpendicular to the axis, then one should find an equal refraction but in the opposite sense; if at 45° to the axis, then zero refraction since the two rings would mutually compensate. The value observed, amounting to one fifth the value for cellulose, is to be expected if the plane of the glucose rings is inclined at 36° to the fiber axis.

Therefore, we have to construct a model which corresponds to this conclusion. Using the Stuart models we find that if the glucose residues are arranged in a chain so that subsequent residues are not screwed digonally

[244] A. Frey-Wyssling, *Tabulae Biologicae*, **B19**, 30 (1948).

with respect to the earlier ones, then the chain is necessarily crumpled, and can, at all events, be screw formed, as was assumed by Hanes[245] and also by Freudenberg.[246] But the position of the glucose rings in this screw do not meet the requirements with regard to the inclination to the fiber axis, so that this interpretation does not seem very probable. On the other hand, if the residues are twisted in a digonal screw then the model gives a chain as in figure 8 (page 473) in which the angle actually is 33°. The water molecules can occupy the spaces between the chains. This arrangement or an arrangement with a trigonal screw axis are at least not in disagreement with any observations and may be considered as highly probable.

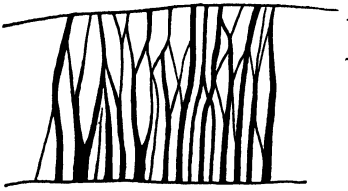


Fig. 9. Arrangement of crystalline regions (heavy lines) and the primary valence chains uniting these in a single layer of a starch grain.



Fig. 10. Network structure of swollen amylopectin. The crystalline regions acting as points of linkage are indicated by thicker lines.

Now the starch grain is no ordinary spherocrystal since it can be elastically deformed. It seems rather that the crystallites are linked together by free segments of the molecular fibers which form an amorphous, elastic cementing material. The branches of different amylopectin molecules are gathered together in crystalline bundles, these bundles being themselves linked one with another by parts of the amylopectin chains which are not included in the crystal structure. Any one amylopectin molecule can thus form part of a number of crystallites, and conversely an individual crystalline region—a micelle—may be composed of segments of several molecules. This concept also explains the fact that, on treatment with dilute acid or saliva, starch grains become brittle and even break up. It is the free chains, responsible for the cementing material, which are first attacked

[245] C. S. Hanes, *New Phytologist*, **36**, 189 (1937).

[246] K. Freudenberg and H. Boppel, *Ber.*, **73**, 609 (1940).

and undergo hydrolytic fissure, so that the micelles can then be easily separated from each other, and the grain as a whole is broken down.

To sum up: The layers contain crystalline micelles arranged perpendicularly to the plane of the layer. The crystalline regions consist of starch chains or portions of such, and the crystallites are united by amorphous chains. It is probable that the crystallites differ in size, that is, there are small crystallites destroyed by warm water, and larger crystallites which occur principally in the outermost layer and are responsible for the integrity of the shells on swelling. This system of crystallites united by molecular threads is built up from the giant branched molecules of amylopectin. Figure 9 shows a diagrammatic radial section through a fragment of one of the concentric layers of a starch grain. Figure 10 illustrates the structure of the swollen layer, held together by the crystalline regions (thick lines). At room temperature, the outer layer does not permit amylose to escape into solution, while a damaged starch grain swells in the injured region and liberates amylose. Probably part of the amylose is stored in a pure form in the interior of the grain, while the remainder is distributed in the amylopectin and there is embedded in mixed crystals with the latter.

5. Starch Pastes

(*a*) *Swelling Phenomena and "Pastes"*

When starch is put into hot water the grains swell very rapidly to many times their original volume (for potato starch one hundred fold); in the course of a few minutes a thin suspension is converted to a highly viscous paste. If the granular structure is changed, by solution, *e.g.*, in hydrazine hydrate, followed by alcohol precipitation, then the swelling properties are completely altered. The particles swell only partially; a cloudy suspension results with a much lower viscosity than that of the real paste. Clearly, therefore, paste formation is connected with the morphological structure of the starch granule.

Paste formation may now be considered in the light of the ideas developed with regard to the fine structure of starch. On treatment with warm water the small, more readily soluble crystallites are first dissolved so that water enters into the granule, dissolved amylose molecules diffuse outward, and the whole structure is loosened up without breaking down. A granule may absorb many times its own volume of water without the amylopectin molecules becoming severed from each other; they remain interlocked here and there in the crystalline regions. In this way each grain gives rise to a three-dimensional spherical or bubblelike net filled with water, or more

correctly a conglomerate of several concentric bubblelike nets. The granule only breaks down when more crystallites have dissolved; complete solution is only reached in water at 110° or with other particular solvents like hydrazine hydrate and aqueous alkali.

In the highly swollen grains as, for example, in a starch paste, the giant branched amylopectin molecules are joined together in a loose three-dimensional network through parts arranged regularly in the lattice, in which segments from many molecules are gathered together. Conversely, the micellar oriented segments are held together by the molecular threads (coherent fringe micelles).

Paste formation depends, therefore, on the size and highly branched structure of the amylopectin molecule which admits the possibility of parts of the molecule being fixed in crystallites while other parts of the same molecule are completely surrounded by solvent. In contrast, the molecule of amylose, the unbranched constituent, is either completely dissolved or is bound as a whole in lattice union with unswollen solid and is therefore not capable of paste formation. The larger the amylopectin molecule the further extended can the network become without breakdown of the granule; this explains why potato starch grains (amylopectin mol. wt. 180,000) can absorb one hundred times their own volume of water, while corn starch grains (amylopectin mol. wt. 45,000) only absorb to the extent of thirty-fold.

The degree of swelling depends on the temperature; for corn starch there is scarcely any visible effect below 35° and at 80° the granule has swollen to twenty-five times its volume. Within the swollen grain there must be an equilibrium between the "dissolved" chain segments, *i.e.*, those surrounded by water, and the "undissolved," *i.e.*, those still bound together as crystalline micelles. The saturation concentration of the dissolved fraction, corresponding to this equilibrium, is very small at room temperature; it increases exponentially with temperature.

The portion of the starch granule which may be described as dissolved, depends not only on the saturation concentration, determined by temperature, but also on the amount of water which has forced its way in, which for its part, is determined by the width of the network. Upon the melting of some crystallites, at the onset of swelling, the structure is loosened; this allows more water to enter and so more crystallites dissolve. Because of this, the temperature interval between the beginning of swelling up to the point of 2000 to 4000% of water absorption is relatively small and amounts to scarcely 30°. At about 90° the granule disintegrates into small fragments.

The heat of swelling[247] amounts to 36.75 cal. per g. This is made up of the heat of fusion and the heat of solution of the *molten* polysaccharide. On melting 39.65 cal. per g. should be absorbed and on solution 2.6 cal. should be given out. The heat of fusion is roughly equal to that of α-glucose.

On cooling, the paste solidifies, provided it is sufficiently concentrated. In the course of the solidification, the amylopectin shells crystallize as well as the dissolved amylose which also forms mixed crystals with the amylopectin, so that the felted crystallites of adjacent granules give a mechanically rigid structure. Crystallization phenomena are also involved, according to Katz, in the changing mechanical properties of bread, the process of becoming stale.

(*b*) *Variations in Physical and Chemical Properties of Starches*

It is well known that swollen corn and rice starches solidify much more quickly than potato starch; the cereal starches have a higher rate of crystallization. The differences in this respect depend chiefly on variations in the structure of the carbohydrate and are only slightly affected, if at all, by the texture of the grains, *i.e.*, the way in which the molecules are assembled together in the granule. In support of this view, it is found that completely dissolved cereal starches, in which no trace of the original grain structure persists, crystallize much more rapidly than potato starch. The former contain high molecular, readily crystallizable amylose, which is lacking in potato starch.[248]

A further difference is observed in viscous properties; a dilute potato starch paste is very much more viscous than one prepared from a cereal starch. But this difference disappears almost entirely on destruction of the granules and so it must be conditioned by their texture. The very considerable increase in viscosity of water brought about by paste formation depends on two different factors: (*1*) the increase in viscosity due to dissolved polysaccharide; and (*2*) the effect of the suspended swollen granules. The viscosity increase due to the latter in dilute solutions can be expressed in the form of the Einstein equation:

$$\eta_{rel} = 1 + 2.5\varphi$$

φ is the volume of the particles divided by that of the solution. The greater the value of φ, *i.e.*, the more swollen are the particles, the higher the viscosity. Now it has been observed that potato starch grains swell one

[247] A. Küntzel and K. Doehner, *Kolloid-Z.*, **88**, 209 (1939).

hundred fold without disintegrating, whereas rice granules swell only thirty-fold. Potato starch grains are transformed into giant bubbles, which shrink on adding salt,[248] behaving as small osmotic cells, and are torn open by vigorous stirring. Corn starch granules are more compact and more stably constructed. If the granules are broken down by vigorous stirring, prolonged heating, or superheating, then the viscosity is diminished, the decrease being very considerable in the case of potato starch, much less for corn or rice starch.

From what has been said, it is clear that a starch paste is a very complex system, much more so than the highly viscous solutions formed by long-chain polymers such as nitrocellulose in acetone. It must always be remembered that a freshly prepared starch paste is not in equilibrium; a whole series of more or less rapid processes are taking their course, which are difficult to disentangle mentally with regard to their several effects; the swelling of the granules, the diffiusion of the amylose which reduces the osmotic pressure within the grains and ultimately results in shrinkage, the breakdown of the granules, and most important of all the diverse phenomena of aging and crystallization.

The following examples illustrate how curious the observed effects may be. If corn starch is treated with several hundred times its weight of water at 70° a definite amount of amylose passes into solution. If instead the grains are heated at 60° for several hours and the temperature is raised to 70°, much less amylose is dissolved after a much longer period of treatment.[249] At 60° a recrystallization of the half-swollen granule already occurs, larger amylose crystallites grow at the expense of smaller ones which disappear, with the result that the amylose becomes appreciably less soluble than it was originally.

In the concentrated starch pastes used technically as adhesives one has to deal with swollen granules which have absorbed practically all the water available. The amylopectin branches of adjacent swollen grains can intertwine and become hooked together—formation of lumps—in crystalline micelle formation so that the whole mass solidifies out as a quite solid jelly.

All that has been said indicates that the adaptability of the different varieties of starch for use as printing pastes, adhesives, the making of puddings, or laundering of linen, is determined by the molecular weight and the polydispersity of amylose and amylopectin, the variation in amylose–amylopectin content, the morphology of the grain, its size, the mesh of the

[248] K. H. Meyer and M. Fuld, *Helv. Chim. Acta*, **25**, 391 (1942).

[249] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **23**, 890 (1940).

network, the magnitude of the crystallites, and the way in which the larger, less soluble, and the smaller, more soluble, micelles are arranged together.

6. Addition Compounds of Starch

(*a*) *Alkali Starch*

At room temperature, in a solution of sodium hydroxide of concentration $> 0.25\ N$ starch swells and sodium hydroxide is taken up by the amylopectin shell. Corn starch first dissolves in $0.25\ N$ sodium hydroxide and subsequently sets to form a jelly. According to Reychler[250] the reaction of amylose with sodium hydroxide obeys the mass action law for homogeneous reactions. The process involves the formation of an alcoholate; the reaction is therefore analogous to the formation of alkali cellulose from cellulose and alkali. Starch reacts in a similar way with lithium and potassium hydroxides. In alkaline solution, starch is very sensitive to the presence of oxygen; the rapid breakdown can be followed viscometrically. Bernfeld has calculated from solubility data the dissociation constant of starch reacting with alkali to be 2.5×10^{-12}. Alkali amylose obtained by saponification of an oriented amylose acetate fiber exhibits a very distinct x-ray fiber diagram.[251]

(*b*) *Compounds with Salts*

The swelling of starch in the presence of strong acids or concentrated solution of salts, such as hydrochloric or phosphoric acids, zinc chloride, potassium mercuric chloride (K_2HgCl_4), or lithium bromide, is entirely comparable with the corresponding phenomena in the case of cellulose. The process consists in the formation of compounds between the cations and alcoholic hydroxyls.

(*c*) *Reaction with Iodine*

The blue color produced by starch solutions in the presence of iodine has been the subject of many investigations. The nature of the compound formed will be best understood if the following facts are taken into account. Not only starch, polyvinyl alcohol, and cellulose swollen with zinc chloride, but also many other substances, in particular, certain colloidal suspensions or finely divided precipitates, show color reactions with iodine. These in-

[250] A. Reychler, *Bull. soc. chim. Belg.*, **23,** 378 (1909).

[251] F. R. Senti and L. P. Witnauer, *J. Am. Chem. Soc.*, **70,** 1438 (1948).

clude basic lanthanum and praesodymium acetates[252] and, according to Barger and Eaton,[253] the sodium compounds of carbethoxyhydrindene, *o*-oxychalcone, benzylidene phthalide, and acetocumarine, and, according to Bergmann,[254] the methyl cycloacetals of hydroxyacetone and acetoine.

None of these substances give the color reaction if molecularly dispersed in solution or in a well-crystallized form; color effects are on the whole only obtained from precipitates with a very much distorted lattice. The conclusion may be drawn that the iodine molecules are deposited in the fissures of such particles, and that the change in light absorption of the iodine is related to its disposition in this way. Colored addition compounds are not limited to iodine systems; ultramarine is a case in point.

Investigation of the equilibrium between starch and iodine and the starch–iodine compound shows that the concentration of the iodine starch is proportional to the starch and to the iodine concentrations to powers higher than the first:[255]

$$K[\text{starch–iodine}] = [\text{starch}]^n(I_2)^m \quad (n, m > 2)$$

This indicates that several molecules of iodine combine with several molecules of amylose to give the blue compound.

Dilute solutions of amylose form a blue-black precipitate with aqueous solutions of iodine in the presence of electrolytes, such as hydrogen chloride or sodium sulfate. The composition of this precipitate varies from I_{2n}-$(C_6H_{10}O_5)_{10n}$ in the presence of excess iodine, to $I_{2n}(C_6H_{10}O_5)_{20n}$ with excess amylose. On addition of iodine to a dilute starch solution the activity of the iodine increases if the amount exceeds more than one iodine molecule for each 8 glucose residues.[256] Experience shows that iodine ions are neither necessary for the production of the blue color with iodine nor for the precipitation of the iodine compound; on the other hand, water is essential, for while air-moist starch is colored by iodine vapors, vigorously dried starch remains colorless. All these observations suggest that iodine starch should be classified with other colored colloidal addition compounds; the iodine enters into the micellar aggregate of the amylose or amylopectin. The more specific suggestion of an amylose–iodine structure should be rejected.

According to Rundle[257] the iodine molecules are oriented with their long

[252] See also D. Krüger and E. Tschirch, *Ber.*, **63**, 826 (1930).
[253] G. Barger and F. J. Eaton, *J. Chem. Soc.*, **125**, 2407 (1924).
[254] M. Bergmann, *Ber.*, **57**, 755 (1924).
[255] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **24**, 389 (1941).
[256] F. L. Bates, D. French, and R. E. Rundle, *J. Am. Chem. Soc.*, **65**, 142 (1943).
[257] R. E. Rundle and D. French, *J. Am. Chem. Soc.*, **65**, 558 (1943).

axes parallel to that of the amylose, the streaming dichroism furnishing evidence of this. Optically, the streaming solution behaves similarly to an iodine crystal oriented with its long axis in the direction of flow.

The color tone of the iodine addition compound depends chiefly on the structure of the polysaccharide as a glance at the following summary shows. Unbranched amylose gives a pure blue reaction, branched varieties like glutinous rice and limit dextrins exhibit red colors, the still more branched glycogen, a red-brown reaction, while the still more structurally irregular compound—the limit dextrin arising from the degradation of glycogen with β-amylase—only causes an intensification of the yellow iodine color to brown. The color tone of the iodine reaction can therefore serve as a useful indication of the degree of branching. Also, it cannot be mere chance that the iodine color reaction shows a definite relationship to the capacity for crystallization, which diminishes from amylose to quite amorphous glycogen. Molecules so shaped that crystallite formation is permissible, combine together in aqueous solution as micelles, in which iodine can take up definite positions with resultant blue coloration; the smaller the tendency for crystallite formation the weaker is the iodine effect. Then, also, there is a connection between color tone and the length of the unbranched chain segments,[255] for the longer the latter, the more readily can crystallization or micelle formation occur. In view of that, it may be surmised that even unbranched amylose would give intermediate products with iodine red color reaction, either by acid or by α-amylase degradation; the chains could be so far broken down that the remaining shortened chains would not suffice for the formation of well-arranged micelles. According to Swanson[257a] the minimum chain length necessary for formation of a colored complex is 4–6 glucose residues; these give a slight brown color. Free chains and segments between branching points of 8–12 residues give red complexes while the typical blue color of amylose appears when the chains are more than 30 residues in length.

The previously much discussed question as to whether an *adsorption* compound or a *chemical* compound is concerned is meaningless, in view of our present knowledge of attractive forces and chemical linkages. Quite certainly iodine is bound to starch by secondary valences.

7. Starch Ethers and Esters

As mentioned before, starch can be methylated in alkaline solution; amylose and amylopectin are similarly converted to their methyl ethers.

[257a] M. A. Swanson, *J. Biol. Chem.*, **172**, 825 (1948).

The methylated products are soluble in chloroform and in cold water but precipitate from hot water. In this respect they resemble methyl cellulose. Methylated derivatives with high or low viscosity can be prepared, depending on the nature of the starch used.

The sodium salt of starch–glycolic acid, prepared by reaction of alkali starch with chloroacetic acid is used as an emulsifying agent.

Starch inorganic esters have been prepared by methods similar to those used for the amylose derivatives.

8. Chemical Breakdown of Starch

(*a*) *Aqueous Cleavage*

Starch is degraded by being heated with water above 100°. According to Schoch, the acids and phosphatides contained in the starch are responsible for this and, by defatting and then buffering to *p*H 7, the breakdown can be prevented even at 110°. Repeated treatment with superheated water at higher temperatures leads to the formation of fragments giving a red iodine reaction.

In the course of such hot-water breakdown, not only are the larger molecules broken down into smaller ones, but also the outer branches of the amylopectin molecules are more especially attacked, as can be shown by an investigation of the enzyme breakdown.[258]

(*b*) *Breakdown by Acids*

In the presence of acids, hydrolysis and breakdown occur at room temperature. So-called Lintner starch is obtained by treating starch for a week with 7% hydrochloric acid; the product still gives a blue coloration with iodine, and is to be regarded as a mixture of breakdown products. As the action of the acid continues, the molecular weight and viscosity of the products of hydrolysis fall[259] and the reducing power increases.

One breakdown product to be mentioned here is amylodextrin, obtained, according to A. Meyer, by prolonged treatment of starch with cold hydrochloric acid; this crystallizes out in water in the form of spherites composed of needle-shaped crystals. On being heated with acids, dextrins are formed from starch. These are shorter chains of the maltose type, the

[258] K. H. Meyer, M. Wertheim, and P. Bernfeld, *Helv. Chim. Acta*, **24**, 378 (1941).

[259] See, among others, W. Biltz, *Ber.*, **46**, 1533 (1913). M. Samec and S. Jeučič, *Kolloid-Beihefte*, **7**, 157 (1915). H. Staudinger and E. Husemann, *Ann.*, **257**, 195 (1937).

molecular weights of which, according to Haworth,[260] are several thousand. The end product of hydrolysis is glucose. Maltose has also been isolated from the products of acid hydrolysis (Ost[261]).

Starch can also be broken down by heating in glycerol; first a soluble starch, Zulkowski starch, is formed (mol. wt. 6600 to 7000), and subsequently products of lower molecular weight which do not reduce Fehling's solution. These have been investigated by Pictet[262] and Pringsheim[263] and are regarded as di- and trihexosans but they are certainly products of appreciably high degree of polymerization in which the aldehylde groups are closed by glycerine.

Karrer[264] obtained a yield of about 30% acetobromomaltose by acetolytic breakdown with acetyl bromide.

In alkaline media starch is degraded by atmospheric O_2; it is thus essential to exclude oxygen when carrying out reactions in alkali such as methylation.

9. Enzymatic Breakdown of Starch

Starch can be broken down by enzymes in various ways; the enzymes effecting starch hydrolysis are called amylases (formerly diastases). The cleavage of the glucosidic linkages is not always hydrolytic, it may be phosphorolytic involving the uptake of phosphoric acid; the effective enzymes here are called phosphorylases. In addition, starch can be degraded enzymatically without any hydrolysis being involved. Of these the amylase reactions are by far the most important both biologically and from the technical point of view. They include the amylolytic breakdown of starch in the intestinal tract and the saccharification of starches by malt in the manufacture of beers and spirits.

(*a*) *Amylases*

Researches on the malt enzymes have revealed that there are two amylases present which react with starch in fundamentally different ways but which are complementary in their effect. On the other hand, starch also is not homogeneous. In the course of malting, therefore, a number of dif-

[260] W. N. Haworth, E. L. Hirst, and A. C. Waine, *J. Chem. Soc.*, **1935**, 1299.

[261] H. Ost, *Angew. Chem.*, **17**, 1663 (1904).

[262] A. Pictet and R. Salzmann, *Helv. Chim. Acta*, **7**, 934 (1924); **8**, 948 (1925). A. Pictet, *ibid.*, **9**, 33 (1926).

[263] H. Pringsheim, *Die Polysaccharide*. 3rd ed., Springer, Berlin, 1931.

[264] P. Karrer and C. Naegeli, *Helv. Chim. Acta*, **4**, 263 (1921).

ferent processes are superimposed which it is only possible to disentangle by the separate study of each one.

Two types of amylase have been distinguished since the investigations of Wijsmann,[265] Kuhn,[266] and Ohlsson[267]: (*1*) The α-amylases or dextrinogen amylases; these first split the polysaccharide into large fragments, dextrins; maltose only appears later in the course of the reaction and then in the α downward mutarotating form. (*2*) The β-amylases or saccharogen amylases, which produce maltose at once, but in the β upward mutarotating form.

α-Amylases are present in animal fluids and organs, *e.g.*, saliva, pancreas, liver, and leucocytes; α-amylase is also found in *Aspergillus* species (taka-diastase) in certain bacteria (*e.g.*, *Bacillus mesentericus subtilis*), and together with β-amylase in malt. By warming the aqueous malt extract at 70° the β-amylase is destroyed and α-amylase can be obtained free from the β enzyme.

Different amylases have been obtained in a crystalline form. α-Amylases from different sources are certainly chemically different; their resistance to heat and chemical attack is not the same. It has also been found[268] that a protein-containing substance found in wheat grains acts as an *amylase inhibitor* with regard to saliva and pancreas amylases but not toward malt amylase.

β-Amylase is contained in cereals; they also contain α-amylase, the amount increasing on germination. The former can be isolated by fractional precipitation with ammonium sulfate, the α component being first precipitated, or by destroying the α-amylase with acetic acid.[269] Another source is *Ipomoea batatas* (sweet potato) from which crystalline β-amylase has been obtained.[270]

(*b*) *Action of α-Amylase*

α-Amylase first brings about fissure of the starch into larger fragments (dextrins) and at the same time destroys the shells; it liquefies—thus causing an immediate diminution in viscosity. The idea that liquefaction can occur without any hydrolytic breakdown is quite wrong and is due to the fact that the small degree of cleavage necessary ($\ll 1\%$ of the glucosidic

[265] H. P. Wijsmann, *Dissertation*. Amsterdam, 1888.
[266] R. Kuhn, *Ann.*, **443**, 1 (1925).
[267] E. Ohlsson, *Z. physiol. Chem.*, **189**, 17 (1930).
[268] E. Kneen and R. M. Sandstedt, *J. Am. Chem. Soc.*, **65**, 1247 (1943).
[269] J. Blom, A. Bak, and Braae, *Z. physiol. Chem.*, **241**, 273 (1936).
[270] A. K. Balls, R. R. Thompson, and M. K. Walden, *J. Biol. Chem.*, **163**, 571 (1946).

links) can easily be overlooked, though it is quite sufficient to effect a fissure of all the amylopectin molecules and so bring about liquefaction.[270a]

From pure amylose larger fragments are produced initially, then maltotriose, maltose, and glucose, the larger fragments slowly breaking down further to give maltose and glucose.[271] It must be assumed that α-amylase is not selective in its attack on the glucosidic linkages, apart from the fact that linkages attached to a terminal residue are more slowly attacked than others. In the limiting case where the linkage is between two terminal residues (*i.e.*, maltose) it is not attacked at all. Both the quantitative results of the breakdown and the kinetics are in agreement with this interpretation. The final products are maltose (87%) and glucose (13%). Crystallization of amylose almost completely inhibits the enzyme attack.

For amylopectin (and therefore also for starch) the enzyme degradation is complicated by the presence of branch points. These are only very slightly if at all attacked by α-amylase, so that besides maltose and glucose branched oligosaccharides[272] are obtained. These so called "limit dextrins" are finally, though very slowly, broken down into isomaltose and isomaltotrioses, glucose, and maltose.

(*c*) *Action of β-Amylase*

β-Amylase only attacks at the nonaldehydic ends of the chains with the immediate production of maltose but no immediate change in viscosity; the primary valency chains, therefore, are not really broken down but simply lose small fragments from their ends. Amylose is completely broken down to maltose as was first shown by Samec and Waldschmidt-Leitz.[273]

The breakdown of amylose may be inhibited or completely prevented by aging of the solution, *i.e.*, the formation of submicroscopic crystallites; the aged solution can, however, be rejuvenated by alkali treatment followed by acidification, and again becomes vulnerable to the β-amylase. Complete hydrolysis can only be achieved if the alkaline-treated solution—which is molecularly disperse—is mixed with a large excess of buffered enzyme solution. Under those conditions each amylose molecule enters into an enzyme–substrate complex so that aging cannot occur.[234a]

Amylopectin is only broken down to the extent of 61–64% by β-amylase, from which it may be deduced that about two thirds of the amylopectin material lies in the outer regions exposed to enzyme attack. A β-amylase-

[270a] K. H. Meyer, F. Duckert, and E. H. Fischer, *Helv. Chim. Acta*, **33**, 207 (1950).
[271] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **24**, 359E (1941).
[272] K. Myrbäck, *Biochem. Z.*, **307**, 132, 140 (1941).
[273] M. Samec and E. Waldschmidt-Leitz, *Z. physiol. Chem.*, **203**, 16 (1931).

resistant high molecular dextrin (limit dextrin, also known as erythrogranulose) is produced, which gives a red iodine coloration. Estimation of the end groups shows that their number is unchanged by conversion to the dextrin[274]; therefore, the branch points must present an obstacle to the further attack of the enzyme, which is only effective for linkages near the chain ends[275] (*cf.* Fig. 11).

The limit dextrin is slowly broken down further[276] by isophosphorylase[276a]; the product obtained is again vigorously attacked by β-amylase,

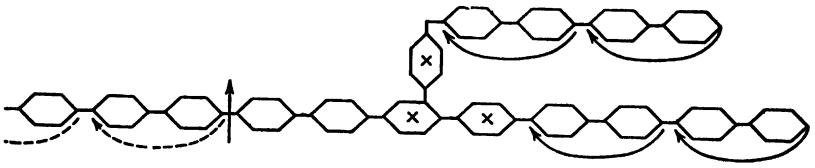


Fig. 11. Enzyme degradation of amylopectin: full curved arrow, attack by β-amylase; x, branch points prevent continued hydrolysis and residual dextrin I remains; straight arrow, cleavage by α-amylase or superheated water; and broken curved arrow, subsequent attack by β-amylase

up to the point where a second limit dextrin, of lower molecular weight, is formed. Amylopectin can therefore be regarded as having a treelike structure with very many branches; the outmost branches are attacked by β-amylase as far as the branch points which can only be removed by the isophosphorylase. By the action of the latter the inner branches become exposed to further β-amylase attack which continues until the next branch points are reached (*cf.* Fig. 6). The outer branches consist of some 13 to 18 glucose residues, the inner segments situated between the branch points contain on the average 8 to 9 residues.

(*d*) *Combined Action of α- and β-Amylases in Malt*

In malt the effects of both enzymes are superimposed; β-amylase attacks only at the ends of the chains but, as the α-amylase attacks the inner parts of the giant molecules, new ends become available for β-amylase attack.

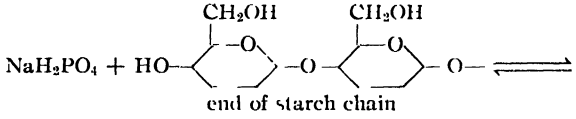
The end products of malt saccharification are chiefly maltose (*ca.* 80%), a small percentage of glucose, and some 20% of limit dextrins which are not

[274] K. H. Meyer, M. Wertheim, and P. Bernfeld, *Helv. Chim. Acta*, **24**, 212 (1941).
[275] C. S. Hanes, *New Phytologist*, **36**, 189 (1937).
[276] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **25**, 399 (1942).
[276a] P. Bernfeld and A. Meutémédian, *Helv. Chim. Acta*, **31**, 1724 (1948).

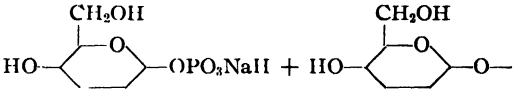
fermentable by brewers' or bakers' yeast. These are essentially branched oligosaccharides, *i.e.*, di-, tri-, and tetrasaccharides arising from the branch points; isomaltose (1,6-glucosidoglucose) has been identified as one constituent of the mixture.[277]

(*e*) *Phosphorolysis and Synthesis*

In conjunction with investigations on the phosphorolysis of glycogen which will be discussed later, Cori[278] and Schäffner[279] have examined the effect on starch of yeast phosphorylase and muscle phosphorylase, respectively. They found that in the presence of phosphate glucose 1-phosphoric ester was produced and that the enzyme catalytic effect is reversible:



end of starch chain



glucose-1-phosphate shorter chain

Later Hanes[280] obtained active phosphorylase preparations from potatoes and from peas, and with them he prepared a polysaccharide from glucose 1-phosphate which gave a blue reaction with iodine, was completely degraded by β-amylase, and exhibited in every way the properties of amylose.[281] This product can be regarded as synthetic amylose. Meyer and Bernfeld have shown [282] that potato phosphorylase is without effect on limit dextrin; its effect both phosphorolytic and synthetic is therefore limited to 1,4 linkages. Like β-amylase it attacks the nonaldehydic ends of the molecules and is inhibited by branch points. The branch points (1,6-α linkages) are formed by lateral addition of glucose residues from glucose 1-phosphate to the amylose chain by the agency of a particular enzyme, a 1,6-phosphorylase, termed *isophosphorylase* by Bernfeld and Meutémédian.[276a]

As plant phosphorylase is found in the same regions as the starch deposits, it is probably concerned in the biosynthesis of starch. If so, then

[277] E. M. Montgomery, F. B. Weakley, and G. E. Hilbert, *J. Am. Chem. Soc.*, **69**, 2249 (1947).

[278] G. T. Cori, S. P. Colowick, and C. F. Cori, *J. Biol. Chem.*, **123**, 375 (1938).

[279] A. Schäffner and H. Specht, *Naturwissenschaften*, **26**, 494 (1938).

[280] C. S. Hanes, *Proc. Roy. Soc. London*, **B128**, 421 (1940); **B129**, 174 (1940).

[281] W. H. Haworth, R. L. Heath, and S. Peat, *J. Chem. Soc.*, **1942**, 55.

[282] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **25**, 399, 404 (1942).

starch is not built up directly from glucose but from glucose 1-phosphate. This is plausible since the equilibrium for starch $+H_2O\rightarrow$ glucose or maltose largely favors sugar formation, for glucose is associated with less energy than starch. But glucose 1-phosphate has about the same energy content as starch so that synthesis from or degradation to the glucose 1-phosphate are energetically nearly equal.

These considerations are, however, not sufficient to explain the simultaneous formation of amylose and amylopectin. *In vitro* a mixture of more or less branched polysaccharides may be obtained by the action of phosphorylase in the presence of isophosphorylase on glucose-1-phosphate.[276a] The degree of branching of the carbohydrate formed initially is dependent on the relative proportions of the enzymes, whereby increasing quantities of isophosphorylase produce more highly branched substances. But as in any reversible system the equilibrium state is independent of this proportion; the final product is a highly branched compound of the glycogen type. Initially formed unbranched or slightly branched mixtures are in solution transformed into this compound. Crystallization of the initially formed polysaccharides may slow down or even inhibit this transformation. It follows that in the formation of polysaccharides in the starch granule crystallization phenomena interfere; if, on the other hand, a polysaccharide of this group is present in dispersed form in living cells it is always of the highly branched glycogen type.

(*f*) *Amylosucrase, Amylomaltase, and Macerans Amylase*

Several enzymes may both degrade and synthesize polysaccharides of the starch group by transglucosidification, that is, migration of glucosidic bonds, no hydrolysis or phosphorolysis being involved. From cultures of *Neisseria perflava* an enzyme termed amylosucrase has been obtained which transforms sucrose into fructose and an amylopectin-like substance (Hehre).[282a]

$$\underset{\text{sucrose}}{n\ C_{12}H_{22}O_{11}} \longleftrightarrows (C_6H_{10}O_5)n + \underset{\text{fructose}}{n(C_6H_{12}O_6)}$$

A similar enzyme, amylomaltase, transforms maltose into a polysaccharide of the starch type and glucose; it occurs in *Escherichia coli* (Monod).[282b]

As early as 1903 Schardinger obtained crystalline dextrins (so-called Schardinger dextrins) by the degradation of starch with *Bac. macerans*:

[282a] E. J. Hehre, *J. Biol. Chem.*, **177**, 267 (1949).

[282b] J. Monod and A. M. Torriani, *Compt. rend.*, **227**, 240 (1948).

actually small amounts of other dextrins are formed at the same time.[283] The same dextrins can also be obtained using an enzyme extract of the dead bacilli.[284] They have no reducing action toward Fehling's solution and can be precipitated from aqueous solution by means of trichloroethylene. Freudenberg and French and Rundle[285] have shown that these dextrins are closed-ring compounds with six, seven, or eight glucose residues, respectively, joined one to another by 1,4-α linkages. X-ray analysis of Schardinger dextrin is in agreement with this view.[286]

The macerans amylase also attacks the molecules from the nonaldehydic end and degradation is prevented at the branch points (*cf.* β-amylase). This at least seems highly probable from observation made by Wilson, Schoch, and Hudson[287] according to which amylose (apparently impure) was broken down to the extent of 90%, amylopectin only being degraded as far as 60%.

On the other hand, the enzyme transforms the cyclic dextrins into amylose up to a certain equilibrium concentration.[287a] This reversible transformation of long chain into cyclic compounds recalls the analogous reactions with sulfur, polyphosphonitrilic chloride, and the polyamides.

10. Glycogen[288]

(*a*) *Occurrence and Preparation*

The term glycogen embraces carbohydrates which are closely related chemically to starch, yielding a violet or brown color with iodine, but not occurring in the form of granules. Glycogen may occur in nearly all animal cells and in several plants. Mushrooms and yeast contain glycogen; it is further found in the immature grains of a variety of maize "golden bantam."[288a] In general, it is dispersed in the protoplasm. In embryos

[283] See also K. Freudenberg and R. Jacobi, *Ann.*, **517**, 102 (1935).

[284] E. B. Tilden and S. Hudson, *J. Am. Chem. Soc.*, **61**, 2900 (1939). E. B. Tilden, M. Adams, and C. S. Hudson, *ibid.*, **64**, 1432 (1942).

[285] K. Freudenberg, G. Blomqvist, L. Ewald, and K. Soff, *Ber.*, **69**, 1258 (1936). K. Freudenberg and W. Rapp, *ibid.*, **69**, 2041 (1936). K. Freudenberg and M. Meyer-Delius, *ibid.*, **71**, 1596 (1938). D. French and R. E. Rundle, *J. A. C. S.*, **64**, 1651 (1942).

[286] O. Kratky and B. Schneidmesser, *ibid.*, **71**, 1413 (1938).

[287] E. J. Wilson, Jr., T. J. Schoch, and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 1380 (1943).

[287a] D. French, J. Pazur, M. L. Levine, and E. Norberg, *J. Am. Chem. Soc.*, **70**, 3145 (1948).

[288] Review: K. H. Meyer, in *Advances in Enzymology*, Vol. III, Interscience, New York, 1943, page 109.

[288a] D. L. Morris and C. T. Morris, *J. Biol. Chem.*, **130**, 535 (1939); **154**, 503 (1944).

and diabetic subjects only it is found in the form of granules or flakes of varying size (1–10 μ) in liver and muscle cells, both in the nucleus and in the cytoplasm. Glycogen is readily formed from glucose, other sugars, sugar alcohols, and various organic substances, and, conversely, is as readily broken down by the living organism.

Glycogen is usually prepared by boiling liver, rich in glycogen, with strong alkali, thus destroying the proteins; the glycogen is then precipitated by the addition of alcohol. It is most easily obtained from the immature grains of golden bantam.[288b]

Only part of the glycogen passes into solution on boiling with water, which led Willstätter and Rohdewald[289] to the conclusion that glycogen is partly present in the organism in a free state *lyoglycogen*, and that it is partly present in combination with cell protein as *desmoglycogen*. Meyer and Jeanloz[290] have shown, however, that insolubility persists after removal of protein and so cannot be dependent on a protein complex formation. The pure polysaccharide consists of water-soluble and water-insoluble fractions, apparently high polymeric, which can be separated from each other by intensive centrifugation or by electrodialysis.

To prepare glycogen from yeast, which has been made rich in glycogen by culture on cane sugar solution, the yeast is ground up with quartz powder; the mass is extracted first with water and then with chloral hydrate, and finally mannan and proteins are removed from the extracts.[291]

The optical rotation of glycogen is $[\alpha]_D = +197°$. X-ray analysis shows that it is amorphous.

(*b*) *Constitution*

Acid hydrolysis yields glucose, whereas, by the action of amylase, maltose is formed in considerable quantities. On methylation and hydrolysis 2,3,6-trimethylglucose is obtained as the chief product together with about 9% of both 2,3-dimethyl- and 2,3,4,6-tetramethylglucose.[291] While admittedly inconclusive evidence provided by D. J. Bell[291a] suggests that the majority at least of the branching bonds are of the 1,3 type, the behavior of enzymes toward glycogen indicates that it has a similar constitution to that of amylopectin which contains only 1,6 linkages.[295]

The molecule is highly branched; both methylation and periodate oxi-

[288b] K. H. Meyer and M. Fuld, *Helv. Chim. Acta*, **32**, 757 (1949).
[289] R. Willstätter and M. Rohdewald, *Z. physiol. Chem.*, **225**, 103 (1934).
[290] K. H. Meyer and R. Jeanloz, *Helv. Chim. Acta*, **26**, 1784 (1943).
[291] F. Stockhausen and K. Silbereisen, *Biochem. Z.*, **287**, 276 (1936).

dation techniques indicating one end group per 11-12 glucose residues for all glycogens with the exception of certain samples from rabbit liver which give a 1:18 ratio.[291b] Only very small amounts of phosphoric acid are present in glycogen in the combined form. The water content of the air-dried product corresponds to the formula $C_6H_{10}O_5H_2O$.

The particle weight of native glycogen amounts to 1 to 10 million.[292] Estimations on inferior preparations gave values of several hundred thousand.[293] The physically determined particles are much larger than the molecules whose weight is obtained by reductometric methods. Here, as in the case of amylopectin, many molecules seem to be mechanically entangled to form the particle.

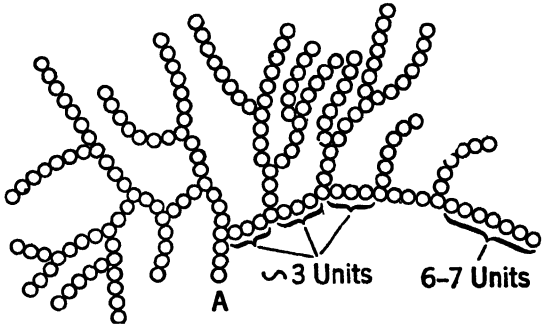


Fig 12. Structure of glycogen: A, aldehydic end; O, glucose units.

Further evidence has been provided by investigating the enzyme breakdown of glycogen with β-amylase (Meyer and Fuld[295]). This shows that only quite short chains of glucose residues with the 2, 3, and 6 hydroxyls free (on an average, three) can lie between residues with the 6 position involved in a branch linkage. Figure 12 above shows an arrangement of glucose residues based on this.

It can readily be understood that, with such a structure, the parallel disposition of longer chain segments to give crystalline bundles is impossible

[291a] D. J. Bell, *J. Chem. Soc.*, **1948**, 992

[291b] T. G. Halsall, E. L. Hirst, and J. K. N. Jones *ibid.*, **1947**, 1399.

[292] A. van der Wyk, *unpublished work.*

[293] S. R. Carter and B. R. Record, *J. Chem. Soc.*, **1939**, 664.

[294] W. N. Haworth, E. L. Hirst, and F. A. Isherwood, *J. Chem. Soc.*, **1937**, 577. W. N. Haworth, E. L. Hirst, and F. Smith, *ibid.*, **1939**, 1914.

[295] K. H. Meyer and M. Fuld, *Helv. Chim. Acta*, **24**, 375 (1941).

and this explains why glycogen is always amorphous in contrast to starch which shows quite definite crystalline interferences.

(c) *Glycogenolysis and Glycogen Synthesis*

The breakdown of glycogen within the living organism is termed glycogenolysis. In muscle, this leads to lactic acid, in yeast to alcohol and carbon dioxide. The reaction chain in each case is known in practically all its details, thanks to the investigations of Embden, Meyerhof, Parnas, Cori, Warburg, and Negelein. It was made possible by isolating each separate reaction, partly with pure enzymes, and partly with enzyme mixtures, in which, by suitable addition, the effect of all enzymes except one was inhibited, so that the effect of each enzyme could be separately studied. The first breakdown product is glucose 1-phosphate produced from glycogen in the presence of phosphate by a phosphorylase as in the breakdown of amylose. Glucose-1-phosphate discovered by Cori, Colowick, and Cori is often termed Cori ester.

Schäffner and Specht,[296] Kiessling,[297] and also Cori[298] have shown that the reaction is reversible. Starting from glucose 1-phosphate and an enzyme prepared either from yeast or from muscle, a polysaccharide can be obtained which shows the same rotation and iodine coloration as glycogen and is undoubtedly branched. The enzyme involved is a mixture of phosphorylase and isophosphorylase; the conditions which favor the formation of branched polysaccharides have been exposed in connection with amylopectin (Chap. F, sect. VII8*e*).

11. Other Glucosans

(a) *Lichenin*

From iceland moss (*Cetraria islandica*) and from many other varieties of lichen, *e.g.*, *Evernia*, *Usnea*, *Parmelia*, etc., a number of carbohydrate substances are obtainable by hot-water extraction. They include lichenin which separates as a jelly on cooling the aqueous extract, and also the so-called isolichenin.

Acetolysis of lichenin gives roughly 6% of cellobiose octaacetate; methylation and hydrolysis yield 0.6% tetramethylglucose[299] and *ca.* 73% of

[296] A. Schäffner and H. Specht, *Naturwissenschaften*, **26**, 494 (1938).
[297] W. Kiessling, *Biochem. Z.*, **302**, 50 (1939).
[298] C. F. Cori, G. Schmidt, and G. T. Cori, *Science*, **89**, 464 (1939).
[299] K. Hess and L. W. Lauridsen, *Ber.*, **73**, 115 (1940).

2,3,6-trimethylglucose, together with *ca.* 27% of the 2,4,6 isomer.[300] The molecular weight determined osmometrically,[301] or by reduction of dinitrosalicyclic acid, is about 40,000. From these facts it can be deduced that lichenin has an unbranched structure and that the chain involves 1,4-α and 1,3-α linkages. In aqueous solution, lichenin is practically optically inactive; correspondingly, the triacetate has a small but definite levorotation. $[\alpha]_D = -32°$ to $-38°$ in chloroform.

Isolichenin is actually a mixture of polysaccharides one of which is a polyglucosan of unknown constitution with $[\alpha]_D = 9.6°$, characterized by giving a blue coloration with iodine, though quite certainly different from starch. The other polysaccharide constituents are structurally more complex, giving rise to glucose, mannose, and galactose on hydrolysis.

After removal of lichenin, isolichenin, and lichen acids, it is possible to extract hemicelluloses from the residues of iceland moss by treatment with alkali. The mixtures of hemicelluloses obtained have been fractionated by Percival[302] and the products of hydrolysis investigated, showing that glucose, mannose, galactose, and uronic acids are present. A polysaccharide similar to lichenin has been found in oat flour.[303]

(*b*) *Soluble Glucosans*

According to Barry,[304] laminarin, the neutral polysaccharide obtained from *Laminaria* is a polyglucosan with repeated 1,3 linkage (see sect. IV 4).

From barley roots Hassid[305] has isolated a water-soluble glucosan (yield

TABLE VIII

Review of Polyglucosans of Known Constitution

Polyglucosan	Linkage	Polyglucosan	Linkage
Cellulose, tunicin	1,4-β	Lichenin	1,4-β and 1,3-β
Laminarin (relatively low molecular)	1,3-β	Floridean starch	1,4-α and 1,3-α
Polysaccharide from the skeletal substance of bakers' yeast (high molecular)	1,3-β	Amylose	1,4-α
Pustulin, luteose	1,6-β	Bacterial dextran	1,6-α
Bacterial levan	1,2-β	Polysaccharide from barley root	1,6-α
		Amylopectin, glycogen	1,4-α and 1,6-α, branched
		Schardinger dextrin	1,4-α, cyclic

[300] K. H. Meyer and P. Gürtler, *Helv. Chim. Acta*, **30**, 751 (1947); R. A. Boissonnas *ibid.*, **30**, 1703 (1947).

[301] H. Staudinger and B. Lantzsch, *J. prakt. Chem.*, **156**, 65 (1940).

[302] H. Granichstädten and E. G. V Percival, *J. Chem. Soc.*, **1943**, 54.

[303] D. L. Morris, *J. Biol. Chem.*, **142**, 881 (1942).

[304] V. C. Barry, *Chem. Zentr.*, **1943**, II, 317.

[305] W. Z. Hassid, *J. Am. Chem. Soc.*, **61**, 1223 (1939).

0.4%) to which he assigns a 1,6-α-polyglucosidic structure similar to that found for certain bacterial dextrans already described. It is strongly dextrorotatory $[\alpha]_D = 201°$ in water. Pustulin obtained from certain lichen by Drake,[306] $[\alpha]_D = 68°$, is also thought to be a 1,6-polyglucosan but in this case repeated β-linkage is involved (Table VIII). The so-called "Floridean starch" is an unbranched glucosan with 1,4-α and 1,3-α bonds.[306a]

12. Galactogen

Hammarsten[307] first discovered a polysaccharide in the so-called protein gland of snails (*Helix pomatia*) and many years later it was shown[308] to give D-galactose on hydrolysis. Later Bell and Baldwin[309] claimed that small amounts of L-galactose were also present. Methylation and identification of the hydrolysis products showed that the molecule must be highly branched, Schlubach[310] giving evidence for 50% of end groups, Bell for only 25%. From their results, Bell and Baldwin have drawn further conclusions with regard to the arrangement of the residues but more investigation is necessary before they can be accepted. It is also desirable that some further confirmation of the L-galactose should be found, since up to now it depends only on the preparation of 2-D,L-galactobenzimidazole from a fraction of the hydrolysis sugars. Another galactogen has been isolated from beef lung.[310a]

13. Mannans

(*a*) *Salep Mannan*

From the bulbs of certain orchids (*Tubera salep*) a carbohydrate has been isolated, which gives a slimy aqueous solution and can be hydrolyzed to mannose. According to Klages and Niemann,[311] its structure is essentially similar to that of the insoluble mannan from ivory nut, *i.e.*, the residues are connected by 1,4-β linkages. Husemann[312] ascribes the water solubility to a partial acetylation (one acetyl group to 12 mannose residues) which prevents regular micelle formation and so increases the tendency to dis-

[306] B. Drake, *Biochem. Z.*, **313**, 388 (1942).

[306a] V. C. Barry, T. G. Halsall, E. L. Hirst, and J. K. N. Jones, *J. Chem. Soc.*, **1949**, 1468.

[307] O. Hammarsten, *Arch. ges. Physiol. Pflügers*, **36**, 373 (1885).

[308] F. May, *Z. Biol.*, **95**, 277, 401 (1934).

[309] D. J. Bell and E. Baldwin, *J. Chem. Soc.*, **1941**, 125.

[310] H. H. Schlubach and W. Loop, *Ann.*, **532**, 228 (1937).

[310a] M. L. Wolfrom, D. I. Weisblat, and J. V. Karabinos, *Arch. Biochem.*, **14**, 1 (1947).

[311] F. Klages and R. Niemann, *Ann.*, **523**, 224 (1936).

[312] E. Husemann, *J. prakt. Chem*, **155**, 241 (1940).

solve. If the acetyl group were removed then the product would be insoluble. The molecular weight determined osmometrically for the nitrate, is very high (~300,000). The intrinsic viscosity of an aqueous solution is some 3.5 times as high as in cuprammonium solution.

(*b*) *Konjak Mannan*

The tubers of *Conophallus konjaku*, a member of the family Araceae, contain a mannan as reserve. This forms the chief constituent of konjak flour, used in Japan as food. The mannan is soluble in hot water and in cold, dilute alkali; it does not reduce Fehling's solution; the optical rotation in dilute alkali: $[\alpha]_D = 42.8°$. The constitution has been studied in various ways; mannose and glucose appear to be present. On methylation and hydrolysis, 2,3,4-trimethyl glucose, 2,3,6-trimethylmannose, and 2,3,4-trimethylmannose have been obtained (Nishida and Hashima[313]). Moreover, an acetylated trisaccharide consisting of two mannose units and one glucose unit was obtained by acetolysis. From this the most probable constitution is that of a long chain in which one glucose residue in 1,6 linkage, a mannose residue in 1,6 linkage, and a mannose residue in 1,4 linkage are periodically repeated.

As Sakurada and Hutino[314] have shown, konjak mannan, the commercial form of which is water-soluble, is transformed into a form insoluble in water as a result of purification by crystallization from water and treatment with chlorine dioxide.

If the aqueous solution is forced through a nozzle into dilute sulfuric acid, threads are obtained which, on drying, yield a fiber diagram. The identity period is 8.5 Å. The lattice contracts on drying and expands once more on moistening; the identity period remains unchanged. The results of x-ray analysis thus appear to contradict the chemical results, since the identity period rather suggests that the unit cell contains not three but two residues of a chain and that this is the structural unit.

From lucerne seeds a galactomannan has been obtained which, according to May,[315] undergoes cleavage to give equal amounts of galactose and mannose.

(*c*) *Carubin*

In the seeds of *Ceratonia siliqua* (carob beans) considerable quantities of a polymeric mannogalactan are found which contains mannose and galactose

[313] K. Nishida and H. Hashima, *J. Dept. Agr. Kyushu Imp. Univ.*, **2**, 277 (1930).
[314] I. Sakurada and K. Hutino, *Z. physik. Chem.*, **B21**, 18 (1938).
[315] F. May and A. S. Schulz, *Z. Biol.*, **97**, 201 (1936).

in the proportions (3 to 4):1. Even at great dilution it gives a highly viscous solution and so finds application as an adhesive and as a thickener in calico printing. This very high molecular compound is composed of long chains of D-mannose residues to which D-galactose residues are laterally attached in 1,6 linkages.[316] The optical rotation indicates the presence of β linkages. The aqueous solution forms a gel on addition of boric acid, the latter forming bridges between two sets of *cis*-hydroxyl groups of the mannose residues.

Similar mannogalactans are found in the seeds of other leguminous plants, *e.g.*, *Gleditschia tricanthos*, *Cyamopsis tetragonoloba*.

14. Inulin and Other Fructosans

Water-soluble fructosans are very widely distributed in the vegetable kingdom. They function as carbohydrate reserves as shown by their increase during tuber formation and decrease when shoot growth begins.

In a series of systematic investigations, Schlubach in particular has shown that fructosans are very varied in structure and degree of polymerization. Branched and unbranched, high and low molecular fructosans are known; also the glucosidic linkage varies. The optical rotation may be positive (phlein) or negative (inulin). Fructosans are found dissolved in cell saps; the solution must generally be supersaturated as many of the pure substances are almost insoluble in water.

(*a*) *Inulin*

Inulin is found in considerable quantities in the tubers of composites, *e.g.*, *Dahlia*, *Helianthus tuberosus*, where it replaces starch as a food reserve. It is present in the cell sap but crystallizes from it very quickly if by chance the sap exudes from a damaged tuber. The solubility of crystallized, purified inulin is very small, amounting to only 0.01%, so that it must be in a state of considerable supersaturation in the cell sap. It can be isolated from an aqueous extract of the tubers by precipitation with alcohol or by freezing out. Alcohol-precipitated inulin is amorphous and water-soluble; it crystallizes slowly from aqueous solution—in this respect, it resembles amylose. The optical rotation: $[\alpha]_D^{20} = -40°$; the molecular weight of dahlia inulin is from 3000 to 5000. Whereas dahlia inulin always shows definite reducing power, that from Jerusalem artichokes (*Helianthus*)

[316] E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, **1948**, 1278; F. Smith, *J. Am. Chem. Soc.*, **70**, 3249 (1948).

is nonreducing, and on that account Schlubach postulates a cyclic structure for it which is not at all probable.

On the basis of methylation and hydrolysis Haworth and Learner[317] formulated inulin as a polyfructofuranose with 1,2-glucosidic linkages.

Fructosans of lower molecular weight are also found with inulin in the tubers.

(*b*) *Other Fructosans*

According to Haworth,[318] the fructosans of barley leaves consist of fructofuranose residues linked in 2,6 positions, the chain being relatively short, ten or twelve residues, and unbranched.

Phlein[319] obtained from the tuber-like root of *Phleum pratense* is very sparingly water-soluble (melting point 215°). On methylation and hydrolysis only 1,3,4-trimethylfructose can be isolated, which indicates that phlein is unbranched and the linkages are 2,6. Schlubach estimated the molecular weight cryoscopically as 2500, but the method cannot be applied to high polymers; it is probably very much higher, corresponding to a degree of polymerization of several hundred, as indicated by the very small reducing power (0.2%). The cyclic formula postulated by Schlubach, involving some 15 residues, is not very probable.

Asphodelin isolated many years ago from the bulbs of *Asphodelus* contains fructose and glucose residues in the ratio 6:1. As for inulin, the fructose residues appear to be linked in the 2,1 positions; it is nonreducing. Schlubach[320] considers the degree of polymerization to be only seven.

Asparagosin[321] from asparagus roots is also nonreducing but contains one end group for eight fructose residues, also having 1,2 linkages. Again, Schlubach postulates a cyclic structure but in this case with side chains; the cryoscopic molecular weight estimation is, however, not acceptable. Secalin[322] prepared from rye straw is considered by Schlubach to consist of a ring of three fructose residues, linked 2,6, carrying a fructose residue as side chain; the graminin obtained from rye meal should contain ten residues; again Schlubach and Koenig[323] ascribe a cyclic structure very much branched. Actually the molecular weight estimations on which their as-

[317] W. N. Haworth and A. Learner, *J. Chem. Soc.*, **1928**, 619.
[318] W. N. Haworth, E. L. Hirst, and R. R. Lyne, *Biochem. J.*, **31**, 786 (1937).
[319] H. H. Schlubach and O. K. Sinh, *Ann.*, **544**, 101 (1940).
[320] H. H. Schlubach and H. Lendzian, *Ann.*, **532**, 200 (1937).
[321] H. H. Schlubach and H. Böe, *Ann.*, **532**, 191 (1937).
[322] H. H. Schlubach and C. Bandmann, *Ann.*, **540**, 285 (1939).
[323] H. H. Schlubach and K. Koenig, *Ann.*, **514**, 182 (1934).

sumptions are based vary between 776 and 1537. Triticin from *Agropyrum* rhizome is also thought to be very much branched and is given a cyclic formula,[324] irisin likewise.[325] For sinistrin,[326] obtained from sea onion (*Scylla maritima*), both a branched cyclic structure and an open chain formulation have been considered, the reducing power of the latter having disappeared by reason of anhydride formation of the terminal fructose residue.

15. Other Soluble Reserve Polysaccharides

Many other poly- and oligosaccharides are found occurring as food reserves. For example, a carbohydrate isolated from unripe wheat has been shown[327] to contain arabinose, xylose, and galactose residues in the proportion 6:8:1. Ford and Peat conclude from the results of methylation and hydrolysis that arabinose residues occupy the terminal positions in the branches, and that the xylose residues form the branch points.

[324] H. H. Schlubach and H. Peitzner, *Ann.*, **530**, 120 (1937).
[325] H. H. Schlubach, H. Knoop, and M. Yin Liu, *Ann.*, **511**, 140 (1934).
[326] H. H. Schlubach and W. Loop, *Ann.*, **523**, 130 (1936).
[327] L. H. Ford and S. Peat, *J. Chem. Soc.*, **1941**, 856.

G. PROTEINS[1]

I. INTRODUCTION: FIBRILLAR AND GLOBULAR PROTEINS

By far the largest group of natural high polymers is that of the proteins. The variety of their properties and the infinite diversity of the functions they fulfil defy description. Silk, horn, keratin, and tendon all consist of protein; in the form of elastic fibers, protein displays rubberlike properties; it also forms the contractile elements of muscle; enzymes themselves are proteins—or at least contain proteins as essential constituents—and complex protein compounds are responsible for the transport of oxygen in the circulation. It would exceed the scope of this book to attempt a complete review of the proteins and their chemical and physical properties, as well as their biological functions. It will be necessary to limit this account to a short summary, considering briefly the constitution, size, shape, and changes in shape of protein molecules. In doing this, the data relative to the molecular morphology of the proteins will be examined, the forces exerted by individual groups of the proteins on each other and on other molecules will be discussed, and, finally, it will be seen to what extent the many remarkable properties of proteins—the peculiarities of their solubility, precipitation, mechanical strength, and contractility, their denaturation and their rôle in enzyme chemistry—can be reduced to general principles. An understanding of these properties will lead eventually to an understanding of the biological functions of these substances.

Our knowledge of the molecular morphology of proteins has been very considerably extended during recent years. Formerly the protein molecule was regarded as spherical; this assumption was supported, for example, by the results of Svedberg's work on hemoglobin.[2] Against this widely accepted view, Meyer and Mark[3] were the first to show that silk threads contained extended protein chains; they were able to interpret the lattice of silk (revealed by the x-ray diagrams of Herzog and Jancke) as a primary

[1] Detailed accounts: C. A. L. Schmidt, *The Chemistry of Amino Acids and Proteins*, Thomas, Springfield, Ill., 1938; *Addendum*, 1944. D. Jordan Lloyd and A. Shore, *The Chemistry of the Proteins*, 2nd ed., Churchill, London, 1938.

[2] T. Svedberg and R. Fåhraeus, *J. Am. Chem. Soc.*, **48**, 430 (1925).

[3] K. H. Meyer and H. Mark, *Ber.*, **61**, 1932 (1928).

valence chain lattice in which the length of a peptide unit in the chain amounted to 3.5 Å.

Later research showed[4,5] that in all fibers composed of protein, such as muscle and tendon fibers, stretched primary valence chains are arranged parallel to the fiber axis, and that under certain conditions these chains are able to change their shape and fold up. This result was amplified by the important work of Astbury and of Boehm and Weber. Since that time, it has been customary to distinguish, on the basis of the shape of the molecules, two classes of proteins: fibrillar and globular—to use Astbury's terminology.[6]

II. CONSTITUTION OF PROTEINS

1. Structural Units and Linkage

Protein molecules are composed of α-amino acids united by peptide linkages. The individual amino acid residues consist of the atoms $\overset{1}{\underset{\|}{C}}\!-\!\overset{2}{C}\!-\!N$—; this unit is linked with neighboring residues. The unit also contains a ketonic oxygen atom attached to carbon atom 1, and substituents attached to carbon atom 2 and to the nitrogen atom. These lateral groups determine the physical properties of the protein. Table I gives a list of the amino acids which have been isolated from proteins.

TABLE I. Structural Units of Proteins

Glycine	$CH_2(NH_2)COOH$
Alanine	$CH_3CH(NH_2)COOH$
Valine	$CH_3CH(CH_3)CH(NH_2)COOH$
Norvaline	$CH_3CH_2CH_2CH(NH_2)COOH$
Leucine	$CH_3CH(CH_3)CH_2CH(NH_2)COOH$
Isoleucine	$CH_3CH_2CH(CH_3)CH(NH_2)COOH$
Norleucine	$CH_3CH_2CH_2CH_2CH(NH_2)COOH$
Serine	$HOCH_2CH(NH_2)COOH$
Threonine	$CH_3CH(OH)CH(NH_2)COOH$
Cysteine	$HSCH_2CH(NH_2)COOH$
Cystine	$HOOCCH(NH_2)CH_2S—SCH_2CH(NH_2)COOH$
Lanthionine	$HOOCCH(NH_2)CH_2SCH_2CH(NH_2)COOH$

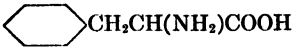
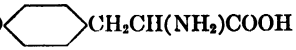
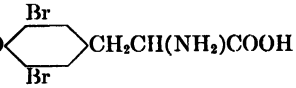
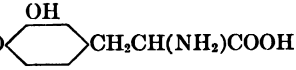
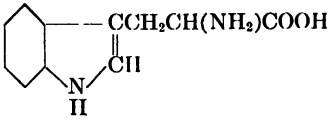
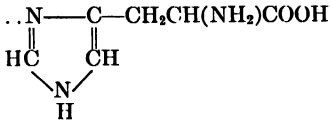
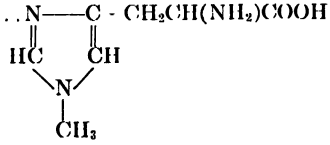
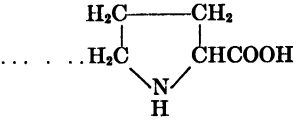
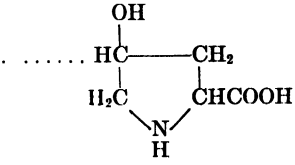
Table continued

[4] K. H. Meyer, *Biochem. Z.*, **214**, 253 (1929).

[5] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930.

[6] See F. Haurowitz who recommends the expressions "spheroproteins" and "linear proteins," *Kolloid-Z.*, **74**, 208 (1936).

TABLE I (*continued*)

Methionine	$CH_3SCH_2CH_2CH(NH_2)COOH$
Phenylalanine	
Tyrosine	
Dibromotyrosine and diiodotyrosine	
Dihydroxyphenylalanine	
Aspartic acid	$HOOCCH_2CH(NH_2)COOH$
Glutamic acid	$HOOCCH_2CH_2CH(NH_2)COOH$
β-Hydroxyglutamic acid	$HOOCCH(OH)CH(NH_2)COOH$
Lysine	$NH_2CH_2CH_2CH_2CH_2CH(NH_2)COOH$
Hydroxylysine	$NH_2CH_2CH(OH)CH_2CH_2CH(NH_2)COOH$
Arginine	$NH_2C(=NH)NHCH_2CH_2CH_2CH(NH_2)COOH$
Canavanine	$NH_2C(=NH)NHOCH_2CH_2CH(NH_2)COOH$
Tryptophane	
Histidine	
N-Methylhistidine	
Proline	
Hydroxyproline	

The natural amino acids isolated from proteins are all (with the exception of glycine) optically active. They all belong to the same stereometrical series, the L-series, whatever the rotation of the individual amino acids—represented by the symbol (−) or (+) may be—so that the natural proteins may be said to be L-proteins. An exception is provided by the specific immunizing capsule substance of anthrax bacilli, a polypeptide consisting exclusively of D(−)-glutamic acid units (Ivánovics and Bruckner).[7] Moreover, according to Hotchkiss and Dubos,[8] two polypeptides, gramicidin and tyrocidin, have been obtained from the culture medium of the soil bacterium, *Bacillus brevis*. These contain amino acids of the D series and because of their bactericidal action have aroused great interest. The interesting observation of Kögl and Erxleben[9] that the proteins of malignant tumors contain D-amino acid residues has not been confirmed.

2. Homogeneity of Proteins

(a) *Methods of Purification*

Pure proteins are only to be obtained after repeated reprecipitation and, if possible, recrystallization. Fractional precipitation with solutions of graded concentrations of ammonium sulfate or sodium chloride, as well as fractional precipitation with acetone or alcohol, is often used. All operations with labile proteins such as enzymes must be carried out at or below 0°C.; this preparation is also taken during the preparation of plasma proteins.[10] By controlling the *p*H, the salt content, the temperature, and the concentration of alcohol (the precipitating agent most commonly used), it has been possible to determine the optimal conditions under which many hitherto unknown protein components can be obtained in a pure and undamaged condition.

Less use has been made of two physical methods of purification: the use of the ultracentrifuge and of electrophoresis. The ultracentrifuge employed for this purpose has been developed (from the centrifuge constructed by Henriot and Huguenard,[11] driven by an air turbine) by Beams, Bauer,

[7] C. Ivánovics and V. Bruckner, *Z. physiol. Chem*, **247**, 28 (1937); *Naturwissenschaften*, **25**, 250 (1937).

[8] R. D. Hotchkiss and R. J. Dubos, *J. Biol. Chem*, **141**, 155, 171 (1941); *Advances in Enzymology*, **4**, 153 (1944).

[9] F. Kögl and H. Erxleben, *Z. physiol. Chem.*, **258**, 57 (1939).

[10] E. J. Cohn *et al.*, *J. Am. Chem. Soc.*, **62**, 3396 (1940); *J. Clin. Invest.*, **23**, 417 (1944).

[11] E. Henriot and E. Huguenard, *Compt. rend*, **180**, 1389 (1925).

Pickels, and Wyckoff.[12,13] The number of revolutions can be increased up to 60,000 per minute and in this way a centrifugal field of 250,000 to 300,000 times that of gravity is obtained. An electrically driven and much improved ultracentrifuge designed by Pickels is manufactured by the Specialized Instruments Corporation in Belmont, California.

The material is first centrifuged at a moderate velocity in order to remove coarser particles. It is then centrifuged at a higher speed, whereupon all molecules of molecular weight more than 30,000 are concentrated at the outer end of the celluloid container. By successive centrifuging with increasing centrifugal force, protein mixtures can be separated into components of different molecular weight. In this way, Wyckoff has purified virus proteins and several other proteins of high molecular weight, including a normal plant protein,[14] and a protein with a molecular weight of three to six million from healthy silk worms.[15]

Various forms of apparatus for the separation of protein fractions in quantity by electrophoresis have been described by Bennhold,[16] Theorell,[17] and Tiselius.[18] The principle involved is the same as that of analytical electrophoresis (chapter A, section I 3). The vessels containing the protein solution are considerably larger but an upper limit is set to their size since the heat generated by the current must be dissipated continually or the temperature will rise and convection occur. Electrophoresis is quite unsuitable, therefore, for the preparation for commercial purposes of large quantities of material in a pure condition.

(*b*) *Tests of Homogeneity*

An indication of the degree of homogeneity of a compound may be obtained from its ability to crystallize. It is true that certain proteins which separate out in a crystalline form frequently show no crystal pattern on x-ray analysis, but in such cases it is usually possible by repeated recrystallization to obtain preparations which yield good powder diagrams. Even

[12] J. W. Beams and E. G. Pickels, *Rev. Sci. Instruments*, **6**, 299 (1935). J. H. Bauer and E. G. Pickels, *J. Exptl. Med.*, **64**, 503 (1936). R. W. G. Wyckoff, *Science*, **84**, 291 (1936). R. W. G. Wyckoff and J. B. Lagsdin, *Rev. Sci. Instruments*, **8**, 74, 427 (1937).

[13] J. W. Beams, F. W. Linke, and P. Sommer, *Rev. Sci. Instruments*, **9**, 248 (1938).

[14] H. S. Loring, H. T. Osborne, and R. W. G. Wyckoff, *Proc. Soc. Exptl. Biol. Med.*, **38**, 239 (1938).

[15] R. W. Glaser and R. W. G. Wyckoff, *ibid.*, **37**, 503 (1937).

[16] H. Bennhold, *Kolloid-Z.*, **62**, 129 (1933).

[17] H. Theorell, *Biochem. Z.*, **223**, 93 (1930); **275**, 1, 11, 19, 30 (1934); in Abderhalden, *Handbuch der biologischen Arbeitsmethoden*, Vol. V, Part 10, page 109.

[18] A. Tiselius, *Kolloid-Z.*, **85**, 129 (1938); *Trans. Faraday Soc.*, **33**, 524 (1934).

undeniably crystalline preparations may be unhomogeneous, however, as experience with crystallized enzymes has shown. Cohn[19] and Northrop[20] have used the constancy of solubility of reprecipitated or recrystallized preparations, as well as the independence of the concentration at saturation of the quantity of substance present, as tests of homogeneity.

Still another method of testing is to determine the velocity of sedimentation in the ultracentrifuge. This method, however, can only indicate whether the particles present are of the same molecular weight, not whether they are chemically similar. Tiselius[18] has shown that an antibody which has the same molecular weight as normal serum globulin, sediments at the same rate although both proteins are by no means chemically identical (sect. VI 3). It is also conceivable that particles differing both in molecular weight and in form may possess the same velocity of sedimentation.

Electrophoretic investigation in the Tiselius apparatus is relatively dependable (see chap. A, sect. I 3), and if electrophoretic homogeneity is observed at different hydrogen ion concentrations the protein is very probably homogeneous.

The purity of biologically active proteins, *e.g.*, enzymes, may be checked in the following way. First of all, a quantitative method for the measurement of the activity is set up and the ratio of the activity to the nitrogen content (or ratio of the activity to the extinction due to tyrosine absorption band) is determined. If after recrystallization the same ratio is found for crystals and supernatant liquid, then the protein is homogenous; otherwise, the concentration of foreign proteins would be greater in the mother liquor than in the crystals, and the ratio would be smaller in the liquor than in the crystals.

(*c*) *Identification of Proteins*

Pure proteins of different origin may be identified by comparing their properties and by testing a mixture with respect to homogeneity. In addition a serological test is generally carried out as follows. One of the proteins is injected into an animal in order to provoke formation of an antibody (see Sect. VI 3) in the serum of the animal. If both proteins give equal positive reactions with the antibody they may be considered as being identical or at least very nearly related.

[19] E. J. Cohn, *Naturwissenschaften*, **20**, 663 (1932); *Ann. Rev. Biochem.*, **4**, 93 (1935).
[20] J. H. Northrop and R. M. Herriott, *Ann. Rev. Biochem.*, **7**, 37 (1938).

3. Molecular Weight of Proteins

(*a*) *Results Obtained with Ultracentrifuge*

The most reliable determinations of molecular weight are those made with the ultracentrifuge. In the hands of Svedberg and his school of workers, this method has provided values for the molecular weight of a large number of proteins. A summary of their results is given in Table II.

Svedberg[21] concluded from his measurements that all proteins are built up from units of molecular weight about 17,600. Recently this conclusion has been criticized, and it has been pointed out that the "Svedberg rule" is not confirmed by a detailed statistical analysis; there is some grouping of the lower molecular weights about the first two Svedberg numbers, but this does not amount to more than the occurrence of two gaps in an otherwise random distribution of log M.[22]

(*b*) *Determination of Molecular Weight from Measurements of Osmotic Pressure*[23]

Before the development of the ultracentrifuge technique, values for the molecular weights of proteins were obtainable only from osmotic pressure measurements. Early measurements usually overlooked the fact that high polymers cease to obey the van't Hoff law at relatively low concentrations, and that this anomalous behavior leads to apparent values for the molecular weight which are mostly too low. As with other polymers, the reduced osmotic pressure, P/c at zero concentrations, must be determined by extrapolation from the pressures measured at different concentrations.

Complications are introduced by the presence of ions, when (as is usually the case) the protein must be brought into solution by the addition of salts, acids, or alkalis. In this case, the distribution of ions (according to the Donnan theory of membrane equilibrium) inside and outside the membrane must be determined, and the pressure due to the difference in ionic concentrations must be subtracted from the measured osmotic pressure, in order to obtain the pressure due to the protein particles. The pressure due to the Donnan effect may be reduced to a minimum by working in the presence of excess salt.

[21] T. Svedberg, *Proc. Roy Soc.*, **B127**, 1 (1939).

[22] I. P. Johnston, H. C. Longuet-Higgins, and A. G. Ogston, *Trans. Faraday Soc.*, **41**, 588 (1945). H. B. Bull, *Advances in Enzymology*, **1**, 1 (1941).

[23] For a review of the material available up to 1933, see W. Pauli and E. Valkó *Kolloidchemie der Eiweisskörper*. 2nd ed., Steinkopff, Dresden, 1933.

TABLE II

MOLECULAR CONSTANTS OF PROTEINS[a]

V_{20} = partial specific volume of the protein. (The values in parentheses are assumed values based on experimental determinations on closely related proteins.)

s_{20} = sedimentation constant = $(dx/dt)(1/\omega^2 x)$, in Svedberg units (1 S = 1×10^{-13} c.g.s. units) reduced to water at 20°C.

D_{20} = diffusion constant in units of 10^{-7} reduced to water at 20°C.

M_s = molecular weight computed from sedimentation velocity and diffusion measurements according to the formula:

$$M_s = RTs_{20}/D_{20}(1 - V_{20}\rho^0)$$

f/f_0 = ratio of experimentally determined molar frictional constant, $f = M(1 - V\rho^0)/s_{20}$, to molar frictional constant calculated for an unsolvated spherical particle of the same mass, $f_0 = 6\pi\eta^0 N (3MV/4\pi N)^{1/3}$.

$du/dp\mathrm{H}_0$ = slope of mobility curve in the vicinity of the isoelectric point.

R = gas constant = 8.313×10^7 ergs per degree Centigrade

N = Avogadro constant = 6.06×10^{23}.

T = absolute temperature.

ρ^0 = density of water at 20°C. = 0.9982.

ρ = density of solution.

η^0 = viscosity of water at 20°C. = 0.010 poise.

x = distance from axis of rotation.

dx/dt = sedimentation velocity.

ω = angular velocity of rotating solution.

Values for M_s beginning with 1 are given to three significant figures; values beginning with 2, 3 or 4 are rounded off to 0 or 5 in the third figure; and values beginning with 5 or greater are given to two significant figures.

Name	V_{20}	s_{20}	D_{20}	M_s	f/f_0	Isoelectric point[b]	$\frac{du}{dp\mathrm{H}_0} \times 10^5$	References
Cytochrome c (cow heart)	0.702	1.83	12.0	12,400	1.17	9.5	—	40,68
Ribonuclease (pig)	0.707	2.00	13.1	12,600	1.07	7.8*	2 2	47
Trypsin (pig)	(0 751)	1 69	10.95	15,000	1.2	—	—	2
Lysozyme (egg yolk)	0 75)	1.9	11.2	16,400	1.12	10.5–11.0*	—	1
Metmyoglobin (human)	0 741	2.0	11.4	16,400	1.11	—	—	38
Myoglobin (beef heart)	0.741	2.04	11.3	16,900	1.11	7.0	7	43,57,68
Bacillus phlei protein	0 748	1 8	10.2	17,000	1.22	—	—	50
Erythrocruorin (*Lampetra*)	0 751	1 87	10.7	17,100	1.16	5 6	3.2	37,43,57,61
Lactalbumin	(0 751)	1 9	10.6	17,400	1.16	5.12	6.7	24,43

Name	V_{20}	s_{20}	D_{20}	M_r	f/f_0	Isoelectric point[b]	$\frac{du}{dpH} \times 10^5$	References
Adrenocorticotropic hormone (sheep)	(0.75)	2 08	10 4	20,000	1 1	—	—	6
Gliadin	0 724	2 1	6 72	27,500	1 59	—	—	13,26,27
Hordein	(0 729)	2 0	6.5	27,500	1 64	—	—	46
Barley globulin α	0 72	2 5	7 4	29,000	1 28	5 00	2 7	9,45
Crotoxin	0 704	3 14	8 6	30,000	1 22	—	—	15
Protein from								
Posterior lobe, ox pituitary	(0 749)	2 7	8 5	30,500	1 20	4 8*	10	76
Human tuberculosis bacillus	0 70	3 3	8 2	32,000	1 25	4 5*	—	50
Pepsin	(0 750)	3 3	9 0	35,500	1 08	—	—	41
Lactoglobulin	0 751	3 12	7 3	41,500	1.26	5 20	11 9	34,43
Concanavalin B	0 73	3 5	7 4	42,000	1 25	—	—	55
Plaquealbumin (egg albumin)	(0 749)	3 44	7 6	43,500	1.19	—	—	38
Ovalbumin	0.749	3 55$_0$	7 8	44,000	1 16	4 55	10 4	27,37,73
Peroxidase (horse-radish)	0 69	3 85	6 8	44,500	1.36	—	—	69
α-Amylase (pig pancreas)	0 70	4 5	8 0	45,000	1 14	—	—	8
Insulin (ox)	(0 749)	3 5$_5$	7 53	46,000	1 18	—	—	30
Fetuin								
From cow fetus	0.69$_2$	3.2$_9$	5.0	48,500	1.80	3 5	1 1	36
From calf	0 71$_4$	3 0$_8$	5.5	51,000	1.60	—	—	36
Zein	0 776	1.9	4.0	51,000	2.1	—	—	13,77
Malt amylase (α and β amylase)	0.69	4 52	6.53	54,000	1.33	5 75*	—	10
Enolase (yeast)	0.735	5 6	8.1	64,000	1 00	—	—	2
Chymotrypsin (pig)	(0 751)	2.7$_4$	4 1$_4$	64,000	1.9	—	—	2
Hemoglobin (horse)	0 749	4 41	6.3	68,000	1.24	6.92	7.2	37,39,63,74
Serum albumin								
Horse	0 748	4 46	6.1	70,000	1.27	4.80	9.1	23,37,65
Human	0.73$_6$	4.6$_7$	5 9	72,000	1.30	—	—	35
Ricin	(0 75)	4.8	(6.0)	77,000	1 2	5.4–5 5*	—	20
Yellow enzyme (yeast)	0.731	5 76	6 3	82,000	1.17	5.22	6.4	25

(*Table continued*)

TABLE II (*continued*)

Name	V_{20}	s_{20}	D_{20}	M_s	f/f_0	Isoelectric point[b]	$\frac{du}{d\rho H_0} \times 10^5$	References
Iron-binding protein (pig serum)	(0.725)	5 8	5.8	88,000	1.25	~4.4*	—	29
Luteinizing hormone (ICSH) from pig	(0 749)	5 4	5 9	88,000	1.2	7 45*	—	51
Diphtheria antitoxin (purified by trypsin) (ox plasma)	(0.749)	5.5	5.8	92,000	1.22	7.0*	—	48
Lactoperoxidase	0.764	5.3_7	$5\ 9_5$	93,000	1.18	7.7*	—	71,72
Concanavalin A	0.73	6.0	5 6	96,000	1.26	—	—	55
Lipoxidase (soybean)	0.750	5.62	5.59	9 000	1.24	~5 4*	—	38,70
Canavalin	0.73	6.4	5 1	113,000	1.31	—	—	55
Myogen A (rabbit muscle)	0.735	7.86	4.78	150,000	1.26	—	—	14
Serum globulin γ (human)	0.71_5	7.1_2	4.0	153,000	1.51	—	—	35
Antipneumococcus serum globulin								
Rabbit	(0.745)	6.5	3.9	158,000	1.52	5 85*	—	19,75
Man	(0.745)	7.4	3.60	195,000	1.53	—	—	19
Vicilin	0 752	8.1_0	4.3	186,000	1.31	—	—	9
Barley globulin γ	0.72	8.7	3 6	210,000	—	5.70	3 2	9,45
Phosphatase (prostata)	(0.749)	4.0_1	1.8_8	212,000	2.9	—	—	2
LS antigen of *Vaccinia* (from infected rabbits)	0 728	6.3	2 48	220,000	2.1	4.8*	—	52
Catalase	0.73	11.3	4 1	250,000	1.25	—	—	53
Phycocyan (*Ceranium*)	(0.746)	11.4	4.05	270,000	1 22	4.85	10.2	11,37,56,74
Visual purple (frog)	(0.752)	11.1	4	270,000	1 2	—	—	17
Phycoerythrin (*Cerium*)	0 746	12 0	4 00	290,000	1.2	4.25	14.2	11,62,73,74
Excelsin	0.743	13.3	4.26	295,000	1.13	—	—	43,62,66
Legumin	0.73_5	12 6	3.5	330,000	1.33	—	—	9
Amandin	0.746	12.5	3.45	346,000	1.32	—	—	3,66
Serum globulin (*Lampetra*)	(0.745)	12	3.2	360,000	1.4	—	—	58
Edestin	0.744	12.8	3.18	381,000	1.39	—	—	3,67

Name	V_{20}	s_{20}	D_{20}	M_s	f/f_0	Isoelectric point[b]	$\frac{du}{dpH_0} \times 10^5$	References
Phosphorylase b (rabbit muscle)	(0.74)	13.7	3.3	385,000	1.33	~5.8*	—	16,32
Hemocyanin (*Palinurus*)	(0.740)	16.4	3.4	450,000	1.23	—	—	12,43,62
Apoferritin (horse)	0.747	17.6	3.6_1	465,000	1.14	—	—	49
Urease (jack bean)	0.73	18.6	3.46	480,000	1.19	—	—	54
Thyroglobulin (pig)	0.72	19.2	2.65	630,000	1.43	4.58	11	18
Hemocyanin (*Homarus*)	(0.740)	22.6	2.78	760,000	1.27	4.95	18	12,37,43
Botulinus A toxin	$0\ 75_5$	17.3	2.10	810,000	1.6	—	—	22,44
Hemocyanin (*Nephrops*)	(0 740)	24.5	2.79	820,000	1.23	4.64	13	12,37,43,57
Antipneumococcus serum globulin								
Cow	(0.715)	18.1	1.69	910,000	1.98	4.8*	—	19,21,75
Horse	0.715	19.3	1.80	910,000	1.86	4.4*	—	19,75
Pig	(0.715)	18.0	1.64	930,000	2.02	5.1*	—	19,75
Erythrocruorin (*Planorbis*)	0 745	33.7	1.96	1,630,000	1.30	4.77	10.6	27,33,61,64
Nucleohistone from calf thymus	0 658	31	0.93	2,300,000	2.7	—	—	7
Hemocyanin								
Eledone	(0.740)	49.1	1 64	2,800,000	1.39	4.6	—	12,37,43,62
Octopus	0.740	49.3	1.65	2,8[illegible],000	1.38	—	—	12,42,59
Erythrocruorin (*Lumbricus*)	0.740	60.9	1 81	3,150,000	1 21	5 28	12.6	37,43,57,60
Hemocyanin								
Rossia	(0.740)	56 2	1 58	3,300,000	1 36	—	—	43,62
Paludina vivipara	(0.738)	102 5	1.09	8,700,000	1.43	4 71	10.8	5,33
Helix pomatia	0.738	103.0	1.07	8,900,000	1 45	5 05	8 1	4,33,73
Tobacco mosaic virus	0 73	185	0.53	31,400,000	1 93	—	—	28
Rabbit papilloma virus	0.754	280	0.59	47,000,000	1 5	—	—	31

* These determinations were carried out under other conditions than specified in footnote *b*.

[a] Data courtesy K. O. Pedersen, Institute of Physical Chemistry, Uppsala University, Sweden. Revised to September, 1948.

[b] Except when otherwise stated, the values refer to experiments carried out at 20° C. in acetate or phosphate buffers with ionic strength of 0.02.

(*Table continued*)

TABLE II (*continued*)

(1) Alderton, G., Ward, W. H., and Fevold, H. L., *J. Biol. Chem.*, **157,** 43 (1945).
(2) Bergold, G., *Z. Naturforsch.*, **1,** 100 (1946).
(3) Bevilacqua, E. M., Bevilacqua, E. B., Bender, M. M., and Williams, J. W., *Ann. N. Y. Acad. Sci.*, **46,** 309 (1945).
(4) Brohult, S., *Nova Acta Regiae Soc. Sci. Upsaliensis IV*, **12,** No. 4 (1940).
(5) Brohult, S., *J. Phys. & Colloid Chem.*, **51,** 206 (1947).
(6) Burtner, E , *J. Am. Chem. Soc.*, **65,** 1238 (1943).
(7) Carter, R. O., *ibid.*, **63,** 1960 (1941).
(8) Danielsson, C.-E , *Nature*, **160,** 899 (1947).
(9) Danielsson, C.-E., *unpublished data.*
(10) Danielsson, C.-E., and Sandegren, E., *Acta Chem. Scand.*, **1,** 917 (1947), and *unpublished data.*
(11) Eriksson-Quensel, I.-B., *Biochem. J.*, **32,** 585 (1938).
(12) Eriksson-Quensel, I.-B., and Svedberg, T., *Biol. Bull.*, **71,** 498 (1936).
(13) Foster, J. F., and French, D., *J. Am. Chem. Soc.*, **67,** 687 (1945).
(14) Gralén, N., *Biochem. J.*, **33,** 1342 (1939).
(15) Gralén, N., and Svedberg, T., *ibid.*, **32,** 1375 (1938).
(16) Green, A. A., *J. Biol. Chem.*, **158,** 315 (1945).
(17) Hecht, S., and Pickels, E. G., *Proc. Natl. Acad. Sci. U. S.*, **24,** 172 (1938).
(18) Heidelberger, M., and Pedersen, K. O., *J. Gen. Physiol.*, **19,** 95 (1935).
(19) Kabat, E. A., *J. Exptl. Med.*, **69,** 103 (1939).
(20) Kabat, E. A., Heidelberger, M., and Bezer, A. E., *J. Biol. Chem.*, **168,** 629 (1947).
(21) Kabat, E. A., and Pedersen, K. O., *Science*, **87,** 372 (1938).
(22) Kegeles, G., *J. Am Chem Soc.*, **68** 1670 (1946)
(23) Kekwick, R. A., *Biochem. J.*, **32,** 552 (1938).
(24) Kekwick, R. A., see Svedberg and Pedersen, *The Ultracentrifuge*, 1940.
(25) Kekwick, R. A., and Pedersen, K. O., *Biochem. J.*, **30,** 2201 (1936).
(26) Krejci, L., and Svedberg, T., *J. Am. Chem. Soc.*, **57,** 946 (1935).
(27) Lamm, O., and Polson, A., *Biochem. J.*, **30,** 528 (1936).
(28) Lauffer, M. A., *J. Am. Chem. Soc.*, **66,** 1188 (1944).
(29) Laurell, C.-B., and Ingelman, B., *Acta Chem. Scand.*, **1,** 770 (1947).
(30) Miller, G. L., and Andersson, K. J. I., *J. Biol. Chem.*, **144,** 459 (1942).

(31) Neurath, H., Cooper, G. R., Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W., *J. Biol. Chem.*, **140,** 293 (1941).
(32) Oncley, J. L., *J. Biol. Chem.*, **151,** 27 (1943).
(33) Pedersen, K. O., *Kolloid-Z.*, **63,** 268 (1933).
(34) Pedersen, K. O., *Biochem. J.*, **30,** 961 (1936).
(35) Pedersen, K. O., "Ultracentrifugal Studies on Serum and Serum Fractions." *Dissertation,* Uppsala, 1945.
(36) Pedersen, K. O., *J. Phys. & Colloid Chem.*, **51,** 164 (1947).
(37) Pedersen, K. O., see Svedberg and Pedersen, *The Ultracentrifuge,* 1940.
(38) Pedersen, K. O., *unpublished data.*
(39) Pedersen, K. O., and Andersson, K. J. I., see Svedberg and Pedersen, *The Ultracentrifuge,* 1940.
(40) Pedersen, K. O., and Andersson, K. J. I., *unpublished data.*
(41) Philpot, J. St. L., *Biochem. J.*, **29,** 2458 (1945). Philpot, J. St. L., and Eriksson-Quensel, I.-B., *Nature,* **132,** 932 (1933). Eriksson-Quensel, I.-B., *unpublished data,* see Svedberg and Pedersen, *The Ultracentrifuge,* 1940.
(42) Polson, A., *Nature,* **137,** 740 (1936).
(43) Polson, A., *Kolloid-Z.*, **87,** 149 (1939).
(44) Putnam, F. W., Lamanna, C., and Sharp, D. G., *J. Biol. Chem.*, **165,** 735 (1946).
(45) Quensel, O., "Untersuchungen über die Gerstenglobuline." *Dissertation,* Uppsala, 1942.
(46) Quensel, O., and Svedberg, T., *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **22,** 441 (1938).
(47) Rothen, A., *J. Gen. Physiol.*, **24,** 203 (1940/41).
(48) Rothen, A., *ibid.*, **25,** 487 (1942).
(49) Rothen, A., *J. Biol. Chem.*, **152,** 679 (1944).
(50) Seibert, F. B., Pedersen, K. O., and Tiselius, A., *J. Exptl. Med.*, **68,** 413 (1938).
(51) Shedlovsky, T., Rothen, A., Greep, R. O., van Dyke, H. B., and Chow, B. F., *Science,* **92,** 178 (1940).
(52) Shedlovsky, T., Rothen, A., and Smadel, J. E., *J. Exptl. Med.*, **77,** 155 (1943).
(53) Sumner, J. B., and Gralén, N., *J. Biol. Chem* , **125,** 33 (1938).
(54) Sumner, J. B., Gralén, N., and Eriksson-Quensel, I.-B., *ibid.*, **125,** 37 (1938).
(55) Sumner, J. B., Gralén, N., and Eriksson-Quensel, I.-B., *ibid.*, **125,** 45 (1938).
(56) Svedberg, T., *Chem. Revs.*, **20,** 81 (1937).
(57) Svedberg, T., *Nature,* **139,** 1051 (1937).
(58) Svedberg, T., and Andersson, K. J. I., *ibid.*, **142,** 147 (1938), and *unpublished data.*
(59) Svedberg, T., and Eriksson, I.-B., *J. Am. Chem. Soc.*, **54,** 4730 (1932).

(Table continued)

TABLE II (*concluded*)

(60) Svedberg, T., and Eriksson, I.-B., *ibid.*, **55**, 2834 (1933).
(61) Svedberg, T., and Eriksson-Quensel, I.-B., *ibid.* **56**, 1700 (1934).
(62) Svedberg, T., and Eriksson-Quensel, I.-B., *Tabulae Biologicae*, **XI**, 315 (1935/36).
(63) Svedberg, T., and Fåhraeus, R., *J. Am. Chem. Soc.*, **48**, 430 (1926).
(64) Svedberg, T., and Hedenius, A., *Biol. Bull.*, **66**, 191 (1934).
(65) Svedberg, T., and Sjögren, B., *J. Am. Chem. Soc.*, **50**, 3318 (1928).
(66) Svedberg, T., and Sjogren, B., *ibid.*, **52**, 279 (1930).
(67) Svedberg, T., and Stamm, A. J., *ibid.*, **51**, 2170 (1929).
(68) Theorell, H., *Biochem. Z.*, **252**, 1 (1932); **285**, 207 (1936).
(69) Theorell, H., *Arkiv Kemi Mineral Geol.*, **B15**, No. 24 (1942).
(70) Theorell, H., Holman, R. T., and Åkeson, Å., *Acta Chem. Scand.*, **1**, 575 (1947).
(71) Theorell, H., and Paul, K. G., *Arkiv Kemi Mineral. Geol.*, **A18**, No. 12 (1944).
(72) Theorell, H., and Pedersen, K. O., in *The Svedberg*, 1884–1944. Uppsala, 1944, p. 523.
(73) Tiselius, A., "The Moving Boundary Method of Studying the Electrophoresis of Proteins," *Dissertation, Nova Acta Regiae Soc. Sci. Upsaliensis IV*, **7**, No. 4 (1930).
(74) Tiselius, A., and Gross, D., *Kolloid-Z.*, **66** 11 (1934).
(75) Tiselius, A., and Kabat, E. A., *J. Exptl. Med.*, **69**, 119 (1939).
(76) van Dyke, H. B., Chow, B. F., Greep, R. O., and Rothen, A., *J. Pharmacol. Exptl. Therap.*, **74**, 190 (1942).
(77) Watson, C. C., Arrhenius, S., and Williams, J. W., *Nature*, **137**, 322 (1936), and *unpublished data*.

The first measurements taking this factor into consideration were those of Sørensen[24]; later Adair[25] undertook a series of careful measurements and obtained values subsequently confirmed by Svedberg. Measurements of the molecular weight of globin (the protein component of hemoglobin) by Roche, Roche, Adair, and Adair[26] showed that the molecular weight increases with increasing concentration from 94,500 to 155,000. The phenomenon is due to a reversible association equilibrium such as has been observed by Sørensen in a number of other proteins.

Using solvents other than water, measurements have been made by Roche and Adair,[26] Burke and Greenberg,[27] and Duclaux and Dobry.[28] The extent of divergence from the van't Hoff relation with increasing concentration varies with the nature of the solvents. In solutions of urea, for example, proteins dissociate as a rule into smaller particles than in water.

(*c*) *Nature of Protein Particles*

Many solvents, as well as changes in *p*H or addition of other substances to the solution, produce a reversible dissociation into smaller units. Hemoglobin of molecular weight 67,000 dissociates, for example, in 6.6 *M* urea solution, into particles of molecular weight 34,000 (Burke and Greenberg); egg albumin of molecular weight 40,000 also dissociates into particles of half this molecular weight. Such reversible dissociation (which has been demonstrated in other ways) shows that the osmotically active particles in protein solutions are not identical with the groups of atoms united by primary valences. The molecular weight determined from osmotic pressure measurements is not identical with the "chemical" molecular weight.

The particles of protein in solution consist of several chemical molecules united to form a relatively stable associated supermolecule. Sørensen, who reached this point of view as a result of years of study, described such particles as systems of reversibly dissociating components.[29] Meyer and Mark used the term "protein micelles." In the case of protein solutions it is customary to use the term molecular weight in the sense of particle weight or micellar weight, not in the sense of the chemical molecular weight.

[24] S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg*, **12** (1917); *Z. physiol. Chem.*, **106**, 1 (1919).

[25] G. S. Adair, *Proc. Roy. Soc. London*, **108**, 627 (1925); **109**, 292 (1925); **120**, 573 (1928); **126**, 16 (1929); *Trans. Faraday Soc.*, **23**, 536 (1927); **31**, 98 (1935).

[26] J. Roche, A. Roche, G. S. Adair, and M. E. Adair, *Biochem. J.*, **26**, 1811 (1932).

[27] N. F. Burk and D. M. Greenberg, *J. Biol. Chem.*, **87**, 197 (1930).

[28] J. Duclaux and A. Dobry, *Compt. rend. trav. lab. Carlsberg*, **22**, 155 (1938).

[29] S. P. L. Sørensen, *Kolloid-Z.*, **53**, 102, 170, 306 (1930); *Compt. rend. trav. lab. Carlsberg*, **18**, No. 5 (1930).

(*d*) *Determination of Molecular Weight from Analyses of Structural Units*

Since each of the amino acids present must occur at least once in each molecule, it is possible to calculate the minimal molecular weight from the amount of the particular acid which is present in lowest concentration. If, for example, horse hemoglobin contains 1 mole of cystein in 34,000 g. of hemoglobin, it follows that the molecular weight is 34,000 or $n \times 34{,}000$.

It is clear that this method can only be used for homogeneous proteins, and not in the case of mixtures. Unfortunately, this is not always taken into consideration, even at the present time.

4. Determination of Constitution

(*a*) *Analysis of Amino Acid Content*[30]

Tyrosine and tryptophane may be determined by means of their extinction in the ultraviolet, both exhibiting characteristic absorption bands at 295 and 277 mμ, respectively. All other amino acids have to be estimated after hydrolysis of the protein. Complete hydrolysis is most frequently carried out in an acid medium, but alkaline and enzymatic breakdown have been used as well. The individual amino acids are then isolated from the mixture. Until recently the amino acids were usually converted into methyl esters and submitted to fractional distillation; fractionation by means of partition between water and butanol (Dakin's method) was also widely used. The fractions thus obtained were further separated into groups of acids or even pure acids by precipitating with specific reagents or by fractional crystallization.

Today separations are being carried out through chromatographic adsorption. Fuller's earth, aluminum oxide, and synthetic ion exchangers have been employed as adsorbents. The separation through several columns in series has been described by several authors, *e.g.*, Tiselius.[31] The aqueous solution of the amino acid mixture flows first through a column of activated charcoal (adsorption of aromatic amino acids), then through Wofatit C (basic amino acids), then through Amberlite IR-4 (glutamic acid and the like), and finally through Wofatit KS (everything that remains).

A very useful method is the *partition chromatography* of Gordon,

[30] See A. J. P. Martin, *Advances in Protein Chem.*, **2**, 1 (1945).

[31] A. Tiselius, B. Drake, and L. Wagdahl, *Experientia*, **3**, 21 (1947).

Martin, and Synge.[32] In this method an inert powder, *e.g.*, moist silica gel or moist starch grains, is used, not as an adsorbent, but as a carrier for the aqueous solution of the amino acids (converted previously into the acetyl amino acids). A small amount of the solution is put into the column; chloroform is then run through it. Partition equilibrium is rapidly established at the interface chloroform–water; the acetyl amino acids, readily soluble in chloroform, migrate most rapidly with the latter and arrive at the base of the column before the less soluble acids.

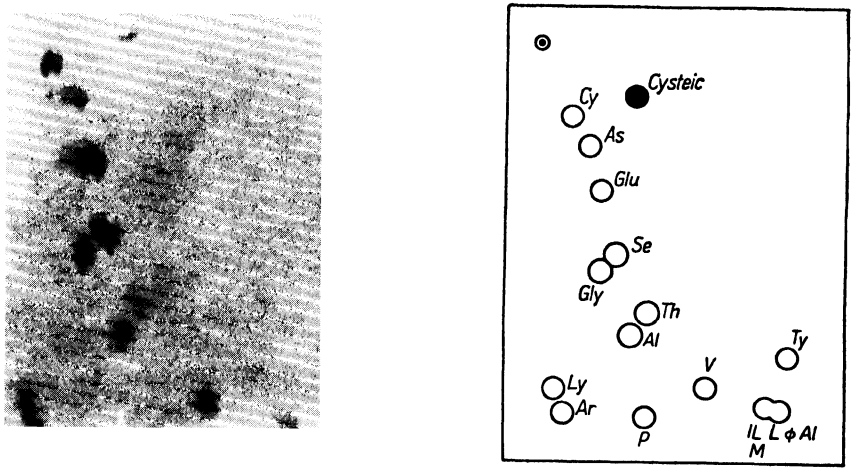


Fig. 1. Two-dimensional paper chromatogram of a wool hydrolyzate and key diagram (Cysteic, cysteic acid; Cy, cystine; As, aspartic acid; Glu, glutamic acid; Se, serine; Gly, glycine; Th, threonine; Al, alanine; V, valine; Ty, tyrosine; Ly, lysine; Ar, arginine; P, proline; IL, isoleucine; L, leucine; φ Al, phenylalanine; M, methionine). (Martin and Synge).

An ingenious variation of partition chromatography has been described by Consden *et al.*[33] A rectangular strip of filter paper is used as a carrier. One margin dips into a trough containing collidine; the strip thus hangs down from the trough. A drop of the aqueous solution containing a few tenths of a milligram of the mixture of amino acids is put on the upper left corner of the strip and the whole is left for two to three days in a closed chamber at constant temperature. Collidine flows slowly down the strip. The amino acids migrate with it, their rate of migration being determined by the respective partition coefficients between collidine and water.

[32] A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, **37**, 79, 86, 92, 93 (1943); **38**, 65 (1944).

[33] R. Consden, A. H. Gordon, and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

Finally, all the amino acids are found on a line parallel and near to the left edge of the strip, those which move most rapidly being located at the lower end. The paper is dried and hung with the edge which formerly was the left one hanging from the trough, which is filled this time with water-saturated phenol. After two to three days the paper is dried and sprayed with ninhydrin solution, dried again, and heated. The individual amino acids thus appear as colored spots. By comparison with chromatograms of amino acid mixtures of known composition the spots may be identified. Figure 1 shows a chromatogram of a wool hydrolyzate.

Moore and Stein[34] have elaborated a very accurate method for the determination of the amino acid content of a few milligrams of protein hydrolyzate. The mixture is fractionated on starch columns into several hundred fractions, and each fraction is estimated for amino acid content by a ninhydrin colorimetric method. Integration of the values obtained gives, after standard corrections, the amount of each amino acid in the mixture.

Electrophoresis of amino acids on paper strips moistened with buffer allows a quantitative separation in basic neutral and acidic components.

(*b*) *Determination of Order of Arrangement of Amino Acid Residues*

How are the amino acids arranged in the protein chain? This question may be answered in part by the investigation of products of incomplete hydrolysis, *e.g.*, di-, tri- and tetrapeptides, etc. These may be separated and identified by chromatographic methods described above. A very promising field for constitutional studies has been opened by the isolation of pure enzymes and the recognition of their specificity. Each proteolytic enzyme can hydrolyze only certain definite groups. An example is provided by the behavior of chymotrypsin toward various synthetic peptides (Bergmann and Fruton[35]). This enzyme does not attack either benzoyltyrosinamide or benzoyltyrosylglycine, but rapidly hydrolyzes benzoyl-L-tyrosyl/glycinamide at the point indicated by the stroke, while the optical antipode is not attacked. One may conclude then that a protein which is attacked by chymotrypsin contains a similar or indeed identical grouping of amino acids. Experiments performed by Calvery[36] also appear to indicate that

[34] S. Moore and W. H. Stein, *J. Biol. Chem.*, **178**, 53(1948).

[35] M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 405 (1937); **127**, 643 (1939). See also M. Bergmann and C. Niemann, *ibid.*, **118**, 781 (1937); J. S. Fruton and M. Bergmann, *ibid.*, **127**, 627 (1939).

each enzyme can hydrolyze only linkages between certain definite amino acids.

Certain enzymes, *e.g.*, carboxypolypeptidase attack the protein chain from one end, splitting off one amino acid residue with free carboxyl groups after the other. This phenomenon has only been used for constitutional analysis in a few cases, *e.g.*, insulin, the end group of which has been determined by Lens.[36a] On the other hand, the nature of the amino acid located at the noncarboxylic end of the chain may be determined in the following way.[36b] The protein is treated with dinitrofluorobenzene; the free amino groups are thus transformed into dinitrophenyl amino groups which resist subsequent hydrolysis of the protein. The labelled amino acid can be identified by chromatography.

(*c*) *Results of Constitutional Analysis*

Most natural proteins contain many (or most) of the amino acids listed in Table I. It has not as yet been possible, however, in any single case to give a complete account of the constitution of a protein and to determine the sequence of the units in the chain. The most promising material for such constitutional studies would appear to be natural polypeptides of low molecular weight, such as those present in the strongly basic protamines occurring in the testes of fish; the molecular weight of these amounts to 2000–4000 and they do not show the characteristics of typical high polymers. Even in the case of clupein (the protamine from the herring), however, no satisfactory formula has as yet been proposed. Kossel suggested many years ago that stoichiometrical relations exist between the structural units of clupein. Waldschmidt-Leitz[37] as a result of a study of the breakdown products set free by various enzymes, suggests the formula:

MAAMAAMAAPAAMAA...

where M = monoamino acid, A = arginine, and P = proline. According to Bergmann[38] this is still incomplete. Clupein contains, according to this worker, 1 alanine group, 1 serine group, 2 valine groups, 4 proline groups, and 16 arginine groups. These groups are supposed to recur periodically (according to his theory, which will be considered shortly), so that after every 2 arginine units a different unit follows:

[36] H. O. Calvery, *ibid.*, **102**, 73 (1933).
[36a] J. Lens, *Biochim. et Biophys. Acta*, **3**, 367 (1949).
[36b] F. Sanger, *Biochem. J.*, **39**, 507 (1945).
[37] E. Waldschmidt-Leitz and E. Kofranyi, *Z. physiol. Chem.*, **236**, 181 (1935).
[38] M. Bergmann, *Ann. Rev. Biochem.*, **6**, 186 (1937); **7**, 111 (1938).

AAPAAVAASAA...

On the other hand, Felix and Mager[39] propose the constitution: 2 alanine + serine, 3 valine + unknown structural unit, 4 proline + hydroxyproline, 22 arginine. They suggest the following schematic formula, in which AA represents arginylarginine, Al represents alanine, S = serine, P = proline, Op = hydroxyproline, and V = valine. The terminal groups of P and A are indicated:

HN < P—AA—AA—V—S—AA—AA—Op—V—AA—AA—P—Al—

AA—AA—P—S—AA—AA—V—Al—AA—COOH

This formula assumes that the structural units of clupein are tripeptides, which consist of one molecule of arginylarginine and one molecule of a monoamino acid, and in which the monoamino acid forms alternately the carboxyl and the amino end. By partial hydrolysis of mugelin, a protamine closely related to clupein, Hirohata[40] has obtained the compound valylvaline anhydride, from which it follows that two valine units are neighbors—a fact which finds expression neither in the formula of Bergmann nor in that of Felix.

Stoichiometrical relationships have also been found in more complex proteins; the molecule of egg albumin contains in all 288 units: 36 of glutamic acid, 16 of aspartic acid, 12 of methionine, 12 of lysine, 12 of arginine, 8 of tyrosine, 4 of histidine, and 4 of cysteine (Bergmann and Niemann[41]). These authors claim to have discovered similar stoichiometrical relationships in other proteins, and they regard these figures as exemplifying a rule of general validity in protein structure, which they formulate in the following way. The number, z, of units of each amino acid present in a protein molecule is equal to $2n \times 3m$ (where $n, m = 0, 1, 2, 3, 4, 5\ldots$). In explanation of this, it is assumed that identical units are spaced at identical distances from each other. If one eighth of all the units are glutamic acid units, it is supposed that every eighth group in the chain is a glutamic acid unit. This interesting assumption is not reconcilable with certain facts which will be discussed in connection with silk fibroin, nor does it agree with the observations on the composition of clupein and mugelin quoted above. Furthermore, the constitution of certain tripeptides obtained

[39] K. Felix and A. Mager, *Z. physiol. Chem.*, **249**, 111 (1937). See also R. J. Block and D. Balling, *Arch. Biochem.*, **6**, 419 (1945), on salmin.

[40] R. Hirohata, *J. Biochem. Japan*, **25**, 519 (1937).

[41] M. Bergmann and C. Niemann, *J. Biol. Chem.*, **118**, 301 (1937); *Science* **86**, 187 (1937).

from wool, gelatin, and edestin by acid hydrolysis does not agree with the theory of Bergmann and Niemann.[42] A whole series of criticisms of this theory have been brought forward by Bull,[43] and it seems probable that it will have to be abandoned.

5. Cross-Linked Proteins

In contrast with those proteins that are soluble in suitable solvents and consist of molecules of determinate size, is the large group of proteins which display limited swelling and are insoluble. These occur in the substance of muscle, in the stroma of red blood corpuscles, in tendons and elastic ligaments, and in keratinoid substances such as hair, feathers, tortoise shell, etc. It is to be supposed that the protein chains present in such substances are for the most part cross linked to form a three-dimensional network. (It is conceivable, however, that a system consisting of excessively long chains (molecular weight ~1,000,000) would behave essentially as a cross-linked system.)

Numerous opportunities for the formation of cross linkages are provided by the side chains. In hair, for example, we have cysteine residues bearing lateral SH groups which, in the oxidized condition, as cystine, may unite two chains by means of an S—S bridge. Lateral carboxyl groups, for example, may be united with basic groups of other chains to form amides. Cross links, so few in number as practically to preclude analytical confirmation, can lead to the formation of networks among long chains.

On the other hand, the cyclol hypothesis advanced by Wrinch,[44] according to which proteins in the imino form unite to form rings, appears extremely improbable (see formula II, Fig. 2). In the case of lower polypeptides, the cyclol form certainly does not occur, even in minimal quantity, as a tautomeric form in equilibrium with the normal peptide form. Meyer and Hohenemser[45] were able to show that a mixture of polypeptides (I) is not transformed in solution into a mixture of other peptides (III), as would be the case if the cyclol (II) were formed. It may be assumed likewise that the peptide linkages in proteins do not isomerize to form "cy-

[42] A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, **35**, 1369 (1941).

[43] H. B. Bull, *Advances in Enzymol.*, **1**, 1 (1941).

[44] D. M. Wrinch, *Nature*, **137**, 411 (1936); **138**, 241 (1936). D. M. Wrinch and D. Jordan-Lloyd, *ibid.*, **138**, 758 (1936). D. M. Wrinch, *ibid.*, **139**, 718, 972 (1937); *Science*, **85**, 566 (1937); *Proc. Roy. Soc. London*, **A160**, 58 (1937); **161**, 505 (1937).

[45] K. H. Meyer and W. Hohenemser, *Nature*, **141**, 1138 (1938). See also F. Haurowitz, *Z. physiol. Chem.*, **256**, 28 (1938).

clols." Calculations of Pauling and Niemann[46] have shown that such isomerization would involve a strongly endothermic reaction.

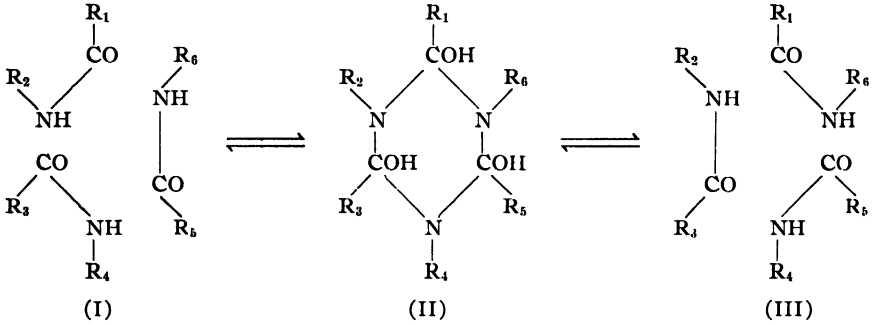


Fig. 2. Cyclol (II) in equilibrium with open peptides (I and III).

The question of the molecular weight of infinite nets or excessively long chains is without meaning; nevertheless, it is repeatedly asked (in the case of collagen and elastin, for example). Only when the network is destroyed—by rupture of bridge linkages (conversion of —S—S— into —SH HS—) or by hydrolysis of peptide linkages—are fragments set free which pass into solution, and of which the mean molecular weight can be determined. The molecular weight is then, of course, dependent on the degree of degradation.

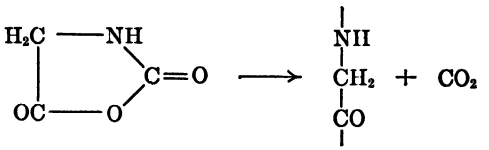
III. FIBRILLAR PROTEINS

1. Higher Peptides of Glycine and Its Homologues

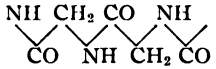
Synthetic polypeptides of higher molecular weight have hitherto only been prepared from a few amino acids. Abderhalden and Fodor,[47] by gradual addition of units, obtained L-leucyltriglycyl-L-leucyltriglycyl-L-leucyltriglycyl-L-leucylpentaglycylglycine. The substance is sparingly soluble and possesses none of the typical properties of proteins. According to Boehm[48] the hexapeptide of DL-alanine displays birefringence of flow in solution; it is to be supposed that needle-shaped submicroscopic crystallites are responsible for this phenomenon.

[46] L. Pauling and C. Niemann, *J. Am. Chem. Soc.*, **61**, 1860 (1939).
[47] E. Abderhalden and A. Fodor, *Ber.*, **49**, 561 (1916).
[48] G. Boehm, *Biochem. Z.*, **284**, 322 (1936).

A compound $(CH_2CONH)_n$ was obtained by Leuchs[49] on heating the cyclic anhydride:



The constitution of this compound was subsequently elucidated by Meyer and Go.[50] It is a polyglycylglycine consisting of about 30 residues. The substance is insoluble in water but soluble in concentrated acids and in concentrated lithium bromide. It yields a powder diagram, and Meyer and Go suppose that the glycyl chains are folded in a zigzag manner:



Go and Tani[51] prepared in the same way polyalanine and polyleucine of molecular weight 1000–6000, as well as a copolymer of glycine and leucine. Woodward and Schramm[52] succeeded in obtaining from phenylalanine and leucine a film-forming, high molecular copolymer which dissolves in benzene to give a very viscous solution. By extruding a concentrated solution into a nonsolvent, *e.g.*, methanol, fibers are formed which display an x-ray fiber diagram similar to that of silk fibroin. Hånby[53] has synthesized polyglutamic acids of molecular weight 15000; the product is very similar to the natural D-polyglutamic acid isolated from *Bacillus anthrax*. Polylysine has also been prepared by the same synthesis.[54]

Protamines, *i. e.*, natural basic polypeptides, are mentioned on page 517.

2. Silk Fibroin

The silk thread spun by the caterpillar of the silk moth, *Bombyx mori*, consists of a central core composed of a water-insoluble, mechanically re-

[49] H. Leuchs, *Ber.*, **39**, 857 (1906). H. Leuchs and W. Manasse, *ibid.*, **40**, 3235 (1907). H. Leuchs and W. Geiger, *ibid.*, **41**, 1721 (1908). See also F. Wessely and M. John, *Z. physiol. Chem.*, **170**, 38 (1927).

[50] K. H. Meyer and Y. Go, *Helv. Chim. Acta*, **17**, 1488 (1934).

[51] Y. Go and M. Tani, *Bull. Chem. Soc. Japan*, **14**, 510 (1939).

[52] R. B. Woodward and C. H. Schramm, *J. Am. Chem. Soc.*, **69**, 1551 (1947).

[53] W. E. Hånby, S. G. Waley, and J. Watson, *Nature*, **101**, 132 (1948).

[54] E. Katchalski, I. Grossfeld, and M. Frankel, *J. Am. Chem. Soc.*, **69**, 2564 (1947).

sistant, fibrous material (known as fibroin) and an outer coating of silk glue (sericin), which causes the fibers to adhere, and which may be removed by boiling in soapy water. The amino acids of sericin are very incompletely known. It contains many residues of dibasic acids and is soluble in alkalis. The composition of fibroin is better known.

(*a*) *Constitution of Silk Fibroin*

Questions concerning the homogeneity, constitution, and molecular weight of silk fibroin can only be partially answered at the present time. Quantitative analyses of the amino acids obtained by hydrolysis have repeatedly been made. Table III shows the quantities (as percentages) of the various amino residues present, according to Vickery and Bergmann[55] and Abderhalden.[56]

TABLE III
Constitution of Silk Fibroin

Amino acid	Amount, %		Amino acid	Amount, %	
	Ref. (55)	Ref. (56)		Ref. (55)	Ref. (56)
Glycine	43.8	40.5	Tyrosine	13.2	11.0
Alanine	26.4	25 0	Arginine	0.95	1.5
Leucine	—	2.5	Lysine	0 25	0 9
Serine	—	1.8	Histidine	0.07	0.8
Phenylalanine	—	1.5	Proline	—	1.0

Bergmann assumes (in accordance with his hypothesis) that the units are repeated periodically in such a way that every second unit is a glycine residue, every fourth unit an alanine residue, every sixteenth unit a tyrosine residue, and every two hundred and sixteenth unit an arginine residue. On the basis of integral proportions, he calculates a molecular weight of 217,700. Bergmann's hypothesis, however, is contradicted by the finding of glycylglycine and alanylalanine among the products of hydrolysis.[57] Furthermore, the fiber diagram of silk indicates the presence of both amorphous and crystalline fractions. The unit cell is small, indicating that only a few units are repeated periodically; large units, such as tyrosine, can scarcely be accommodated, therefore, in this unit cell. The crystalline regions con-

[55] M. Bergmann and C. Niemann, *J. Biol. Chem.*, **122**, 577 (1937). H. B. Vickery and R. J. Block, *ibid.*, **93**, 105 (1931).

[56] E. Abderhalden, *Z. physiol. Chem.*, **120**, 207 (1920).

[57] E. Abderhalden and A. Suwa, *Z. physiol. Chem.*, **66**, 13 (1910).

sist of chains of at least 10 successive units, GX, in which G is glycine and X alanine or serine, or perhaps even glycine, but not tyrosine, lysine, arginine, or any similar large amino acid residue. Such residues are rather to be found in the amorphous fraction. It is quite possible that part of a given chain may belong to a crystalline region, the rest to an amorphous region, in the following way (where T = tyrosine, L = lysine, and A = arginine):

—GXGXGXGXGXGXGXTGLGATG—
may form part of the lattice amorphous

(*b*) *Primary Valence Chain Lattice of Silk*

As is shown by the fiber diagram (first observed by Herzog and Jancke[58]) a part of the silk fibroin is crystalline (Fig. 3). This diagram was subse-

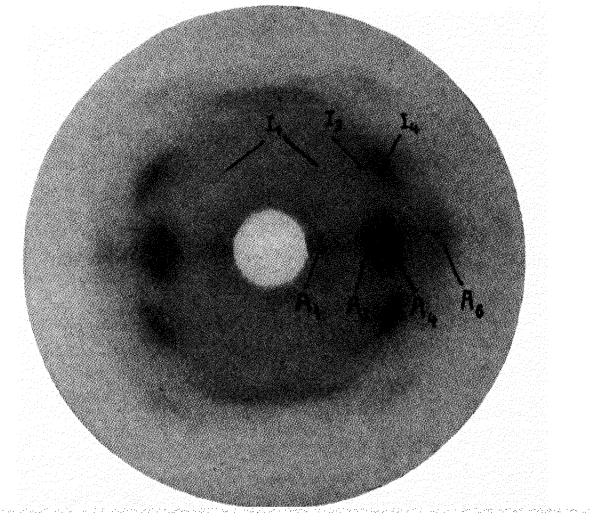


Fig. 3. Fiber diagram of silk.

quently investigated in greater detail by Brill (under Polanyi's direction). Brill[59] found the identity period along the fiber axis to be 7 Å. and put forward a tentative unit cell containing 4 glycine and 4 alanine units. Concerning the arrangement of these nothing could be said.

[58] R. O. Herzog and W. Jancke, *Ber.*, **53**, 2162 (1920).
[59] R. Brill, *Ann.*, **434**, 204 (1923).

Further x-ray investigations were made by Kratky and Kuriyama[60] and by Trogus and Hess[61]; the spacings obtained are given in Table IV. According to Kratky and Kuriyama several unit cells are compatible with these results. A plausible interpretation has been proposed by Brill.[62]

TABLE IV

Comparison of Reflections from Natural Silks

	Bombyx mori		Tussore silks		Nest silks		Spider silks	
Reflection	Intensity	*d*, Å.	Intensity	*d*, Å.	Intensity	*d*, Å	Intensity	*d*, Å
A_1	MS	9.5	—	—	—	—	—	—
A_2	W	4.8	S	5 3	VS	5.0	VS	5.3
A_3	VS	4.3	VS	4.3	VS	4 3	VS	4.3
A_4	VW	3.0	—	—	VW	2.3	W	2.6
A_5	EW	2.4	—	—	—	—	—	—
I_1	VW	5.5	—	—	EW	4.0	S	4.2
I_2	S	3.6	MS	3.6	S	3.6	MS	3 6
I_3	EW	2.2	—	—	—	—	—	—
II_0	W	3.5	W	3.4	—	—	W	3.4
II_1	W	3.3	—	—	—	—	—	—
II_2	W	2.7	W	2.8	MS	2 8	MS	2.9
II_3	EW	2.2	—	—	—	—	—	—
$III_{0,1}$	VW	2.3	VW	2.2	W	2.2	VW	2.3
III_2	W	2.1	VW	2.0	W	2.0	W	2.1
M_1	—	—	—	—	MS	6.8	—	—
M_2	—	—	—	—	MS	7.0	—	—

MS, moderately strong. W, weak. S, strong. VS, very strong. VW, very weak. EW, extremely weak.

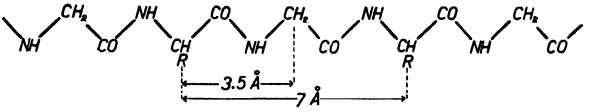
If the equatorial interferences are indexed in the following way: A_1 = (001), A_2 = (002), A_3 = (200) and (202), A_4 = (003),[63] A_5 = (402), then we obtain the following unit cell for silk from *Bombyx mori*: a = 9.65 Å., b (fiber axis) = 6.95 Å., c = 10.4 Å., and β = 62.4°; while for silk from *Antheraea mylitta*: a = 9.5 Å., b = 6.95 Å., c = 11.8 Å., and β = 66°. The lattice of silk was interpreted by Meyer and Mark[64] as a primary valence chain lattice; it was shown that the facts revealed by x-ray analysis and the physical properties of the substance can only be explained on the assumption that long polypeptide chains lie in the direction of the fiber axis and form crystallites, as do the chains in cellulose. The similarity in fine

[60] O. Kratky and S. Kuriyama, *Z. physik. Chem.*, **B11**, 363 (1931).
[61] C. Trogus and K. Hess, *Biochem. Z.*, **260**, 376 (1933).
[62] R. Brill, *Z. physik. Chem.*, **B53**, 61 (1942).
[63] In our opinion A_4 also includes the index $(20\bar{2})$.
[64] K. H. Meyer and H. Mark, *Ber.*, **61**, 1932 (1928).

structure readily explains the similarity in physical properties of natural and artificial silk.

The dimensions of the unit cell imply that the chains are built up from glycine and alanine residues, without there necessarily being any strict alternation of the two types of residue; serine, for example, may occur in the place of an alanine residue. On the other hand, the presence of larger residues, such as those of tyrosine or arginine, in the crystalline regions is extremely improbable. It follows from the sharpness of the diatropes that the crystalline regions have a length at least equivalent to that of 20 amino acid residues united in sequence; portions of chains of at least this length consist, therefore, only of glycine, alanine, and serine.

The form of the chains as determined from the fiber axis period is as follows:



Chain structure of the crystalline fraction of silk fibroin (Meyer and Mark)

Uneven orders of the diatropes are lacking. The half fiber period, 3.5 Å., is equal to the length of an amino acid residue; this periodicity has been found in other proteins.

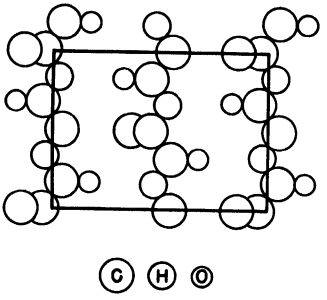
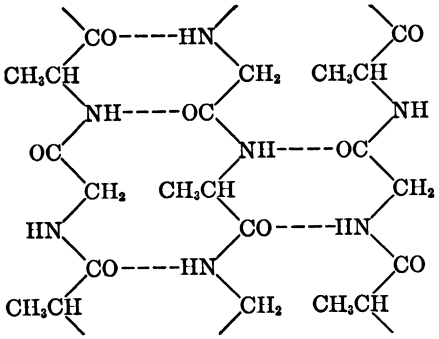


Fig. 4. Arrangement of chains in the lattice of silk (according to Brill).

One may suppose, along with Astbury,[65] Lotmar,[66] and Brill,[62] that the chains are united laterally by hydrogen bonds between CO— and NH—

[65] W. T. Astbury, *Fundamentals of Fibre Structure*, London, 1933.
[66] W. Lotmar and L. E. R. Picken, *Helv. Chim. Acta*, **25**, 538 (1942).

groups, and so form laminae or grids which are themselves associated by rather weaker secondary valence forces:



Hydrogen bonds in the lattice of silk (Brill)

Since the *a* axis is the same in different kinds of silk, while the *c* axis is different and increases along with the relative alanine content, the laminae built up by hydrogen bonds must lie in the *ab* plane, while the alkyl residues are arranged at right angles to this (Brill[62]) (Fig. 4).

(*c*) *Inhomogeneity in Fine Structure of Silk*

It follows from what has been said that the texture of silk is not homogeneous: regions of considerable length, with an orderly crystal lattice structure, composed only of smaller residues (such as glycine and alanine, perhaps also serine and leucine) are embedded in amorphous material which must contain the larger residues.

The silk micelles resist the action of swelling agents and in this respect they are comparable to the micelles of cellulose. When acids and dyes penetrate silk, they leave the lattice structure unchanged (Fikentscher and Meyer[67]); this shows that, within the crystalline micelles, there are no free amino or carboxyl groups and therefore no hexone bases or dicarboxylic acids.

(*d*) *Physical Properties of Silk*

Even without the evidence of x-ray analysis the parallel arrangement of molecular chains along the fiber axis can be inferred from the uniaxial birefringence of the fibers, by their high tensile strength in the direction of the

[67] H. Fikentscher and K. H. Meyer, *Melliand Textilber.*, **8**, 781 (1927).

fiber axis, and by the difference in swelling properties parallel, and at right angles, to the fiber axis.

As is well known, the tensile strength is considerable, *ca.* 35 kg. per sq. mm., and is therefore of the same order as that of moderately well-oriented artificial silk. The tensile strength indicates the existence of strong forces of attraction between the chains and the micelles; in particular hydrogen bonds between —NH and —CO groups of neighboring chains may well contribute to the considerable micellar forces.

Silk displays positive uniaxial birefringence: $\Delta = 0.04$–0.06; the refractive index parallel to the fiber axis, $n_D = 1.584$, while at right angles to this axis, $n_D = 1.529$ (Ohara[68]). If allowed to swell, the fibers also show structural birefringence; from this it follows that fluid penetrates between the bundles of chains and separates these from each other.

(*e*) *Formation of Silk Thread*

The fully grown caterpillar has two silk glands, each consisting of a narrow tube 12–15 cm. long and about 1.6 mm. wide, folded like a hairpin. The thin yellow membrane contains about 0.3 g. of a viscous fluid, the dried contents of which amount to about 30% of the wet weight. As Foà,[69] Ramsden[70] and Meyer and Jeannerat,[71] have shown, the viscous, water-soluble contents become solid and insoluble as a result of mechanical action—stretching, pressing, or shearing. According to these workers, the stretching of the thread during spinning is responsible for its solidification. Meyer and Jeannerat have shown that, if the contents of the gland are stretched, the thread is at first elastic, like rubber. If the tension is removed immediately after stretching, the thread contracts like a rubber band. If, however, it is held in the stretched condition for about 10 seconds, the thread loses its elasticity and then yields an x-ray diagram. This behavior recalls that of rubber stretched at 0°C. After a short time, elasticity is lost and the rubber crystallizes. Pure, amorphous rubber is metastable at 0°C. and crystallizes only after many weeks; stretching leads to the parallel orientation of the chains, and, in this way, the speed of crystallization is very considerably increased, with the result that the state of "supersaturation" is removed. Amorphous selenium behaves similarly;

[68] K. Ohara, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **21**, 104 (1933): **22**, 216 (1933).

[69] C. Foà, *Kolloid-Z.*, **10**, 7 (1912).

[70] W. Ramsden, *Nature*, **142**, 1120 (1938).

[71] K. H. Meyer and J. Jeannerat, *Helv. Chim. Acta*, **22**, 22 (1939).

in the supercooled condition it may be kept indefinitely without crystallizing. On warming to 72°C., it becomes elastic like rubber and, if held in the stretched condition, crystallizes and becomes inelastic.

In all cases, therefore, orientation accelerates the crystallization of a rubberlike body. By analogy, one may conclude that the contents of the gland constitute a metastable system, that is, a supersaturated solution of protein, the crystallization of which may be accelerated by stretching and thus orienting the chains. The formation of an insoluble thread from the soluble contents of the gland offers an example of a specialized "denaturation." The denaturation of many soluble proteins by shaking (discovered by Ramsden[72]), and the denaturation of surface films, are other examples of the same phenomenon.

The contents of the gland are soluble in water and dissolve still more readily in a solution of 6 cc. of 0.1 *N* ammonium chloride, 24 cc. of 0.1 *N* ammonia, and 70 cc. of water at *p*H 10. On dissolving 2 g. of the gland contents, the *p*H falls to 7, indicating that the alkali has been neutralized by acid groups of the protein. The solution is unstable. It is rapidly covered by a solid film of insoluble protein, gradually becomes viscous, and finally solidifies. On stirring, shaking, or bubbling gases through the solution, a fibrous protein is precipitated, while another protein remains in solution and gelatinizes after a short period of time. The gland therefore contains at least two proteins, which remain side by side in supersaturated solution, and which are probably identical with fibroin and sericin (Meyer and Jeannerat).

Coupling of the dissolved material with diazobenzene sulfonic acid prevents coagulation. The absence of protein precipitable by acids after coupling indicates that hydrophilic sulfonic groups are attached to all the chains. Since coupling takes place at the phenol group of tyrosine, it is probable that all chains contain this residue as a structural unit.

(*f*) *Chemical Behavior of Silk and Affinity for Dyes*

Silk dissolves in concentrated lithium bromide, concentrated acids, and in ammoniacal copper oxide solution. According to Sakurada and Hutino,[73] the reprecipitated silk shows the same x-ray pattern as the initial material. The behavior of silk in concentrated formic acid is very char-

[72] W. Ramsden, *Arch. ges. Physiol. Pflügers*, **1894**, 517.

[73] I. Sakurada and K. Hutino, *Sci. Papers Inst. Phys. Chem. Research Tokyo*, **21**, 266 (1933).

acteristic: the thread swells very considerably, shortens, and becomes elastic like rubber; the fiber diagram disappears and, in its place, an amorphous ring appears.[74] This behavior recalls that of cellulose in the presence of concentrated alkalis. Silk, however, does not form an addition compound with a characteristic crystal lattice and, in that respect, differs from cellulose.

The normal silk thread is not attacked by trypsin, whereas silk peptone, obtained by precipitation from acid solution, is attacked. Waldschmidt-Leitz[75] to whom this observation is due, ascribed this behavior to "structural" differences. He suggests as a possibility that the peptide chains of silk form long stretched rings which are split open on solution in acid. During this process, groups are set free which expose the chains to enzyme attack. In favor of this hypothesis is the observed action of trypsin on the aqueous solution of the contents of the silk gland. Sericin alone is attacked, while the fibroin is precipitated in fibrous form and in this condition resists the action of trypsin (Meyer and Jeannerat[76]). Papain, on the other hand, behaves differently. According to Ambros and Harteneck,[77] the dried contents of the gland are digested by papain, and Meyer and Jeannerat have shown[76] that the fibroin in the aqueous solution of the gland contents is also hydrolyzed. In contrast to this the finished silk thread resists the action of papain. This may be due to the inability of the large enzyme molecules to penetrate between the closely packed crystallites.

Silk is capable of uniting with a certain maximal quantity of acid. This quantity is independent of the nature of the acid. The process involves therefore, the formation of a salt with the basic groups of the amorphous substance. One equivalent of acid unites with 4000 g. of silk (Meyer and Fikentscher[77,78]). Assuming that the mean molecular weight of an amino acid unit is 100, this means that every fortieth residue carries basic groups.

The affinity of silk for acid dyes depends on the basic groups present, which unite with the sulfonic groups of the dye to form insoluble, saltlike complexes[77,79] and are adsorbed (at saturation) in the same molar proportions as are acids of lower molecular weight. Silk is also able to adsorb neutral substances such as nitroaniline, and, like nitrocellulose, to "dissolve"

[74] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, page 224.

[75] E. Waldschmidt-Leitz and G. von Schuckmann, *Ber.*, **62**, 1891 (1929).

[76] K. H. Meyer and J. Jeannerat, *Helv. Chim. Acta*, **22**, 22 (1939).

[77] O. Ambros and A. Harteneck, *unpublished work.*

[78] K. H. Meyer and H. Fikentscher, *Melliand Textilber.*, **7**, 605 (1926); **8**, 781 (1927)

[79] E. Elöd and E. Piper, *Angew. Chem.*, **41**, 16 (1928). E. Elöd, *Trans. Faraday Soc.* **29**, 327 (1933); **31**, 305 (1935).

these. The partition coefficient[77] of *o*-nitroaniline between silk and water was found to be 25.

3. Collagen and Gelatin

(*a*) *Constitution and Fine Structure*

Collagen fibers[80] occur in the skin, in connective tissue, and elsewhere. They form the principal constituent of tendons. On being heated in water, they are gradually transformed into a water-soluble substance—gelatin. The structural units of this substance, which are also those of collagen, are well known. Small quantities only of histone bases and dicarboxylic acids occur as products of hydrolysis; the proportions of glycine, proline, and hydroxyproline, on the other hand, are high (see Table V). According to

TABLE V

Analysis of Structural Units of Gelatin[81]

Unit	Amount, % by wt.	Unit	Amount, % by wt.
Glycine	25.5	Methionine	1.0
Alanine	8.7	Arginine	8.2
Leucine	7.1	Lysine	5.9
Serine	0.4	Hydroxylysine	0.1
Phenylalanine	1.4	Histidine	0.9
Glutamic acid	5.8	Proline	19.4
Aspartic acid	3.3	Hydroxyproline	14.1
		Ammonia	0.4

Bergmann[82] every third residue is a glycine residue, every sixth a proline residue, and every ninth an oxyproline residue. The other units are less abundant. According to Grassmann[83] 0.65% (glucose + galactose) is built into the collagen framework. Nothing is known of the arrangement

[80] An excellent summary of the morphology and fine structure of collagen fibers is given by A. Küntzel and F. Prakke, *Biochem. Z.*, **267**, 243 (1933). See also E. Wöhlisch, *Ergeb. Physiol. biol. Chem. exptl. Pharmakol.*, **34**, 405 (1932).

[81] From H. D. Dakin, *J. Biol. Chem.*, **44**, 499 (1920). A. Hunter and J. B. Pettigrew, *Enzymologia*, **1**, 341 (1936). B. W. Town, *Biochem. J.*, **30**, 1833 (1936). H. D. Boernstein, *J. Biol. Chem.*, **97**, 663 (1932). M. Bergmann, *ibid.*, **110**, 471 (1935). D. D. van Slyke, A. Miller, and D. A. MacFadyen, *ibid.*, **141**, 681 (1941) (oxylysine).

[82] M. Bergmann, *J. Biol. Chem.*, **110**, 471 (1935). M. Bergmann and C. Niemann, *ibid.*, **115**, 77 (1936).

[83] W. Grassmann and H. Schleich, *Biochem. Z.*, **277**, 320 (1935). W. Grassmann, J. Janizki, L. Klenk, and F. Schneider, *ibid.*, **294**, 95 (1937).

of the amino acid units. An indication is provided by the isolation of lysylprolylglycine (Grassmann[84]).

The fibers show a marked fibrillar structure. The fibrils themselves are strongly birefringent (birefringence positive and uniaxial), mechanically strong in the direction of the fiber axis, and show characteristic anisotropy of swelling (increase in width but not in length). These facts all point to a structure composed of parallel primary valence chains. The chains are held together in the direction at right angles to the chain axis by intermolecular forces and probably by occasional chemical cross linkages. Trypsin does not attack the fibers. It is to be concluded that the particular

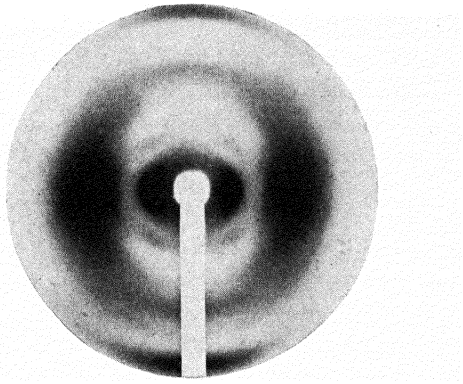


Fig. 5. X-ray diagram of a tendon from the tunny (*Germo alalonga*) (Champetier and Fauré-Fremiet).

grouping which is attacked by trypsin is absent; on hydrolysis, however, this grouping is formed. In all media which have no hydrolytic action, collagen is insoluble and swells to a limited extent only. The molecular weight of "native" collagen cannot, therefore, be determined.

As in the case of silk, the structure of collagen is unhomogeneous. Amorphous regions are present along with regions with a crystal lattice yielding a fiber diagram. This was first observed by Herzog and Jancke.[85] Gerngross and Katz[86] made the important discovery that stretched gelatin yields the same diagram as collagen. This is a further example of the fact that cross-linked chains may show the same latticelike orientation as free chains.

An x-ray diagram obtained from tunny fish tendon is shown in figure 5.

[84] W. Grassmann and K. Riederle, *Biochem. Z.*, **284**, 177 (1936).

[85] R. O. Herzog and W. Jancke, *Ber.*, **53**, 2162 (1920).

[86] O. Gerngross and J. R. Katz, *Kolloid-Z.*, **39**, 181 (1926).

According to Clark[87] an arc corresponding to a spacing of 2.8 Å. is present on the meridian. Reflections from an oblique plane corresponding to a fiber period of 9.8 Å. are distinct (Herrmann, Gerngross, and Abitz[88] on stretched gelatin; Trillat,[89] Küntzel and Prakke,[90] and Clark and Schaad[91] on tendon fibers). These may perhaps be due to a system other than that giving rise to the arcs already mentioned. The spacing of 2.8 Å. might also represent the fourth order of the identity period, 9.8 Å. We shall meet this meridional interference corresponding to a spacing of 2.8 Å. again in the muscle diagram. It corresponds to the length of an amino acid residue in a peptide chain which is not folded in a zigzag manner, but twisted in a spiral about the fiber axis (see Fig. 10*b*). It is possible that the collagen chains are similarly twisted.

As Clark[91] has found, treatment with alkali leads to the formation of a compound with a fiber period of 6.5 Å., corresponding to two residues of a zigzag folded chain, such as is present in silk fibroin; this structure presumably represents a transition from the *muscle chain* to the *silk chain* (β-keratin form).

A larger periodicity (330 Å. in the direction of the fiber axis) has been recorded by Wyckoff[92]; and Kratky[93] and Bear[94] observed an even larger *fundamental* period of 642 Å. in different kinds of tendons. This large period increases in the presence of water and diminishes as a result of tanning, without the smaller periodicities being unaffected. Under the electron microscope Schmitt has observed transverse striation; darker stripes, of relatively greater density, were distributed at the same spacing of 642 Å.[95]

Astbury[96] supposes that the chain is folded in the *cis* configuration, and that this is determined by the presence of 30% of proline and oxyproline residues. We are at a loss to understand why proline residues should provoke *cis* configuration more easily than any other amino acid residue.

[87] G. L. Clark and J. A. Schaad, *Radiology*, **27**, 339 (1936).
[88] K. Herrmann, O. Gerngross, and W. Abitz, *Z. physik. Chem.*, **B10**, 371 (1930).
[89] J. J. Trillat, *Ann. inst. Pasteur*, **48**, 400 (1932).
[90] A. Küntzel and F. Prakke, *Biochem. Z.*, **267**, 243 (1933).
[91] G. L. Clark and J. A. Schaad, *Radiology*, **27**, 339 (1936).
[92] R. G. W. Wyckoff and R. B. Corey, *Proc. Soc. Exptl. Biol. Med.*, **34**, 285 (1936).
[93] O. Kratky and O. Sekora, *J. makromol. Chem.* [3] **1942**, 112.
[94] R. S. Bear, *J. Am. Chem. Soc.*, **64**, 727 (1942); **66**, 1293 (1944).
[95] F. O. Schmitt, C. E. Hall, and M. A. Jakus, *Biol. Symposia*, **10**, 261 (1943).
[96] W. T. Astbury, *J. Intern. Soc. Leather Trades' Chemists*, **24**, 69 (1940); *Nature*, **145**, 421 (1940).

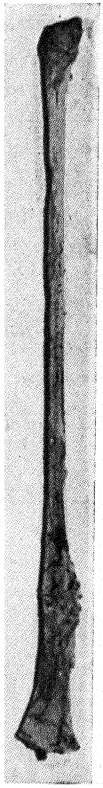


Fig. 6*a*. Tendon.



Fig. 6*b*. The same piece of tendon after thermal contraction.

Furthermore, it will be shown later that the identity period of crystalline myosine which contains only 2% of proline is also 2.8 Å.

The spacing corresponding to the innermost equatorial reflection, A_1, varies with the water content from 10.7 to 17.4 Å. The strong reflection A_2, corresponds to a spacing of 4.6–6.1 Å.

The amount of amorphous material present is considerable. It is to be supposed that the chains which pass through the crystallites also form part of the amorphous substance, so that the crystallites are *fringed*, as Gerngross, Herrmann, and Abitz have suggested.[97]

(*b*) *Behavior of Collagen Fibers on Warming*

On warming, tendon behaves in a remarkable way: it contracts in the direction of the fiber axis and becomes elastic like rubber. At the same time the x-ray diagram disappears. Fine tendon fibers which have been caused to contract by cautious heating may, as a result of prolonged stretching in the cold, be brought back to their original inelastic condition, in which they again show a fiber diagram. The behavior of unchanged tendon recalls that of stretched, frozen rubber; that of the contracted tendon recalls the behavior of amorphous rubber, obtained from stretched and frozen rubber by warming (see Figs. 6 and 7).

[97] K. Herrmann, O. Gerngross, and W. Abitz, *Z. physik. Chem.*, **B10**, 371 (1930). K. H. Meyer, *Biochem. Z.*, **214**, 253 (1924).

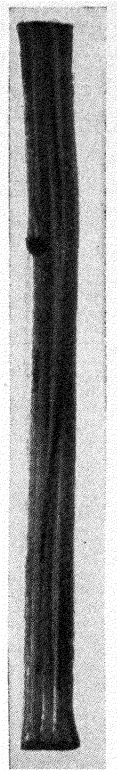


Fig. 7*a*. Stretched and frozen rubber.

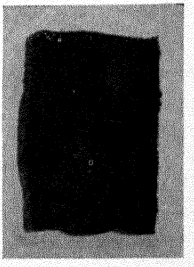


Fig. 7*b*. The same piece of rubber after thermal contraction.

The analogy with rubber is even closer. Wöhlisch[98] determined the stress–strain curve for contracted tendon, and showed that this is reminiscent of that for rubber—as the extension increases, the object becomes more rigid. The tensile stength is similar, as is clear from the values in Table VI obtained by Valkó for untreated, contracted tendon and for the same material after stretching, at −60°C.

The restoring force of stretched tendon in the rubberlike condition has the same origin as that of stretched rubber. The chains which have been induced to abandon probable twisted configurations for the less probable stretched conformation, tend to resume the statistically favored contracted positions and configurations, under the influence of thermal agitation. If fully stretched and at a sufficiently low temperature, the chains snap into position in the crystal lattice—with the evolution of heat of crystallization (Meyer and Ferri[99]).

Collagen chains provide an example of the fact that protein chains may exist in the stretched condition under certain circumstances, while under other circumstances they are contracted (Meyer).[100]

TABLE VI

Comparison of Contracted and Stretched Tendon

Material	Tensile strength, kg./mm.²
Pig's foot tendon: cross section *ca.* 5 mm.²	11.0
Contracted at 80°C.	3.0
Stretched to its original length	10.6
Dried Tendon at Room Temperature	
Pig's foot tendon	9.2
Contracted at 80°C.	2.2

The contraction is to be regarded as a fusion, similar to the fusion of rubber. It is more correct, however, to speak of a two-dimensional solution of the crystallites in the water of imbibition, since the melting point of water-free tendon lies at about 140°C. (Meyer, von Susich, and Valkó[101])

[98] E. Wöhlisch, *Z. Biol.*, **85**, 325 (1927).
[99] K. H. Meyer and C. Ferri, *Arch. ges. Physiol. Pflügers*, **238**, 78 (1936).
[100] K. H. Meyer, *Biochem. Z.*, **214**, 253 (1929).
[101] K. H. Meyer, G. von Susich, and E. Valkó, *Kolloid-Z.*, **59**, 208 (1932).

and is lowered in the presence of water to about 60°C. With regard to this observation, the following remarks may be made:

A binary system consisting of a crystalline substance and solvent at constant pressure possesses a definite saturation concentration for each temperature; conversely, at each concentration there is a definite saturation temperature above which the crystallites disappear and below which crystallites are formed. In the case of collagen, we are dealing with crystallites built up from chains or segments of chains, and linked to form a loose three-dimensional network which can absorb only a limited quantity of water. As a result, a lower limit is set to the concentration of chain segments in water. Accordingly, there exists for each solvent a temperature at which this concentration corresponds to saturation and above which the crystallites disappear. In all chain polymers the temperature at which the product is sparingly soluble is separated by only a very small interval from that at which it dissolves in considerable quantities (as much as 20%, for example). For this reason, "solution" then appears like fusion.[102]

The greater the solubility of the collagen chains in the solvent in question, the lower is the temperature of fusion. Salts which increase the solubility of peptides diminish the temperature of fusion. Salts which lead to salting out raise this temperature. These relationships have been studied in detail by Küntzel. He observed also that many salts lead to solution, that is, to a lowering of the melting point, when present in low concentration, while at higher concentrations, they have a salting-out effect such as has been observed in many proteins. Holleman[103] and, later Küntzel[104] have determined calorimetrically the "heat of fusion" of collagen; this amounts to 12.5 cal. per gram.

Alkalis, acids, and lithium bromide or calcium thiocyanate lower the melting point. If the fibers are soaked in formamide—which has a particularly marked solvent action on peptides—contraction takes place at 35°C. and, on cooling, the fibers return spontaneously to two thirds of their original length.

(*c*) *Formation of Gelatin*

On prolonged treatment with hot water, collagen fibers are converted into water-soluble gelatin. The nature of this reaction has often been discussed, and, in particular, the attempt has been made to explain its relationship

[102] E. H. Buchner, *Rec. trav. chim.*, **42**, 787 (1923). K. H. Meyer and A. van der Wyk, *Helv. Chim. Acta*, **20**, 1331 (1937).

[103] L. W. J. Holleman, H. G. Bungenberg de Jong, and R. S. Tjaden Modderman, *Kolloid-Beihefte*, **40**, 211 (1934).

[104] A. Küntzel and K. Doehner, *Angew. Chem.*, **52**, 175 (1939).

to the transformation of native collagen into the contracted amorphous form. A possible means of detecting even slight chemical changes is provided by the behavior of the substance in the presence of trypsin; native fibers are unattacked, whereas gelatin is digested.

Collagen which has been converted into the contracted condition at 35°C. in the presence of formamide is not attacked by trypsin (Cherbuliez, Jeannerat, and Meyer[105]). The contraction can also take place in water without the fibers losing their resistance to trypsin attack, if the fibers are dipped for only a few seconds into water at *p*H 8 and at 55°C. If heating takes place for a longer period, chemical changes appear and the fiber becomes less resistant to trypsin. From this it may be concluded that the peptide chains undergo hydrolysis in the presence of water, and that certain groups are set free which expose the chains to trypsin attack. The degradation of the chains by hydrolysis is shown by the change in mechanical properties: the specimen very soon displays plastic flow, that is, the stretched tendon treated with warm water relaxes. As heating continues, more and more substance passes into solution until finally the whole is converted into water-soluble gelatin.

Two superimposed processes are, therefore, concerned in the formation of gelatin: (*1*) reversible fusion in the water of imbibition; and (*2*) irreversible hydrolysis of peptide linkages, leading gradually to the formation of soluble products consisting of chain fragments. This point of view has been put forward by Cherbuliez and Meyer; Küntzel,[106] on the other hand, regards fusion without contraction as the only reversible part of the process, the contraction itself being irreversible. Such an interpretation is irreconcilable with the behavior of all rubberlike elastic materials.

Gelatin formation may occur without fusion as a result of prolonged treatment with cold alkali. Under these conditions, the fibers retain their original length and the crystalline regions remain unmelted. Swelling takes place only at right angles to the fiber axis. On washing out the alkali, the swelling persists. In this way an oriented gelatin gel is formed which is rapidly attacked by trypsin and, on warming, passes into solution.

According to Scatchard,[107] in the formation of gelatin certain readily hydrolyzable peptide linkages are split, and the resulting product is relatively homogeneous. If hydrolysis is continued, however, molecular weights of all magnitudes appear.

[105] E. Cherbuliez, J. Jeannerat, and K. H. Meyer, *Z. physiol. Chem.*, **255**, 241 (1938).

[106] A. Küntzel, *Stiasny-Festschrift*, Darmstadt, 1937, page 191.

[107] G. Scatchard, J. L. Oncley, J. W. Williams, and A. Brown, *J. Am. Chem. Soc.*, **66**, 1980 (1944).

Prolonged heating of soluble gelatin in water leads to progressive diminution in chain length and hence to the familiar reduction in viscosity. If the fibers are boiled, the viscosity first increases, since the chains pass into solution and the compact micelles gradually break up; later the viscosity diminishes as a result of the shortening of the chains (Sheppard and Houck[108]).

(*d*) *Properties of Gelatin and Its Solutions*

Gelatin and glue (which differs from gelatin chiefly in its lower molecular weight and greater impurity) are prepared from collagen-containing material (principally hide waste, tendons, and bones) by hydrolytic breakdown with boiling or superheated water. In the case of gelatin, a product is required which shall be pure and odorless and which will gelate in aqueous solution. In the case of glue, value is laid on the adhesive properties.

Osmotically active particles of molecular weights from 20,000 to 70,000 have been found in commercial samples of gelatin.[109] Gelatin contains free basic and acidic groups, and the equivalent weight of gelatin with respect to both bases and acids amounts to *ca.* 800–900.

By fractional precipitation of the aqueous solution with alcohol, commercial gelatin can be split up into various components (Liepatow and Putilowa[110]), the acid-binding power of which increases, according to Mosimann,[111] as the solubility, viscosity, and molecular weight diminish. Presumably, basic end groups are set free by hydrolysis, and these are responsible for the higher acid-binding powers of the constituents of lower molecular weight. The viscosities and equivalent weights of various fractions investigated by Mosimann are collected in Table VII.

TABLE VII

Properties of Gelatin

Fractions		Equivalent weight	η_{rel} in 0.4% formamide
Least soluble	I	1000 ± 40	1.110
	II	870 ± 30	1.104
	III	650 ± 20	1.084
Most soluble	IV	580 ± 20	0.062

[108] S. E. Sheppard and R. C. Houck, *J. Phys. Chem.*, **34**, 273 (1930).

[109] R. S. Lillie, *Am. J. Physiol.*, **20**, 127 (1907). J. Eggert and T. Reitstötter, *Z. physik. Chem.*, **123**, 363 (1926). M. Frankel, *Biochem. Z.*, **249**, 476 (1932).

[110] S. M. Liepatow and J. N. Putilowa, *Kolloid-Z*, **71**, 83 (1935).

[111] H. Mosimann, *Dissertation*. Bern, 1937.

In aqueous solution the viscosity shows a minimum at the isoelectric point. Hot, dilute, aqueous solutions of gelatin display no marked structural viscosity, whereas, at room temperature, they display a number of properties which can be explained only by the simultaneous presence of single molecules, molecular aggregates, and larger micelles, a system which only reaches equilibrium extremely slowly.

The relation between the viscosity of aqueous solutions of gelatin and their concentration is somewhat different from that observed in the case of pure chain polymers, since the viscosity increases more rapidly with increasing concentration. This rise is the more marked the lower the temperature (Bincer,[112] Mosimann[111]). At temperatures above 40°C. the behavior of a sample of gelatin with intrinsic viscosity $[\eta] = 0.5$ follows the Fikentscher equation (page 36) up to a concentration of 15%. At higher concentrations, the relative viscosity increases more rapidly; this is due to the formation of micelles. The very gradual approach to a state of equilibrium between the various types of molecular aggregate present is shown by the following observations. The properties of a warmed and recooled aqueous solution of gelatin of sufficient concentration change with time: the optical rotation falls, ultramicroscopic particles appear,[113] the viscosity rises, and finally the solution sets to a jelly; in these processes heat is evolved. The changes in the solution must be referred to the association of molecules to form micelles—a first stage in gelation. The association of the chain molecules to form rodlets is indicated by the birefringence of flow of aqueous gelatin solutions (Boehm and Signer[114]).

(e) *Gelation*

The higher the molecular weight of a gelatin sample, the lower the concentration and the higher the temperature at which gelation takes place. The process has been interpreted by Katz as one of crystallization. According to Gerngross, portions of the tangled chains present in solution associate to form micelles, and thus act as points of linkage in the meshwork which, as Hardy[115] first suggested, constitutes the solidified gelatin gel. Other parts of the chains remain free and solvated, that is, surrounded by water. These amorphous regions are responsible for the rubberlike properties of the gel. If the chains are desolvated by the addition of alcohol or

[112] H. Bincer, *Kolloid-Z.*, **59**, 82 (1932).

[113] K. Krishnamurti, *Nature*, **124**, 690 (1929).

[114] G. Boehm and R. Signer, *Helv. Chim. Acta*, **14**, 1370 (1931); *Klin. Wochschr.*, **11**, 599 (1932).

[115] W. B. Hardy, *Proc. Roy. Soc. London*, **66**, 95 (1900).

certain salts, the gel separates into water and solid substance. On freezing the gel, a microscopically visible net structure appears (Dhéré[116]).

From the melting points of various concentrations of gelatin, determined by Pleass and Mardles,[117] Meyer and van der Wyk[118] estimated the temperature coefficient of solubility, and from this calculated the molecular heat of solution, which is approximately equal to the molecular heat of fusion. The value obtained, *ca.* 21,000 cal., together with the calorimetrically determined heat of fusion, 12.5 cal. per gram, shows that the segments of chains coming together to form crystallites have a molecular weight of about 2000, that is, they consist of only about 15–20 amino acid units, as opposed to the several hundred units of the whole chain, whose molecular weight amounts to 20,000 to 70,000.

Alcohol-containing gelatin gels of water content 20–30%, as well as glycerol-containing gels, can be stretched several hundred per cent[119]; the x-ray diagram of collagen appears, and the gelatin becomes solid in the direction of stretching, but splits readily at right angles to this (Bergmann[120]; Katz and Gerngross[121]).

(*f*) *Swelling of Collagen and Gelatin*

Collagen and gelatin swell, that is, take up solvents, of which the most important is water. In collagen, swelling is limited; equilibrium is established between the swelling pressure and the compression exerted by the elastically stretched network. The swelling is limited even when the solvent (for example, formic acid) penetrates into the micelles. At suitable temperatures, gelatin also swells to a limited extent, swelling being intermicellar. The crystalline regions, or regions held together by forces of association, act as points of linkage. If the solvent penetrates into the micelles, swelling continues until the gelatin dissolves.

An indication of the nature of the fundamental process involved in swelling is provided by measurements of the heat of swelling. Initially, this is high and determines predominantly the work of swelling. As soon as about

[116] C. Dhéré, *Arch. sci. biol. Italy*, **A7**, No. 3 (1925).

[117] E. W. J. Mardles, *Kolloid-Z.*, **57**, 183 (1931). W. B. Pleass, *Proc. Roy. Soc. London*, **A126**, 406 (1930). See also A. Lottermoser and W. Mathaes, *Z. physik. Chem.*, **141**, 129 (1929).

[118] K. H. Meyer and A. van der Wyk, *Helv. Chim. Acta*, **20**, 1331 (1937).

[119] J. R. Katz and O. Gerngross, *Naturwissenschaften*, **13**, 901 (1925); *Kolloid-Z.*, **39**, 180 (1926).

[120] M. Bergmann and B. Jacobi, *Kolloid-Z.*, **49**, 46 (1929).

[121] J. R. Katz, *Angew. Chem.*, **40**, 1443 (1927).

0.2 g. of water has been adsorbed by 1 g. of gelatin (corresponding to about 1 mole of water per peptide unit), the heat of swelling becomes much smaller, and further swelling and solution occur without forces of attraction being involved, as a result of the tendency of chain segments and whole chains to diffuse away from each other.

When swelling takes place in acids, bases, or certain salts, the tendency of the ions to diffuse also plays a part in the phenomenon. This has been shown by Procter and Wilson[122] in their classical investigations, based on the early work of Loeb. If hydrochloric acid, for example, acts on gelatin, the protons unite with the basic groups of the chains to form cations, which are attached to the network and unable to diffuse away. These are neutralized by an equal number of chloride ions. Equilibrium is reached when the conditions of the Donnan relation are satisfied, that is, when the products of the concentrations of diffusable ions in the gel and in the external liquid are the same. We can represent the concentrations of the ionized gelatin groups and of H^+ and of Cl^- as follows:

Gel	External solution
$z\ G^+$	
$y\ H^+$	$H^+\ x$
$(y + z)\ Cl^-$	$Cl^-\ x$

The condition for equilibrium is that:

$$x^2 = y(y + z)$$

The number of diffusible ions in the gel $(2y + z)$ is greater than that of the ions outside, $2x$. There arises, therefore, a difference in osmotic pressure which tends to inflate the gelatin framework. This pressure is proportional to the difference, e, between internal and external ionic concentrations:

$$e = 2y + z - 2x = 2\sqrt{x^2 + 0.25z^2} - 2x$$

The concentration, z, of the ionized amino groups of the gelatin is dependent on the hydrogen ion concentration. If acid is added to pure gelatin, z increases from zero to a value corresponding to complete saturation of the basic groups present; as a result, e increases. If still more acid is added, z does not increase appreciably, whereas x increases, so that e now diminishes; e passes, therefore, through a maximum value, shown by a swelling maximum. Extensive experiments by Procter and Wilson resulted in satis-

[122] H. R. Procter and J. R. Wilson, *J. Chem. Soc.*, **109**, 307 (1916). See also T. R. Bolam, "Das Donnangleichgewicht," *Kolloid-Beihefte*, **39**, 139 (1934), *et seq.*

factory agreement between theory and experimental observations. The swelling maximum was found to occur at *p*H 2.65.

The process of swelling in gelatin and in collagen has frequently been studied[123] and the theory of Procter and Wilson confirmed and, in detail, refined.

(*g*) *Tanning*[124]

Collagen fibers are "tanned" by various reagents, that is, they are converted into a form in which they swell less and display greater mechanical strength. This result may be obtained in many different ways, but only with reagents that precipitate dissolved gelatin in insoluble form. Vegetable tannins are multivalent phenols of high molecular weight, which unite with the basic side groups of gelatin to form insoluble or sparingly soluble salts. Since each tannin molecule can react with several collagen chains, a kind of three-dimensional network is formed. The formation of such a network and the closing of the hydrophilic amino groups appear to be the essential features of the process of tanning (Meyer, 1929). Chromium salts also have a tanning action, since they are able to unite with several hydrophilic groups to form a complex and thus link together several chains. The nature of this complex formation has been discussed in detail by Küntzel. Both types of tanning are reversible. Tannins may be removed by suitable solvents (at least immediately after tanning), and chromium may be removed by oxalic acid. On the other hand, formaldehyde tanning is irreversible; the cross linkages produced by the aldehyde:

$$—NH_2 + CH_2O + NH_2— = —NH—CH_2—NH— + H_2O$$

cannot be undone without destroying the molecule. In this reaction the amino groups of the lysine residues are probably attacked.[125]

To a certain extent, tanning resembles vulcanization, which also influences mechanical properties and swelling by the formation of cross linkages. The analogy with vulcanization is seen most clearly perhaps in the effect of small quantities of formaldehyde on tendon fibers. Fibers treated in this way contract on heating and become elastic. In contrast to untreated fibers, however, such fibers cannot be plastically extended, and

[123] E. Elöd and E. Silva, *Z. physik. Chem.* **A137,** 142 (1928). D. Jordan Lloyd and W. B. Pleass, *Biochem. J.*, **21,** 1352 (1927); **22,** 1007 (1928). D. Jordan Lloyd, *ibid.*, **24,** 1460 (1930). A. Küntzel, *Biochem. Z.*, **200,** 326 (1929).

[124] See, in particular, A. Küntzel and F. Prakke, *Biochem. Z.*, **267,** 242 (1933). A. Küntzel and C. Riess, *Collegium*, Darmstadt, **1938,** 433. K. H. Gustavson, *Svensk Kem Tidskr.*, **52,** 75 (1940); **58,** 2 (1946).

[125] K. H. Gustavson, *Kolloid-Z.*, **103,** 229 (1943).

swell much less. The mechanical properties closely resemble those of slightly vulcanized rubber.

The solubility of gelatin is considerably reduced by tanning with chromium salts, and extensive use is made of this in the preparation of line cuts. A thin layer of gelatin containing a few per cent of bichromate is spread over a zinc plate. Since the reduction of the bichromate to chromium salts is accelerated in the presence of light, tanned gelatin is formed where the gelatin film is illuminated. On soaking the plate in lukewarm water the tanned areas do not pass into solution. If, therefore, a film of gelatin–bichromate is illuminated through a photographic negative, the white areas of the negative will appear on the zinc, after development, as regions coated with gelatin. These protect the metal from attack by the etching agent (dilute acid) subsequently applied and thus lead to the formation of a relief in metal.

4. Elastoidin

Elastoidin is a substance related to collagen and obtained in the form of long, thin fibers from the fins of cartilaginous fish (*Carcharias, Mustelus, Scylliorhinus*, etc.). According to Champetier and Fauré-Fremiet[126] the fibers yield an x-ray diagram very similar to that of collagen.

Like collagen, elastoidin contracts on heating (Schmidt,[127] Fauré-Fremiet[128]); the reflections of the x-ray diagram disappear, and the contracted fibers show rubberlike elasticity. According to the observations of Picken[129] on the temperature coefficient of the elastic force, the mechanism of elastic return is the same as in rubber: contracted elastoidin is in the rubberlike state.

On cooling, contracted elastoidin lengthens spontaneously—a sign that a completely disordered arrangement of the chains, as in amorphous rubber, does not occur. The contracted elastoidin fiber must possess a structure similar to that which we shall discuss for elastic fibers. According to Schmidt, the birefringence of the contracted fiber is evidence that the structure is not completely amorphous. The birefringence of the normal fiber is positive and uniaxial, that of the contracted fiber negative and uniaxial.

[126] G. Champetier and E. Fauré-Fremiet, *J. chim. phys.*, **34**, 197 (1937).

[127] W. J. Schmidt, *Z. Biol.*, **81**, 193 (1924); *Die Doppelbrechung von Karyoplasma, Cytoplasma und Metaplasma*, Borntraeger, Berlin, 1937.

[128] E. Fauré-Fremiet, *Arch. anat. micr.*, **33**, 81 (1937). E. Fauré-Fremiet and R. Woelfflin, *J. chim. phys.*, **33**, 801 (1936).

[129] L. E. R. Picken, *J. chim. phys.*, **34**, 764 (1937).

5. Ichthyocol

Ichthyocol may be obtained from the swim bladder of fish by digestion with trypsin. According to Fauré-Fremiet[130] that obtained from the commercial product "coeur de Cayenne" (from *Arius luniscutis*) forms fine, glistening needles 0.25–0.3 mm. long. These yield an x-ray diagram corresponding to that of collagen. They dissolve in water at 62°C. Formamide and concentrated sodium thiocyanate solution slowly dissolve the needles at room temperature. Ichthyocol may be precipitated from these solutions by the addition of acetone, or acetone and alcohol. In this condition, it is amorphous and soluble in water; it is not attacked by trypsin. In the case of ichthyocol, therefore, the destruction of the lattice on solution can be separated from the irreversible process of hydrolysis (Cherbuliez, Jeannerat, and Meyer[131]). The destruction of the lattice is reversible to the extent that aqueous solutions of ichthyocol (if not too dilute) gelatinize after a short period; this is equivalent to crystallization.

Whereas formamide leads to solution without hydrolysis, warm water and cold calcium thiocyanate solution lead to breakdown. Papain digests the solution, which is resistant to trypsin.

6. Elastic Fibers

Elastic fibers, which display rubberlike elasticity, occur in the walls of blood vessels and are particularly abundant in the *ligamentum nuchae* of the ox; they consist principally of units of simple amino acids—glycine, alanine, and leucine (see Table VIII). The fibers are therefore poor in basic or acidic groups, as is also shown by their slight staining properties.

TABLE VIII

Amino Acids of Elastin[132]

Amino acid	Amount, % by wt.	Amino acid	Amount, % by wt.
Leucine fraction	30.0	Tyrosine	1.6
Glycine	29.4	Arginine	1.0
Valine	13.5	Cystine	0.23
Proline	15.2	Methionine	0.38
Phenylalanine	3.34	Amide N	0.04
Hydroxyproline	2.0		

Not found: alanine, dicarboxylic acids, histidine, and tryptophane.

[130] E. Fauré-Fremiet and A. Cougny, *Bull. muséum hist. nat. Paris*, **9**, 188 (1937).
[131] E. Cherbuliez, J. Jeannerat, and K. H. Meyer, *Z. physiol. Chem.*, **255**, 241 (1938).
[132] W. H. Stein and E. G. Miller, Jr., *J. Biol. Chem.*, **125**, 599 (1938).

Unstretched elastic fibers are not birefringent (Schmidt[133]); they may be stretched up to 100–150%, and in this condition show positive uniaxial birefringence and an indefinite fiber diagram. They are therefore built up from peptide chains which, in the unstretched condition, are for the most part unoriented and not extended—like the chains in amorphous rubber. On stretching, the chains are straightened and brought to parallelism (Meyer, 1929). From this position, the chains are induced by thermal agitation to return to the disordered and thermodynamically more probable

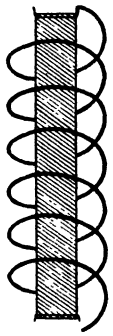


Fig. 8*a*. Model exhibiting thermoelastic properties of the elastic ligament.

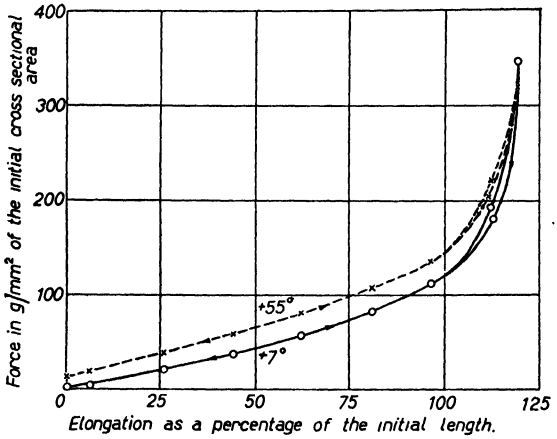


Fig. 8*b*. Stress–strain curves of elastic ligament (Meyer and Ferri).

twisted configuration (Wöhlisch; Meyer, von Susich, and Valkó). Later experiments by Meyer and Ferri[134] have confirmed this interpretation of the nature of the elastic properties. On drying, the fibers lose their elastic properties.

Even when unstretched, the fibers show a negative coefficient of expansion in the direction of the fiber axis. It is suggested that two systems under tension are present, and that their tensions are opposed. If the systems possess different temperature coefficients, the structure will not be in equilibrium on warming, but will contract or expand. A model displaying such properties is provided by a system consisting of a rubber band which is kept stretched by means of a steel spring (Fig. 8*a*). Since the tension of

[133] W. J. Schmidt, *Die Doppelbrechung von Karyoplasma, Cytoplasma und Metaplasma*. Borntraeger, Berlin, 1937.

[134] K. H. Meyer and C. Ferri, *Arch. ges. Physiol. Pflugers*, **238**, 78 (1936).

the rubber increases on warming, while that of the spring remains nearly constant, the system will contract as the temperature rises. The same behavior would be shown by a rubber band, maintained in the stretched position by transverse bands, exerting compression at right angles to the long axis. Histological examination shows that such a structure does, in fact, exist in the *ligamentum nuchae*; the ligament contains abundant collagen fibers arranged transversely to the direction of the elastin fibers. The temperature coefficient of the elastic force of collagen fiber is negative, while that of the elastic fibers is positive. As a result, the ligament contracts on warming and expands once more on cooling.

Such a dual system might also exist at the molecular level: a system consisting of parallel primary valence chains maintained in the parallel position by transverse linkages may exhibit the properties of the elastic ligament. With rising temperature, the contraction will increase, while the constraint exerted by the cross linkages either diminishes or remains constant. Such a system exists in tendon which has been treated with formaldehyde; the parallel protein chains are held together by methylene (CH_2) bridges.

If the ligament is stretched under considerable tension, the temperature coefficient of the elastic force becomes negative. The chains are then fully stretched, and further deformation leads to the distortion of valence angles, and, hence, to a rise in potential energy, which determines the change in sign of the temperature coefficient (see Chap. I, sect. I 2*b*). Figure 8*b* shows the stress–strain curve (elastic force as a function of elongation) of a piece of elastic ligament at 7°C. and at 55°C. The extensibility varies from ligament to ligament. In this case the reversal in the sign of the temperature coefficient of the elastic force occurred at 115% elongation.

An x-ray study of the structure of elastin has been made by Kolpak.[135] He finds that the unstretched ligament yields only diffuse rings while, when strongly stretched and dried, a diffuse fiber diagram appears.

7. Keratins

(*a*) *Chemical Composition*

The keratins of wool, hair, whale bone, horn, etc., are insoluble in all solvents, swell to a limited extent, and are relatively resistant to the action of hydrolytic agents. The amino acids present in the products of hydrolysis of wool are shown in Table IX on page 546.

[135] H. Kolpak, *Kolloid-Z.*, **73**, 129 (1935).

It is natural to associate the high cystine content,[140] which in undamaged wool is almost equivalent to the total sulfur content, with the characteristic insolubility of the keratins, and to assume that the peptide chains of keratins are linked together by S—S bridges (Stary,[141] Astbury,[142] and, above

TABLE IX

Amino Acids of Wool

Amino acid	Amount, % by wt.	Amino acid	Amount, % by wt.
Glycine	0.6[136]	Aspartic acid	2.3[136,137]
Alanine	4.4[136]	Glutamic acid	12.9[136,137]
Valine	2.8[136]	Oxyglutamic acid	—
Leucine	11.5[136]	Arginine	10.2[138]
Serine	0.1[136]	Lysine	2.8[138]
Cystine	7.3, 13.1[138]	Histidine	0.7[139]
Lanthionin	0.5[138a]	Proline	4.4[136]
Tyrosine	4.8[138]	Tryptophane	1.8[138]

all, Speakman[143]). In good agreement with this is the observed sensitivity of keratin toward alkali sulfides, which reduce, and so rupture, S—S bridges. The solubility of hair in media containing alkali sulfides is, therefore, chemically comparable to the solubility of sulfur dyes in alkali sulfides.

The significance of S—S bridges for the cohesion of the chains has been questioned by Elöd[144] who found that, if wool is treated with water at 80°C. in the presence of mercury, sulfur is liberated and reacts with the mercury,

[136] E. Abderhalden and A. Voitinovici, *Z. physiol. Chem.*, **52**, 348 (1907).

[137] According to J. B. Speakman, and F. Townsend, *Nature*, **139**, 411 (1937), aspartic acid 7.25%, glutamic acid, 15.3%.

[138] H. R. Marston, *Bull. Council Sci. Industrial Research, Australia*, No. 38 (1928).

[138a] M. J. Horn and J. B. Jones, *J. Biol. Chem.*, **139**, 473 (1941).

[139] H. B. Vickery and R. J. Block, *J. Biol. Chem.*, **86**, 107 (1930).

[140] For more recent analyses see H. Glafey, D. Krüger, and G. Ulrich, *Technologie der Wolle*. Springer, Berlin, 1938, page 15.

[141] Z. Stary, *Z. physiol. Chem.*, **175**, 178 (1928).

[142] W. T. Astbury and A. Street, *Trans. Roy. Soc. London*, **A230**, 75 (1931). W. T. Astbury and H. J. Woods, *ibid.*, **232**, 333 (1933).

[143] J. B. Speakman, *J. Soc. Chem. Ind.*, **48**, 321T (1929); *Proc. Roy. Soc. London*, **A132**, 167 (1931); *J. Soc. Dyers Colourists*, **52**, 335, 423 (1936). J. B. Speakman and C. S. Whewell, *ibid.*, **52**, 380 (1936).

[144] E. Elöd, H. Nowotny, and H. Zahn, *Melliand Textilber.*, **21**, 385, 457, 617 (1940); **23**, 58 (1942); *Kolloid-Z.*, **93**, 50 (1940); **95**, 81 (1941). E. Elöd, *ibid.*, **96**, 284 (1941).

without change in the mechanical properties of the wool. Elöd's results are to some extent at variance, however, with those of Schöberl.[145]

(*b*) *Morphology of Wool Fibers*

Three different cellular layers can be distinguished in wool fibers: the cuticle (flattened, scalelike cells), the cortex (spindle-shaped cells), and the medulla (cylindrical cells). The fibers may be separated into their cellular elements: (*1*) by prolonged treatment with concentrated ammonia at room temperature; (*2*) by the action of buffered aqueous solutions of trypsin[146]; or (*3*) as a result of the activity of certain species of bacteria.[147] Taking advantage of the differential action of fuming nitric acid, concentrated hydrochloric acid, etc., on wool, Unna and Golodetz[148] and Trotman and Sutton[149] have separated two types of keratin from wool. According to Trotman, the cuticle consists primarily of the form with the higher sulfur content. Haller and Holl[150] report that a definite cementing substance—a protein, to which they give the name "lanain"—is present between the cells of the cortex and the other cellular structures. The molecule of lanain contains tryptophane, tyrosine, and histidine; cysteine or cystine groups are absent.[151] Between the scale cells and the fiber cells there is a carbohydrate-like or carbohydrate-containing substance, known as the "elasticum,"[152] which is responsible for the characteristic appearance of swelling in chlorinated water, used for the detection of damaged fibers (the von Allwörden reaction).

It is clear from this that "wool keratin" is by no means a homogeneous

[145] A. Schöberl, *Angew. Chem.*, **54**, 313 (1942); *Klepzig's Textil-Z.*, **45**, 41 (1942). For the action of sulfites on wool, see, among others, W. R. Meddlebrook and H. Phillips, *Biochem. J.*, **36**, 428 (1942).

[146] R. Burgess, *J. Textile Inst.*, **25**, 289T (1934).

[147] J. Bartsch, *Melliand Textilber.*, **12**, 760 (1931); **13**, 21 (1932).

[148] P. G. Unna and L. Golodetz, *Monatsh. prakt. Derm.*, **44**, 399, 459 (1907); **47**, 62 (1908).

[149] S. R. Trotman, E. R. Trotman, and R. W. Sutton, *J. Soc. Chem. Ind.*, **45**, 20T (1926).

[150] R. Haller, *Melliand Textilber.*, **17**, 645 (1936). R. Haller and F. W. Holl, *Kolloid-Z.*, **75**, 212 (1936).

[151] Concerning the properties of an intercellular substance isolated by pancreatic digestion, see also C. Strachejewa-Kaverznewa and N. Gavrilow, *Bull. soc. chim.* (5) **4**, 647 (1937).

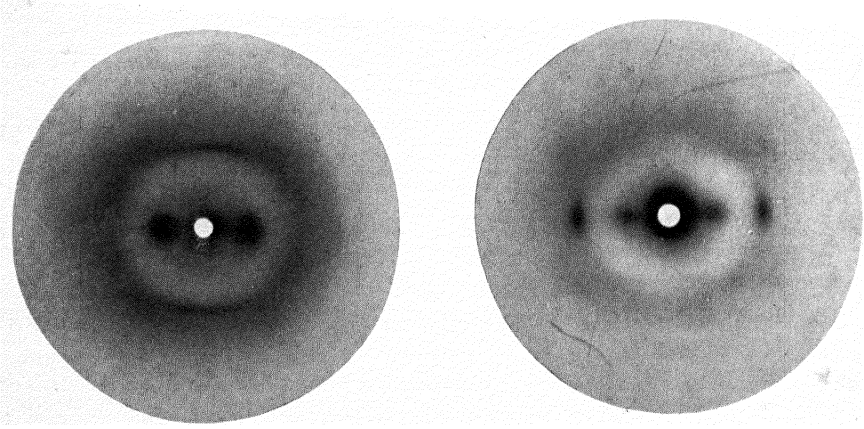
[152] K. von Allwörden, *Angew. Chem.*, **29**, 77 (1916). K. Schirm and H. Collé, *Melliand Textilber.*, **16**, 585, 667, 795 (1935).

substance, and conclusions concerning the structure of "keratin" must, from this point of view, be accepted with caution.

The characteristic scale structure of the wool cuticle and the peculiar elastic properties of wool are probably responsible for the ability of wool to form felt in a fulling mill, that is, under the influence of heat, pressure, and impact at a suitable *p*H.[153] The state of the cuticle (whether damaged or intact) determines the accessibility of the fiber to various reagents and hence, in many cases, the possibility of, or the velocity of, reaction.

(*c*) *The X-Ray Diagrams of Wool and Hair*

The microscopic structure of the cortex which forms the main part of the fibers in hair and wool, that is, the assemblage of long, spindle-shaped cells showing positive and uniaxial birefringence, suggests an oriented molecular



α-keratin
(Cotswold wool unstretched)

β-keratin
(Cotswold wool, 80% stretched)

Fig. 9. X-ray diagrams of keratin (Astbury).

structure. This was proved by Astbury's important observation that hair and wool yield a fiber diagram, which he ascribed to a modification of keratin, distinguished by him as α-keratin (Fig. 9). By no means all sulfur-containing, insoluble proteins called keratins yield the x-ray diagram of α-

[153] J. B. Speakman, *Am. Dyestuff Reptr.*, **21**, 214, 235 (1932). J. B. Speakman, E. Stott, and H. Chang, *J. Textile Inst.*, **24**, 273T (1933).

keratin. Fauré-Fremiet and Champetier[154] found no trace of the reflections characteristic of α-keratin in observations on the egg cases of skates and dogfish.

If hair or wool is allowed to soften in hot water, dilute alkali, or pure formic acid,[155] it can be stretched up to 100% and shows rubberlike elasticity: when the tension is removed, contraction takes place, and the hair returns approximately to its original length. From the thermodynamic analysis made by Woods[156] for wool keratin and by Lundgren[157] for fibers from feather keratin, it follows that the restoring force, as in rubber and other elastic high polymers, arises from thermal agitation of segments of the protein chains oriented by stretching.

The best softener for hair is an aqueous solution of sodium thioglycolate;[157a] it is used in "cold permanent waving." Hair swollen in thioglycolic acid exhibits "ideal" rubberlike behavior (see Chap. I, sect. I2 *a*).

Hair which has been steamed for a short period in the stretched condition, contracts to a length even shorter than the original length, while hair which has been heated for a longer period loses the power of shortening (permanent set). These phenomena have been investigated in detail by Astbury, Street, and Woods.[158] The most important result of this work was the discovery of the fiber diagram of stretched hair, described by Astbury as the fiber diagram of β-keratin (Fig. 9). From this discovery Astbury concluded that β-keratin, like silk, contains stretched polypeptide chains, and that these are held together laterally, in part by secondary valence linkages, in part by primary valence linkages. In α-keratin, on the other hand, the chains are supposed to be folded up in a definite way and hence to be shortened. We shall discuss this hypothesis in detail later.

The remarkable phenomenon of permanent set is ascribed by Astbury and Speakman[159] to the formation of cross linkages during heat treatment, so that the hair structure is strengthened and, to a certain extent, vulcan-

[154] G. Champetier and E. Fauré-Fremiet, *Compt. rend.*, **207**, 1133 (1938). See also C T. Baudouy, *J. chim. phys.*, **36**, 41 (1939).

[155] J. B. Speakman, *Proc. Roy. Soc. London*, **A132**, 167 (1931).

[156] H. J. Woods, *Nature*, **157**, 229 (1946).

[157] H. P. Lundgren, A. M. Stein, V. M. Koorn, and R. A. O'Connell, *J. Phys. & Colloid Chem.*, **52**, 180 (1948).

[157a] K. H. Meyer and C. Haselbach, *Nature*, **164**, 33 (1949).

[158] W. T. Astbury and A. Street, *Trans. Roy. Soc. London*, **A230**, 75 (1931). W. T Astbury and H. J. Woods, *ibid.*, **232**, 333 (1933). W. T. Astbury, *Chem. Weekblad*, **33**,777 (1936); *J. Textile Inst.*, **27**, 281 (1936); *Melliand Textilber.*, **76**, 1 (1935), etc. See also *The Fundamentals of Fiber Structure*, Oxford Press, London, 1933.

[159] J. B. Speakman, *J. Soc. Dyers Colourists*, **52**, 423 (1936).

ized. These authors have put forward certain views on the nature of these bridge linkages. It is supposed that —SNH—, —SBaS— (after subsequent treatment with salt solutions) or —SS— linkages (from the oxidation of —SH— linkages) may be formed. It is conceivable, however, that the hair may lose its contractility as a result of a kind of crystallization, such as occurs in the formation of the silk thread.

Elöd's observations, already referred to, showed that with half the —S—S— linkages destroyed wool neither undergoes supercontraction nor loses the ability to supercontract on treatment with bisulfite solution. But these results and, in particular, the proportion of —S—S— linkages destroyed, are disputed by Schöberl (see sect. 7*a* above).

Harrison[160] has criticized the tendency to refer the phenomena of supercontraction and permanent set to the folding of the keratin chains and to the formation or rupture of cross linkages. According to Harrison, it is certain that the swelling and shrinking powers of the various keratin phases also play a part in these phenomena.

If acids or oxidizing agents such as mercuric or silver nitrate solutions are allowed to act for a prolonged period on wool, the x-ray diagram obtained is that of a breakdown product to which Elöd[161] has given the name of d-keratin (denatured keratin) because of the similarity of the diagram to that of denatured egg albumin (Astbury).

Electron photomicrographs of wool and mercury–wool indicate values for the dimensions of the micelles in agreement with those of Speakman and Astbury.[162]

(*d*) *Fine Structure of α- and β-Keratin*

The characteristic differences between the α- and β-keratin diagrams are as follows: The α diagram shows an arc on the meridian corresponding to a spacing of 5.1 Å., while the β diagram shows an arc corresponding to a spacing of 3.4 Å.; the α-keratin diagram also shows a definite reflection on the equator corresponding to a spacing of 9.8 Å. This reflection persists in the β diagram, and, in addition, a strong equatorial reflection appears, corresponding to a spacing of 4.5 Å. Moreover, a number of reflections from oblique planes are visible, so that the diagram of β-keratin resembles that of silk fibroin.

[160] W. Harrison, *J. Soc. Chem. Ind.*, **56**, 731 (1937).

[161] E. Elöd, H. Nowotny, and H. Zahn, *Kolloid-Z.*, **93**, 50 (1940). E. Elöd, *ibid.*, **96**, 284 (1941).

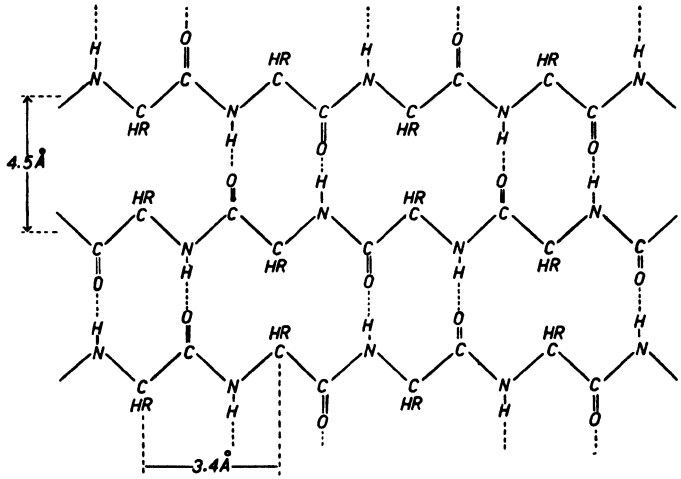
[162] H. Zahn, *Melliand Textilber.*, **21**, 50 (1940).

If stretched horn or horse hair is pressed in steam, the flattened product is found to be the β form, but of a higher degree of orientation. X-ray photographs, taken with the beam at right angles to, and parallel to, the plane of the flattened specimen, show that two sets of net planes parallel to the fiber axis occur, approximately at right angles to each other; the spacing of the one set is 9.8 Å., that of the other 4.5 Å. Astbury interprets these spacings and the fiber period of 3.4 Å. as follows:

b = 3.4 Å. = the length of a peptide unit along the fiber axis, equal to the length of a unit in silk fibroin.

a = 4.5 Å. = the spacing of the peptide chains from each other (backbone spacing).

c = 9.8 Å. = the spacing of the planes in which the peptide chains lie. According to Astbury, this is determined by the length of the side chains standing approximately at right angles to these planes (mean length of side chain, side-chain spacing).



Structure of β-keratin (Astbury)

Here Astbury makes the oversimplifying assumption that the spacing of the chains, that is to say, the spacing of the scattering centers, is equivalent to a net plane spacing. If we postulate for the spacing of the chains a value of 4.8 Å., such as we should expect if hydrogen bonds are formed, and such as has in fact been found in the case of other substances, we have an arrangement such as that shown in figure 10*a* with molecules spaced at 4.8 and 10.4 Å. As Nowotny and Zahn[163] justifiably point out, the strongest

[163] H. Nowotny and H. Zahn, *Z. physik. Chem.*, **B51**, 265 (1942).

bond between the chains is the hydrogen bond. One may therefore assume, that the grids of wool keratin lie in the plane a. The lateral residues will lie obliquely to this in the c plane.

A comparison of the diagrams of α- and β-keratin shows that the macroscopic lengthening of the fiber is accompanied by profound rearrangement of the atoms; as Astbury points out, the shape of the protein chains has changed. Whereas contracted elastic collagen (in the contraction of which a similar change in molecular form occurs) shows no regular, crystal-like arrangement of the atoms, in this case a certain degree of orientation persists even in the contracted condition, as the x-ray diagram of α-keratin shows.

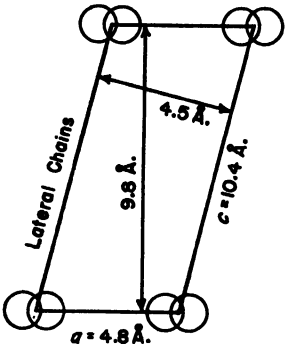


Fig. 10a. Projection of probable arrangement of peptide chains in β-keratin.

Any model of the crystalline structure of α-keratin must not only agree with the x-ray diffraction data, but must allow for a simple rearrangement which will permit an elongation of about 100% without any change in density. Astbury's first interpretation was that primary grids were folded into hexagonal rings in the α configuration, in such a way as to bring the carboxyl groups of the amino acid residue opposite the imino groups of the next, with the formation of a hydrogen bond between them. Under tension, the CO...NH bonds were supposed to break, so that the grids could be extended to the usual zigzag form. This structure, however, requires a distance of only 1.3 Å. for the CO...NH bond, which is less than half its usual value and does not allow room for the side chains (Neurath, Meyer[164]). To meet these objections, Astbury and Bell[165] have proposed a new model for α-keratin in which the grids are bent into square folds, with side chains directed alternately up and down. We are, however, of the opinion that at the present time the experimental data are far from being sufficient for a molecular model.

(*e*) *Form of Chains in Fibrillar Proteins*

From his studies of various proteins Astbury[166] has drawn certain general conclusions, which in many quarters are regarded as beyond question and

[164] H. Neurath, *J. Phys. Chem.*, **44**, 296 (1940). K. H. Meyer, *Die hochpolymeren Verbindungen*, Akadem. Verlagsgesellschaft, Leipzig, 1940, page 446.

[165] W. T. Astbury and F. O. Bell, *Nature*, **147**, 696 (1941).

[166] W. T. Astbury, *Nature*, **157**, 321 (1946).

must therefore be given careful consideration here. According to Astbury, there are two groups of fibrous proteins: the keratin-myosin-fibrinogen group and the collagen group. "These groupings exist despite marked variations in amino acid composition in both groups. The keratin-myosin-fibrinogen group is distinguished by long-range elasticity, based on a particular folding of the main polypeptide chains of the proteins... The

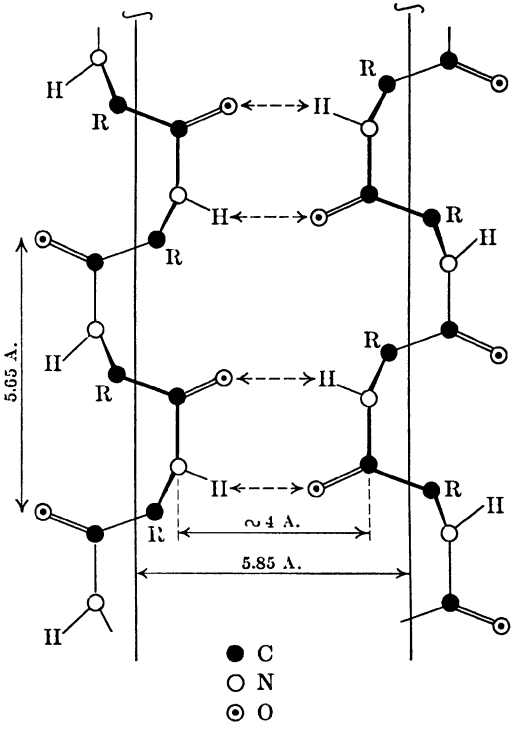


Fig. 10*b*. Hydrogen bonds between spiral peptide or protein chains.

proteins of the collagen group have the chains extended in a manner differing from that which occurs in the keratin group."

Against this, is the objection that long-range reversible extensibility may be observed under suitable conditions in all fibrillar proteins, just as in the case of all other chain polymers: in silk fibroin as it emerges from the silk gland; in collagen after it has been heated, etc. As is clear from the thermoelastic behavior, this reversible extensibility has the same origin as that of other elastomers: oriented segments tend to return to a disoriented condition under the influence of thermal agitation. The phe-

nomenon does not, therefore, involve specific forces of attraction, leading to a definite folding of the chains in the contracted condition. Only in the stretched condition are the extended chains arranged in a definite way. The presence or absence of long-range reversible extensibility provides, therefore, no basis for the classification of the proteins. Still less can such a classification be justified by the results of x-ray analysis.

A valuable result of Astbury's work has been the observation that stretched myosin, as well as considerably stretched keratin, gives an x-ray diagram similar to that of silk, and therefore presumably contains peptide chains similar in shape to those of silk. Fibrin also (see sect. III*g*) has the same fiber period and without doubt essentially the same structure. Zigzag protein chains, the presence of which in silk was postulated by K. H. Meyer, occur, therefore, in several fibrillar proteins. In these the chains are probably united by hydrogen bonds to form lamellae, as Astbury assumes in the case of β-keratin and Brill in the case of silk.

In addition to the zigzag type of chain, another type occurs, the existence of which was postulated by Lotmar and Picken[167] in their analysis of an unusual x-ray photograph obtained from a stretched and dried muscle (see Fig. 11*b*). In this type the peptide unit has a length of only 2.8 Å. and the chain is in the form of a spiral as shown in figure 10*b*, so that as compared with that of silk it is somewhat shortened. Here again, according to Lotmar and Picken, the presence of hydrogen bonds may be assumed, but the interdigitation of the chains is different from that in the grids of the silk chains. The two types of chains are shown schematically in figure 10*b* and in figure 4.

This second form is apparently not limited to muscle; as mentioned previously it probably also occurs in collagen, since the latter is converted into the silk form under the influence of alkali. Presumably the choice of one or the other chain configuration is determined by the substituents, as well as by external factors, such as tension. We are dealing, from all appearances, with an instance of polymorphism—a phenomenon widespread among long-chain polymers (*cf.* gutta-percha, cellulose, amylose).

(*f*) *Physicochemical and Chemical Behavior of Hair and Wool*[168]

Hair and wool absorb considerable quantities of water. The absorption of the first fraction of water is accompanied by the evolution of consider-

[167] W. Lotmar and L. E. R. Picken, *Helv. Chim. Acta*, **25**, 538 (1942).

[168] E. Valkó, *Kolloidchemische Grundlagen der Textilveredlung*, Springer, Berlin, 1937, pages 506, 555, 559. H. Glafey, D. Krüger, and G. Ulrich, *Technologie der Wolle*, Springer, Berlin, 1938, page 15.

able heat, and by a noticeable diminution in volume. The partial molar heat evolved amounts to about 3000 cal. per mole both for wool (Hedges,[169] Schoesing[170]) and for human hair (Fricke and Lüke[171]) at about 4% of water, and falls to zero after 30% water uptake. The water which is first absorbed has an apparent density of 2, and the partial entropy of the water diminishes during absorption. These are indications that water is bound to definite groups of the keratin chains (see Chap. H, Table V). During the uptake of water, the length increases by 1–2% while the cross-sectional area increases by 35–39%. This anisotropic swelling is in itself an indication of the anisotropic, primary valence chain structure of the hair.

Wool fibers absorb acid to the extent of one equivalent of acid to 1200 g. of wool—independent of the nature of the acid (Meyer and Fikentscher,[172] Speakman[173]). Assuming a mean amino acid residue weight of 120, this means that 10% of the amino acid units carry basic groups. This is in agreement with the results of analysis of the products of hydrolysis. One third of the basic nitrogen is primary amino nitrogen.

According to Elöd[174] the isoelectric point of wool keratin lies at *p*H 4.9, while Speakman,[175] as a result of experiments on the relation between the *p*H of the solution and: (*1*) the acid–base binding powers, and (*2*) the resistance to stretching, finds an "isoelectric zone" of minimal action between *p*H 5 and *p*H 7. In order to avoid damaging the wool as far as possible during manufacture, it is now customary to perform all operations (in particular, washing) at a *p*H in the neighborhood of the isoelectric point.

Alkalis also react with keratin, forming salts with the acid groups of the keratin molecule. It has not as yet been possible to determine a limiting value for the alkali uptake, or equivalent proportions between the amount of alkali taken up in the case of various alkalis, since the process of salt formation is masked by adsorption phenomena and by hydrolytic breakdown, inasmuch as wool is extremely sensitive to alkalis.[176] The maximal base-

[169] J. J. Hedges, *Trans. Faraday Soc.*, **22**, 188 (1926); *J. Textile Inst.*, **18**, 350 (1927).

[170] Quoted by E. Valkó, *Kolloidchemische Grundlagen der Textilveredlung*. Springer, Berlin, 1937, page 101.

[171] R. Fricke and J. Lüke, *Z. Elektrochem.*, **36**, 308 (1930).

[172] K. H. Meyer and H. Fikentscher, *Melliand Textilber.*, **7**, 605 (1926).

[173] J. B. Speakman, *J. Soc. Dyers Colourists*, **49**, 180 (1933). J. B. Speakman and S. G. Smith, *ibid.*, **52**, 121 (1936).

[174] E. Elöd and E. Silva, *Z. physik. Chem.*, **A137**, 142 (1928).

[175] J. B. Speakman and M. C. Hirst, *Trans. Faraday Soc.*, **29**, 148 (1933). J. B. Speakman and E. Stott, *ibid.*, **30**, 539 (1934). See also J. B. Speakman, *J. Soc. Dyers Colourists*, **49**, 180 (1933).

[176] A. Lottermoser and W. Ettl, *Melliand Textilber.*, **11**, 627, 709 (1930). See also J. Powney and C. C. Addison, *Trans. Faraday Soc.*, **34**, 1439 (1938). M. Harris and H. A. Rutherford, *J. Research Natl. Bur. Standards*, **22**, 535 (1939).

binding capacity certainly exceeds 0.78 milliequivalent of alkali per gram of wool.[177]

Speakman and Stott[178] found a swelling maximum for wool in dilute strong acids as required by the theory of Procter and Wilson (sect. III 3*f*) at about *p*H 1. In solutions of weak acids, such as chloroacetic acid, the swelling increases steeply and continuously with the concentration of the acid, as the theory predicts, and reaches very high values. Wool does not show a sharp swelling minimum at a definite *p*H (the isoelectric point) but a zone of minimal swelling from *p*H 5 to *p*H 7.[179]

The swelling also increases with increasing alkalinity; in contrast to swelling in acid, however, the osmotic swelling effect is complicated by structural changes in the hair and hydrolysis of the keratin, even at moderate *p*H values, as is shown by a diminution in mechanical strength. After short treatment with strong sodium hydroxide (34–45%) at room temperature or at lower temperatures, however, it is true that an increase in the tensile strength may occur (Buntrock, 1898); this may be due to superficial gelation of the fiber.[180]

The uptake of acid and alkali is accompanied by changes in the mechanical properties of wool. Above *p*H 7 the extensibility increases rapidly and continuously with the *p*H, while at *p*H <5 the extensibility increases at first and then—like the swelling—passes through a maximum at *p*H ~ 1.[181] According to Speakman, a straight line is obtained if the fall in extensibility is plotted against the quantity of hydrochloric acid taken up. The contractility of wool passes through a maximum at *p*H 10, while acids increase extensibility without influencing the contractility; these relationships, together with the relation between *p*H and swelling, determine, according to Speakman, the influence of *p*H on the velocity of shrinkage during acid and alkaline fulling.[182]

Wool dissolves in concentrated solutions of strongly hydrated salts with considerable hydrolysis and with more difficulty than other proteins.

[177] J. Steinhardt and M. Harris, *J. Research Natl. Bur. Standards*, **24**, 335 (1940). J. Steinhardt, C. H. Fuggitt, and M. Harris, *ibid.*, **25**, 519 (1940).

[178] J. B. Speakman and E. Stott, *Trans. Faraday Soc.*, **30**, 539 (1934); **34**, 1203 (1938).

[179] D. Jordan-Lloyd, *Nature*, **130**, 34 (1932). J. B. Speakman, *J. Soc. Dyers Colourists*, **49**, 180 (1933). J. B. Speakman and M. C. Hirst, *Trans. Faraday Soc.*, **29**, 148 (1933).

[180] J. B. Speakman, *J. Soc. Chem. Ind.*, **48**, 321T (1929).

[181] J. B. Speakman and M. C. Hirst, *Trans. Faraday Soc.*, **29**, 148 (1933). J. B. Speakman, *J. Soc. Dyers Colourists*, **49**, 180 (1933); *J. Intern. Soc. Leather Trades' Chemists*, **17**, 229 (1933).

[182] J. B. Speakman, *Am. Dyestuff Reptr.*, **21**, 214, 235 (1932); *Textile World*, **83**, 1629 (1933). J. B. Speakman and E. Stott, *J. Textile Inst.*, **22**, 339T(1931). J. B. Speakman, E. Stott, and H. Chang, *ibid.*, **24**, 273T (1933).

According to von Weimarn,[183] on heating with saturated lithium thiocyanate solution to 170–200°C. wool forms a transparent jelly and eventually a colloidal solution. Wool is only slowly attacked by cuprammonium solution, and even in quaternary organic ammonium compounds it only dissolves at 150–180°C. and then with profound modification.[184] On the other hand, according to Grafe, viscous solutions containing about 20% of protein can be obtained by treatment with sodium sulfide solutions under suitable conditions. But in these solutions also, the wool protein has undergone considerable breakdown.

The marked reactivity of the —S—S— linkage[185] plays a decisive role in the chemical transformations which wool and hair undergo in the presence of various reagents and which are of considerable practical significance in relation to damage done to wool in the usual methods of textile manufacture. Even with application of heat, wool is relatively slowly attacked by acids; on the other hand, even very dilute solutions of alkalis and alkali sulfides rupture the —S—S— bridges with the formation of —SH groups.[186] Hydrolysis of the —S—S— linkages with initial formation of —SH groups also takes place as a result of prolonged treatment with hot water only. The —SH groups are later transformed into other sulfur compounds.[187] In the presence of heavy metals or of the salts of heavy metals, wool becomes colored on being heated in water, owing to precipitation of metallic sulfides on the fibers, due to the liberation of hydrogen sulfide.[188,189] The S—S groups may be reduced by treatment with thioglycolic acid, and the —SH groups so liberated may then be alkylated.[190]

(g) *Behavior of Wool toward Dyes*[191]

The dyes used for staining wool are mostly so-called acid dyes, that is, sodium salts of organic sulfonic acids which are applied in an acid bath,

[183] P. P. von Weimarn, *J. Textile Inst.*, **17**, 642T (1926).

[184] K. Grafe, *Angew. Chem.*, **49**, 303 (1936).

[185] A. Schöberl, *Angew. Chem.*, **53**, 227 (1940).

[186] M. Harris and J. A. Crowder, *J. Research Natl. Bur. Standards*, **16**, 475 (1936). A. Schöberl and P. Rambacher, *Biochem. Z.*, **306**, 269 (1940). A. Schöberl, *Angew. Chem.*, **54**, 313 (1941).

[187] A. Schöberl, *Angew. Chem.*, **54**, 313 (1940).

[188] J. B. Speakman, *Nature*, **132**, 930 (1933). A. Schöberl, *Collegium*, **7**, 412 (1936).

[189] E. Elöd, H. Nowotny, and H. Zahn, *Melliand Textilber.*, **21**, 385, 456, 617 (1940); **23**, 58 (1942). A. Schöberl, *Klepzig's Textil-z.*, **45**, 41 (1942).

[190] W. I. Patterson, W. B. Geiger, L. R. Mizell, and W. Harris, *J. Research Natl. Bur. Standards*, **27**, 89 (1941).

[191] Detailed accounts will be found in E. Valkó, *Kolloidchemische Grundlagen der Textilveredlung*, Springer, Berlin, 1937. H. Glafey, D. Krüger, and G. Ulrich, *Technologie der Wolle*, Vol. 8 (3B), of *Technologie der Textilfasern*, by R. O. Herzog, Springer, Berlin, 1938.

where they act as free acids and unite with basic groups to form insoluble salts. The formation of salts was first recognized by Knecht,[192] who observed that at saturation the amounts of various acid dyes taken up are proportional to their equivalent weights. It was then shown by Vorländer and Perold[193] that there is no essential difference between the capacity of wool to absorb colorless acids, such as sulfuric acid, and its capacity to absorb acid dyes. Other important arguments in favor of the interpretation of dyeing as salt formation were advanced by Suida[194] who showed that the affinity for dyes is influenced by the saturation or chemical modification of the groups capable of salt formation. Bader[195] later confirmed the result that at saturation wool fibers absorb equivalent quantities of acid dyes, and this result was then expressed in a more general form, and extended to acids which are not dyes, by Meyer and Fikentscher.[172]

Dyestuffs containing chromium bound in a complex manner are taken up with particular avidity by wool in a strongly acid bath; it seems probable that the chromium unites with the basic groups of the wool in a complex fashion.

The density of wool measured in helium decreases after dyeing with acid dyes,[196] while the x-ray pattern remains unchanged.[197] Heertjes concludes from this that the dye does not penetrate into the crystalline regions of the fiber, and that this fraction only amounts to a small proportion of the total fiber substance.

The relationship between the *p*H of the dyeing bath and: (*1*) the velocity of dyeing, (*2*) the amount of dye absorbed, is what would be expected if the Donnan equilibria between cations and colorless and colored anions, and the dependence of swelling on *p*H, are taken into account.[198] At the present time it is impossible to define exactly the effect of physical and histological factors on the dyeing of wool. The undamaged cuticle, for example, is extremely resistant to the penetration of dyes[199] and, according to

[192] E. Knecht, *Ber.*, **37**, 3479 (1904).

[193] D. Vorländer and A. J. Perold, *Ann.*, **345**, 288 (1906).

[194] W. Suida, *Monatsh.*, **26**, 413, 855 (1905). P. Gelmo and W. Suida, *ibid.*, **27**, 225 (1906). See also K. Gebhardt, *Angew. Chem.*, **27**, 297 (1914).

[195] M. Bader, *Dissertation.* Lausanne, 1916.

[196] P. M. Heertjes, *Rec. trav. chim.*, **60**, 91 (1941).

[197] W. T. Astbury and J. A. F. Dawson, *J. Soc. Chem. Ind.*, **54**, 6 (1938).

[198] E. Elöd and E. Silva, *Z. physik. Chem.*, **A137**, 142 (1928); *Melliand Textilber.*, **10**, 707 (1929). E. Elöd and F. Böhme, *ibid.*, **13**, 365 (1932). E. Elöd, *ibid.*, **12**, 260 (1931); **17**, 67 (1936); *Trans. Faraday Soc.*, **29**, 327 (1933). J. B. Speakman, *J. Soc. Dyers Colourists*, **52**, 121 (1936).

[199] R. Haller, *Melliand Textilber.*, **18**, 5 (1937).

Haller,[200] the various types of cells in the wool fiber behave differently with respect to dyes.

The molecular weight of acid wool dyes is on the whole considerably lower than that of the substantive dyes used for cotton.[201] In general, acid wool dyes show no affinity for wool when in a neutral salt bath, since under these conditions they are too soluble in water. On the other hand, substantive dyes are insoluble or highly associated as free acids, so that they are unable to penetrate the pores of the wool fiber.

The uptake of basic dyes depends at least in part on salt formation between acid groups of the keratin molecule and basic groups of the dye. This point of view also dates from Knecht.[202]

(*h*) *Feather Keratin*

A particularly fine fiber diagram has been obtained from feather keratin (Astbury[203]). It appears very desirable to extend the study of this substance on the chemical side as well, since there is some hope in this case of obtaining a picture of the arrangement and shape of the chains from a knowledge of chemical composition and physical properties. The same is true of porcupine quill which, according to McArthur, gives a very beautiful diagram with many interferences.[204]

8. Muscle Proteins and Their Significance in Process of Muscular Contraction[205]

(*a*) *Muscle Proteins*

Four distinct proteins are present in muscle: (*1*) water-soluble myogen (von Fürth[206]), albuminoid in character; (*2*) muscle globulin (also called globulin-X), which is obtained in expressed muscle juice, and is precipitated on dialysis against pure water; (*3*) myosin, extracted from muscle by

[200] R. Haller, *Helv. Chim. Acta*, **19**, 15 (1936).

[201] See also E. Valkó, *J. Soc. Dyers Colourists*, **55**, 173 (1939).

[202] E. Knecht, *Ber.*, **21**, 1556 (1888). E. Knecht and J. R. Appleyard, *ibid.*, **22**, 1120 (1889).

[203] W. T. Astbury and H. J. Woods, *J. Textile Inst.*, **23**, 17T (1932).

[204] I. McArthur, *Nature*, **152**, 38 (1943).

[205] General accounts: H. H. Weber, *Naturwissenschaften*, **27**, 33 (1939); *Biochim. et Biophys. Acta*, **4**, 12 (1950). G. Boehm, in L. Lichtwitz, R. E. Liesegang, and N. S. Spiro, *Medizinische Kolloidlehre*, Steinkopff, Dresden, 1933. W. F. H. M. Mommaerts, *Muscular Contraction*, Interscience, New York, 1950.

[206] O. von Fürth, *Arch. exptl. Path. Pharmakol.*, **36**, 231 (1895).

0.6 *N* potassium chloride solution at *p*H 8–9 (Muralt and Edsall[208]); and (*4*) insoluble and, presumably, cross-linked stroma protein. Table X (after H. H. Weber) summarizes the data available concerning the isoelectric point, relative viscosity, molecular weight, and amount of these proteins. For comparison, a few data referring to serum albumin and globulin are added.

TABLE X

Proteins of Rabbit Muscle

Protein	Per cent of muscle protein		Isoelectric point	η_{rel} 1% sol.	Mol wt. (from osmotic pressure measurements)
	White muscle	Red muscle			
Myogen	22	17	*p*H 6 3	1 04	81,000
Globulin X	22	17	*p*H 5 1	1.14–1 20	140,000–180,000
Myosin	39	39	*p*H 5.4	$\geqq 10$	$\sim 10^6$
Stroma	17	27	—	—	—
Serum albumin	—	—	—	1.04	70,000
Serum globulin	—	—	—	1.18	104,000–175,000

TABLE XI

Rabbit Myosin[209]

Amino acid	Amount, g./100 g. of protein	Amino acid	Amount, g./100 g. of protein
Arginine	7.0	Tryptophane	0.8
Histidine	1 7	Glycine	1.9
Lysine	10.3	Alanine	6.5
Glutamic acid	22.1	Leucine	15.6
Aspartic acid	8 9	Valine	2.6
Tyrosine	3.4	Proline	1.9
Serine	3 9	Phenylalanine	4.3
Threonine	4.95	Total N	16.6–16.8
Cystine	1.4	Amide N	1.19
Methionine	3.4	Total S	1.10

The composition of myosin is given in Table XI; very little is known about the other muscular proteins. With the aid of the ultracentrifuge, Gralén[210] has shown the molecular weight of a crystallized myogen, myogen A, to be 150,000. The viscosity of myogen (like that of serum albumin)

[208] A. L. von Muralt and J. T. Edsall, *J. Biol. Chem.*, **89**, 366 (1931).

[209] K. Bailey, *Advances in Protein Chem.*, **1**, 310 (1944).

[210] N. Gralén, *Biochem. J.*, **33**, 1341 (1939).

suggests that the molecules are spherical (H. H. Weber; see also sect. X 4*b*). Two crystalline myogens, A and B, have been obtained from rabbit muscle.[211] The molecular weight of myogen A amounts to *ca.* 150,000.

In addition to high viscosity, myosin displays marked birefringence of flow (Muralt and Edsall; Boehm and Signer[212]); both phenomena suggest the presence of long-chain molecules. Since birefringence of flow is observed when the shearing forces are quite small (on shaking the solution, for example), it may be assumed, as Weber suggests, that the protein particles possess high intrinsic birefringence and are present, not as tangled molecular threads, but as rodlets consisting of parallel, oriented primary valence chains. The observation that the molecular weight (determined osmotically) is lowered to 100,000 on the addition of a little urea also suggests that several molecules are associated to form a myosin particle. Under the electron microscope, fine filaments several thousand mμ long and 5 to 10 mμ thick are to be seen in preparations made from myosin solutions.[213] The intact fiber consists of a bundle of these fibrils.

Since, as we shall see later, the birefringent, contractile elements of muscle consist chiefly of myosin and since, on this account, myosin has come to be regarded as of great physiological significance, its chemical and physical behavior have been the subject of detailed study during the last few years. The results, however, do not as yet suffice to enable one to form a clear picture of the contractile process or, indeed, even to relate the process of contraction to the reactions observed.

The presence of free —SH groups, corresponding to a cysteine content of 0.4%, in solutions of myosin can be demonstrated by titration methods commonly used in protein chemistry. The addition of small quantities of certain substances, such as glycine, ammonium chloride, and arginine hydrochloride, reduces this value to zero, while guanidine hydrochloride and urea double or treble the value.[214] According to Edsall and Mehl,[215] flow birefringence and viscosity are very easily influenced; glycine has no effect, but guanidine hydrochloride, urea, arginine hydrochloride, calcium chloride, magnesium chloride, and potassium iodide reduce viscosity and destroy flow birefringence. There is no connection between the presence of cysteine —SH groups and these physical properties. According to Need-

[211] T. Baranowski, *Z. physiol. Chem.*, **260**, 43 (1939).

[212] G. Boehm and R. Signer, *Klin. Wochschr.*, **11**, 599 (1932); *Helv. Chim. Acta*, **14**, 1370 (1931).

[213] M. von Ardenne and H. H. Weber, *Kolloid-Z.*, **97**, 322 (1941); M. A. Jakus and C. E. Hall, *J. Biol. Chem.*, **167**, 705 (1948).

[214] J. P. Greenstein and J. T. Edsall, *J. Biol. Chem.*, **133**, 39 (1940).

[215] J. T. Edsall and J. W. Mehl, *J. Biol. Chem.*, **133**, 409 (1940).

ham[216] if adenosine triphosphoric acid is added to a solution of myosin the flow birefringence first diminishes and subsequently increases. In this reaction the latter acid is said to be split catalytically by myosin; myosin is therefore supposed to behave as an enzyme. The real enzyme can, however, be separated from myosin.[216a]

According to Schramm and Weber[217] two homodisperse components can be detected in purified myosin solutions in the ultracentrifuge. The heavier of these consists in all probability of aggregates of the lighter, molecularly dispersed component. Szent-Györgyi[218] finds that myosin is composed of two proteins, which constitute the muscular fibril: (*1*) homogenous myosin which can be obtained in a crystalline form, and (*2*) actin. Neither of these proteins is contractile without the other one; together they form a complex actomyosin. In the living fibril 2.5 parts of myosin are combined with one part of actin. Actomyosin may be brought to contraction by means of salts or adenosine triphosphoric acid; the contraction is reversible. In the course of the contraction swelling decreases. If action is extracted from acetone dried muscle by means of KCl solution it dissolves in its globular form with low viscosity. The solution gradually becomes more viscous and finally turns to a gel of the fibrous form. Similar observations had been made before by Meyer and Jeannerat[70] with the proteins of the gland of the silk worm.

Bailey[219] has shown that a more readily soluble component, tropomyosin, can be separated from a solution of myosin by precipitation with 41% saturated ammonium sulfate. In general, the inadequacy of our knowledge of the composition of the proteins of muscle is particularly clear from the electrophoretic analyses of Dubuisson.[220] According to these, frog muscle contains at least 6 proteins, of which 2 undergo change if the muscle is fatigued by continuous stimulation. Similar extracts from rabbit muscles differ markedly in the distribution and in the mobilities of the protein components.

On being squirted into salt-free water, myosin solutions solidify to form threads, which, on drying, yield a fiber diagram, are strongly positively birefringent (birefringence $= 6 \times 10^{-3}$), and display the typical mechanical

[216] D. M. Needham, *Biochem. J.*, **36**, 113 (1942).

[216a] W. H. Price and C. F. Cori, *J. Biol. Chem.*, **162**, 393 (1946). B. D. Polis and O. Meyerhof, *ibid.*, **163**, 339 (1946).

[217] G. Schramm and H. H. Weber, *Kolloid-Z.*, **100**, 242 (1942).

[218] A. Szent-Györgyi, *J. Colloid Sci.*, **1**, 1 (1946).

[219] K. Bailey, *Nature*, **157**, 369 (1946).

[220] M. Dubuisson and J. Jacob, *Experientia*, **1**, 273 (1945).

anisotropy characteristic of fibrous materials (Weber and Boehm). Myosin threads consist, therefore, of parallel oriented primary valence chains.

(*b*) *Fine Structure of Resting Muscle Fibril*

The fiber diagram of myosin is the same as that of a stretched muscle under tension; this similarity together with other considerations leads to the view that myosin is to be regarded as that chemical fraction of muscle

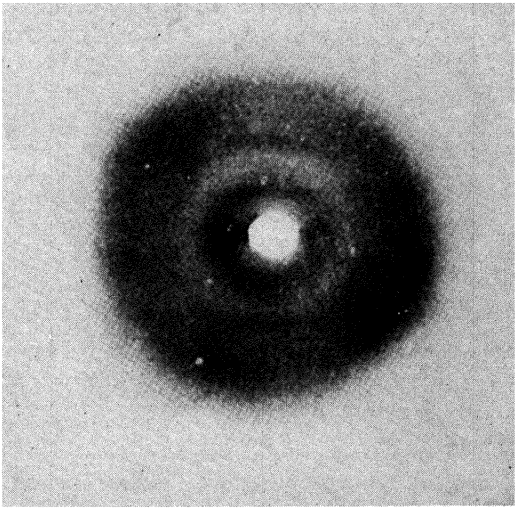


Fig. 11*a*. Fiber diagram of living resting muscle (Meyer and Picken[227]).

responsible for the birefringence and for the x-ray diagram of the resting muscle (Weber[221]). It may be assumed, therefore, that parallel oriented primary valence chains are present in the birefringent regions of resting muscle fibrils—a conclusion reached by Meyer[222] before the x-ray study of myosin, as a result of the following observations. The resting muscle fiber shows positive, uniaxial birefringence and a fiber diagram; frozen in liquid air, the muscle splits along the fiber axis as a result of a sudden blow, while the contracted muscle, similarly frozen, breaks up into small, irregular fragments. Parallel or approximately parallel protein chains are, therefore, present in the muscle. Similarly, the anisotropic swelling of the fibers,

[221] H. H. Weber, *Ergeb. Physiol. exptl. Pharmakol.*, **36**, 109 (1934); *Arch. ges. Physiol. Pflügers*, **235**, 206 (1934).

[222] K. H. Meyer, *Biochem. Z.*, **214**, 253 (1929); **217**, 443 (1930).

which results in an increase in volume at right angles to the fibril axis only, points in the same direction.

Resting muscle yields a fiber diagram (Herzog and Jancke; Meyer; Boehm), shown in figure 11*a*. An equatorial reflection corresponding to a spacing of 9–10 Å. and a meridional arc corresponding to a spacing of 5 Å. are visible. The equatorial spacing diminishes on drying, indicating that water is present in the regions possessing a rudimentary lattice structure. The greater part of the water present (*ca.* 90%) occurs outside the micellar threads, and is for the most part held in the intermicellar spaces (Boehm).

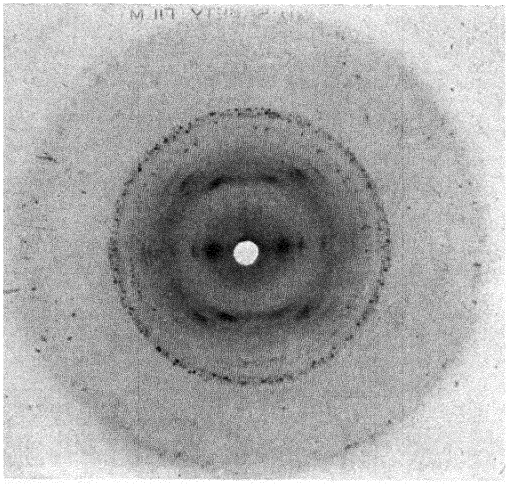


Fig. 11*b*. X-ray diagram of a dried stretched muscle (*Mytilus edulis*) (Lotmar and Picken). Radiation Cu Kα, 30 kV, 20 mA, distance 30 mm., time of exposure 2 hours.

Dried under tension muscle sometimes crystallizes and on x-ray examination yields a well-developed fiber diagram with many interferences (Fig. 11*b*). This was first shown by Herzog and Jancke,[223] for the sartorius of the frog, and later by Picken,[224] for the adductor muscle of *Mytilus edulis*. The x-ray diagram is due presumably to the presence of crystalline myosin. It is the most highly developed fiber diagram hitherto observed in a protein.

According to Lotmar and Picken,[225] analysis yields a monoclinic unit cell in which $a = 11.70$ Å., $b = 5.65$ Å. (fiber axis), $c = 9.85$ Å., and $\beta =$

[223] R. O. Herzog and W. Jancke, *Naturwissenschaften*, **14**, 1223 (1926).

[224] This diagram was first published on page 485 in the first edition of this book.

[225] W. Lotmar and L. E. R. Picken, *Helv. Chim. Acta*, **25**, 538 (1942).

73° 30′. The space group is $P2_1$—the same as that on which the cellulose model is based. The only symmetry element in this space group is a set of digonal screw axes along *b*. Two polypeptide chains traverse the unit cell in the *b* direction; their long axes lie in the screw axes. In constructing a three-dimensional model, Lotmar and Picken take into consideration the rules regarding spacings of primary and secondary valences; the smallest spacing between the carbon and nitrogen atoms of the groups of neighboring chains is assumed to be 4 Å., the spacing of a hydrogen bond. The resulting three-dimensional model is shown schematically in figure 12. The chains are spirally twisted.

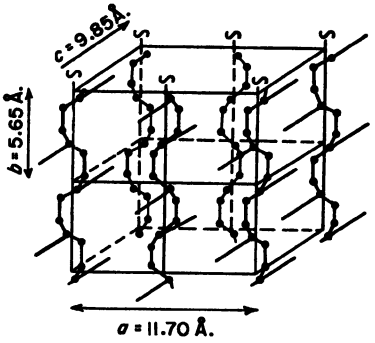


Fig. 12. Arrangement of myosin chains in crystallized muscle (Lotmar and Picken).

The lateral groups (methyl residues of alanine, isopropyl residues of valine, isobutyl residues of leucine, etc.) lie approximately along the *c* axis and interdigitate with those of neighboring chains. The distribution of the various amino acid residues of the myosin in the crystallites must be statistical in the sense that, while the various positions in the lattice are defined, their occupation by one or other amino acid is only statistically determined. It is impossible to decide whether this is also true of the individual peptide chains, or whether more or less regularly constructed chains are present which suffer statistical translation along the *b* axis.

From the sharpness of the interferences, the bundles of chains arranged in a regular crystalline lattice must be of considerable thickness—much thicker than the myosin bundles in living muscle. In muscle fibrils very

[226] M. A. Jakus, C. E. Hall, and F. O. Schmitt, *J. Am. Chem. Soc.*, **66**, 313 (1944). F. O. Schmitt, *Advances in Protein Chem.*, **1**, 25 (1945). M. A. Jakus and C. E. Hall, *J. Biol. Chem.*, **167**, 705 (1947).

[227] K. H. Meyer and L. E. R. Picken, *Proc. Roy. Soc. London*, **B124**, 29 (1937).

large periods can be observed, both by x-ray diffraction and electron micrographs;[226] the period in fibrils stained with osmic acid is 720 Å.

Concerning the condition of the chains in living muscle one can only say that if they are maximally stretched the myosin chains may be assumed to have the same configuration as they have in the crystallites. The chains present in the resting muscle are not completely straightened, since the muscle can be stretched passively up to a considerable fraction of its length. It has been found that the elastic force exerted by a passively stretched muscle increases with rising temperature, as in the case of stretched rubber. Meyer and Picken[227] conclude from this that the chains present in the resting muscle are, in part at least in the rubberlike condition. Under tension, the chains are fully stretched and, as a result, the fraction possessing a rudimentary lattice is increased—as the change in the x-ray diagram shows. The arrangement of the chains is, therefore, comparable to that in rubber which has been vulcanized in a partially stretched condition: a certain parallelism is maintained. If tension is applied, complete parallelism results, but as soon as the external force is removed the system returns to the less complete parallelism of the resting condition. There is thus a certain similarity with the structure of elastic fibers, which also appear to contain a mixed system. This is in agreement with the observation that resting muscle contracts on heating.[227a]

For the rest, regions which can be regarded as possessing a rudimentary lattice do not appear to occur in all muscles. The diagrams from arthropod muscle are very indistinct, and in the case of *Octopus* muscle no crystal-like reflections could be detected. In every case, however, birefringence and mechanical properties indicate a similar structure of parallel peptide chains. The study of the birefringent portions of muscle fibers by means of the polarizing microscope has shown the presence of structural birefringence in addition to the intrinsic birefringence of the oriented micelles, which amounts to 6×10^{-3}. The myosin filaments are therefore surrounded by an aqueous medium of lower refractive index. Weber[228] estimates that the myosin bundles of the living muscle consist of 70% myosin and 30% water; the diameter of each bundle is, at most, 50 Å. and the bundles cannot be more than 70 Å. apart. The space in between is filled with protein-free water. Fibrils with axes 100–150 Å. distant from each other are observed on metal-shadowed electron micrographs[226] of muscle fibers; this is in good agreement with Weber's results. The myogen is present in the sarcoplasm, that is, in the isotropic substance.

[227a] E. Wöhlisch, *Kolloid-Z.*, **96**, 261 (1941).

[228] H. H. Weber, *Naturwissenschaften*, **27**, 33 (1939).

(c) *Process of Contraction*[228a]

During active shortening a number of changes occur which indicate underlying changes in molecular organization. The fiber diagram disappears and the contracted muscle shows only an "amorphous" diagram (Fig. 13); the anisotropy of mechanical properties disappears—the contracted, frozen muscle breaks up into irregular fragments. This indicates that a change in shape of the chains has taken place; these are no longer arranged parallel or approximately parallel to each other, but are in some way twisted or rolled up (Meyer, 1929). The change in shape of the chains is indicated most clearly by the changes in birefringence. The careful work of Hürthle[229] has shown that there can no longer be any doubt that the birefringent portions of the fibrils (in striated fibers) are the actual contractile substance. The total birefringence is the resultant of two components: positive uniaxial structural birefringence and positive uniaxial intrinsic birefringence.[230] The fibrils therefore contain elongated micelles (consisting of parallel or approximately parallel oriented primary valence chains) and these are embedded in a medium of lower refractive index than that of the micelles.

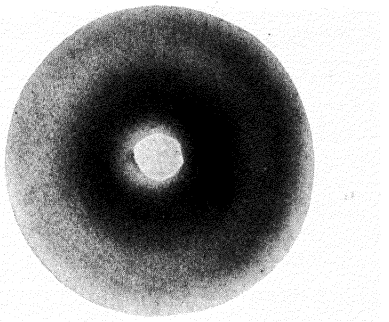


Fig. 13. X-ray diagram of contracted muscle (Meyer and Picken).

Since the birefringence diminishes during contraction, it may be concluded that the parallel, stretched primary valence chains of the resting fibrils lose their parallelism during contraction.

The change in shape of the chains is accompanied by a change in the state of aggregation. In the resting muscle the links of the chains can move over each other like the links of the chains in rubber, that is, the muscle is in the

[228a] A. V. Hill *et al.*, "A Discussion on Molecular Contraction and Relaxation," *Proc. Roy. Soc.*, **B137**, 40 (1950).

[229] K. Hürthle, *Über die Struktur der quergestreiften Muskelfaser.* Cohen, Bonn, 1909.

[230] D. Noll and H. H. Weber, *Arch. ges. Physiol. Pflügers*, **285**, 234 (1935).

rubberlike state and is comparable to unstretched rubber or elastic ligament. On the other hand, the contracted muscle is "solid," that is, the contracted chains are held together by forces of attraction between certain groups. This is indicated by the thermoelastic behavior of the contracted muscle, which is comparable to that of a normal solid body, and not to that of a rubberlike substance. The change of the state of aggregation from the one-dimensional solid, two-dimensional liquid, rubberlike state to the three-dimensional solid state is also shown by other observations. The viscosity of the contracted muscle, measured by the damping of a vibrating steel spring (Gasser and Hill[231]), is considerably higher than in the uncontracted, rubberlike condition. The solidification of the muscle as a result of the chemical processes occurring in contraction is also shown by the experiments of Carvallo and Weiss[232] on the tensile strength. During isometric tetanus, the tensile strength is considerably increased.

We may say that the chains in the resting muscle are surrounded by liquid and, to a certain extent, are "dissolved"; those in the contracted muscle are "coagulated."[233] Support is given to this view by the observation that the translucent muscles of invertebrates become turbid during a contraction, just as happens when a protein coagulates.[234] The change in the light-scattering properties of striated muscle during contraction has been interpreted by Buchthal[235] as indicative of an increase in the size of the micelles; it might also be interpreted as coagulation.

Contraction of the chains and simultaneous changes in the state of aggregation are the molecular phenomena which have been detected during contraction and to which macroscopic contraction is to be ascribed.

Concerning the form of the chains it may be assumed that in the passively and maximally stretched living muscle these have a stretched configuration similar to that postulated by Lotmar and Picken.[236] According to Meyer and Picken[237] some of the chains are associated throughout part of their length in micelles ("fringed micelles"); elsewhere they contribute to the amorphous component. The amorphous regions of the chains are somewhat disoriented with respect to the fibril axis and even the regions with

[231] H. S. Gasser and A. V. Hill, *Proc. Roy. Soc. London*, **B96**, 398 (1924).

[232] J. Carvallo and G. Weiss, *Arch. ges. Physiol. Pflügers*, **75**, 591 (1899).

[233] K. H. Meyer and L. E. R. Picken, *Proc. Roy. Soc. London*, **B124**, 29 (1937). H. Mirsky, *J. Gen. Physiol.*, **26**, 455 (1937).

[234] Biedermann, *Ergeb. Physiol.*, **8**, 26 (1909) (retractor muscle of *Sipunculus nudus*). E. Fischer, *Biol. Symposia*, **3**, 211 (1941) (coelenterate muscles).

[235] F. Buchthal and G. G. Knappeis, *Skand. Arch. Physiol.*, **83**, 282 (1940).

[236] W. Lotmar and L. E. R. Picken, *Helv. Chim. Acta*, **25**, 538 (1942).

[237] K. H. Meyer and L. E. R. Picken, *Proc. Roy. Soc. London*, **B124**, 29 (1937).

micellar organization are not arranged strictly parallel (as is shown by the observations of Buchthal[235] on the scattering of visible light). Regarding the manner in which the chains are folded in the contracted condition, there is as yet no adequate basis on which to erect a theory, and it is deemed advisable to omit all reference to the various opinions which have been advanced.

What chemical processes are responsible for these changes, we do not know and can only guess. It is still impossible to relate directly the complex chemical changes occurring during contraction with the behavior of the

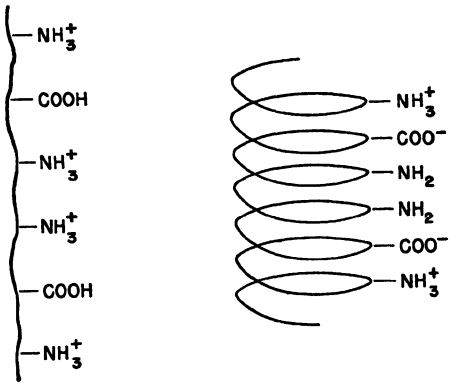


Fig. 14. Weakly basic protein chain, in acid medium (left), straightened by equally charged groups, and at the isoelectric point (right).

chains (contraction and coagulation). It is possible, however, to suggest the sort of relationship which may exist between the chemical changes in the medium surrounding the chains and the contraction itself.

Many free basic and acidic groups are attached to the myosin chains since the structural units include glutamic acid, on the one hand, and arginine and lysine, on the other. At the isoelectric point it is to be regarded as a system of dipolar ions (*Zwitterion system*) and one may suppose that oppositely charged ions approach each other. On either side of the isoelectric point the condition will be otherwise. Thus, for example, the dissociation of the carboxyl groups will be suppressed in an acid medium, while the amino groups will be charged and will repel one another. As a result, the chains will become elongated. A return to the isoelectric point brought about by neutralization of hydrogen ions will lead to coagulation and contraction. The two conditions of the chains are shown schematically above

(Fig. 14), which represents the behavior of a polypeptide chain with free carboxyl and amino groups. Experimental evidence with synthetic polyelectrolytes supports this theory. According to Kuhn[237a] an oriented film of slightly cross-linked polyacrylic acid swells and lengthens in alkali and contracts on acidification. Reversible contractions up to one third of the greatest length have been recorded.

As we shall see later (in section V2) it is probable that the action current which slightly precedes and accompanies the contraction is caused by a diminution of H^+ ion concentration, *i.e.*, an alkalinization in the sarcoplasm. It is also known that during the contraction creatine phosphate is split into phosphoric acid and creatine; as a result of this scission alkali is liberated. One may also demonstrate directly the alkalinization during the contraction by means of indicators. Since the isoelectric point of myosin[237b] in presence of Ca ions is situated above *p*H 8.7, alkalinization must provoke contraction of the chains. We therefore consider that contraction is caused by alkalinization, and relaxation by acidification.

This, however, is only one possible scheme relating chemical processes and contraction, but the important feature of this theory of muscular contraction (Meyer, 1929) is not the particular chemical change involved but the assumption that protein chains may be induced to contract or expand as a result of chemical processes.

There are indications that molecular processes similar to those inferred in muscle also occur in protoplasmic movement. In particular, the birefringence detectable in pseudopodia may indicate that here also primary valence chains are at one moment stretched and oriented parallel to each other, at another brought to contraction.[238]

Attempts have often been made, using various reagents, to induce Weber's myosin threads to contract, thus imitating *in vitro* the process of muscular contraction. Until now an unquestionably positive result has not been obtained. According to Szent-Györgyi[239] fresh, strongly swollen filaments of actomyosin may be caused to contract by adding muscle extracts or adenosine triphosphoric acid. From the description which he gives of this phenomenon (shortening to 50% of the original length, shrink-

[237a] W. Kuhn, *Experientia*, **5**, 318 (1949).

[237b] O. Snellman and Th. Erdös, *Nature*, **161**, 526 (1948).

[238] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930, page 243. W. J. Schmidt, *Naturwissenschaften*, **26**, 481 (1938).

[239] A. Szent-Györgyi, *The Chemistry of Muscular Contraction*, Academic, New York, 1947.

age, and opalescence) the effect is only one of syneresis (expulsion of imbibed fluid).

(*d*) *Relaxation of Muscle*

When the stimulated and contracted muscle ceases to be excited, the tension falls, and the rise in birefringence shows that the chains are transformed from the contorted condition to the more or less straightened condition of the resting muscle. If a resting striated muscle is passively stretched and the deformation remains constant, the tension gradually diminishes to a certain extent. The phenomenon is more marked in smooth than in striated muscle. This diminution in tension is to be ascribed to a different molecular process from that responsible for the fall in tension when the excited state disappears, for, as Picken[237] showed in the sartorius muscle of the frog and in the buccal retractor muscle of *Helix pomatia*, relaxation is accompanied by a fall in birefringence. Smooth muscle behaves, therefore, like relaxing polyvinyl acetate (Chap. I, Fig. 16). If external tension is removed when relaxation is complete, the muscle slowly returns to its original length. In explanation of this phenomenon, Meyer and Picken suppose that interpenetrating cross-linked and non-linked systems are present in muscle. The non-linked chains are able to glide over each other and so lead to relaxation, during passive extension, and to "creeping" (elastic recovery, elastic after-effect), when the tension is removed.

According to Jordan[240] the phenomenon of relaxation is particularly marked in the body wall musculature of coelenterates; the gradual fall in tension observed is similar to that in unvulcanized rubber stretched at constant length. These muscles can be stretched to many times their resting length. In contrast to unvulcanized rubber, however, the muscle is able to return completely to its initial unstretched condition. In our opinion, this indicates the presence of an "ideally cross-linked" system. The analogy between rubber and muscle is incomplete to the extent that rubber does not respond to a stimulus by contraction, shortening of the chains, and coagulation. These phenomena in muscle are superimposed on those of relaxation and elastic after-effect. Since it is possible, however, to distinguish between relaxation and the fall in tension due to straightening and orientation of contracted chains (physiological "relaxation"), by observing birefringence changes, it would be profitable to examine by this means the phenomena of contraction and relaxation in such muscles.

[240] H. J. Jordan, *Naturwissenschaften*, **25**, 17, 34 (1937).

9. Fibrin, Fibrinogen, and Stroma Protein

The coagulation of blood,[241] as is well known, is preceded by an enzyme reaction—the action of thrombin (the enzyme discovered by Alexander Schmidt) on the fibrinogen dissolved in the blood—as a result of which fibrinogen is converted into insoluble fibrin. The fibrin precipitates out and forms the clot.

Fibrinogen can be salted out from oxalated plasma and can be obtained in a microcrystalline condition after purification in various ways.[242] In solution it exhibits flow birefringence[243] which disappears on the addition of urea. It appears that elongated micelles are present in solution and that these dissociate when urea is added. From sedimentation and diffusion constants a molecular weight of 700,000 has been deduced.[241] If, on the other hand, the dimensions of the molecule are evaluated from flow birefringence and viscosity, a length of 700 Å. and a width of 38 Å. are found, corresponding to a molecular weight of 400,000.[245]

Fibrin, on the other hand, undoubtedly has a fibrillar structure. The chain molecule structure is evident in considerably stretched fibrin fibers obtained (according to Katz and de Rooy[246]) from blood plasma to which 2% of citrate has been added, followed by calcium nitrate. The clotting plasma can then be drawn out into threads from which the salts can be washed out. According to these authors, such fibrin threads yield a fiber diagram with a fiber period of 6.7 Å. and two equatorial reflections corresponding to spacings of 4.7 and 10.1 Å.; this perhaps indicates an arrangement of the polypeptide chains similar to that in silk and β-keratin. On the basis of the analysis of the products of hydrolysis, Bergmann and Niemann[247] suppose that every eighth residue is a glutamic acid unit and every twelfth a lysine unit; the periodicity of arginine, aspartic acid, and proline is 18, etc. These results, however, are open to criticism.

The precipitation of fibrin takes place particularly readily at interfaces, and is favored by mechanical agitation of the blood—by violent stirring with a glass rod, for example. The phenomenon seems analogous to

[241] For a review of the complicated processes involved in clotting see E. Chargaff, *Advances in Enzymology*, Vol. V, Interscience, New York, p. 31; W. H. Seegers, *J. Phys. and Colloid Chem.*, **51**, 198 (1947).

[242] K. Laki, *Z. physiol. Chem.*, **237**, 95 (1942).

[243] G. Boehm and R. Signer, *Klin. Wochschr.*, **11**, 599 (1932).

[244] C. G. Holmberg, *Arkiv Kemi Mineral. Geol.*, **A17**, No. 28, (1944).

[245] J. L. Oncley, G. Scatchard, and A. Brown, *J. Phys. & Colloid Chem.*, **51**, 197 (1947),

[246] J. R. Katz and A. de Rooy, *Naturwissenschaften*, **21**, 559 (1933); *Rec. trav. chim.*, **52**, 742 (1933).

[247] M. Bergmann and C. Niemann, *J. Biol. Chem.*, **115**, 77 (1936).

the precipitation of fibroin from solutions of silk fibroin: supersaturation is removed by orientation of the chains either at a surface or by mechanical agitation. According to Boehm[248] stroma protein also shows birefringence of flow. Human fibrin may be obtained in the form of elastic film; they can be used as dura substitute in brain operations for these films are slowly resorbed.

10. Other Fibrillar Proteins

The list of fibrillar proteins is by no means completed by those so far described. Boehm and Signer[249] have shown that a solution of egg globulin exhibits birefringence of flow, indicating the presence of long, rodlet or thread-shaped molecules. One may also assume that the numerous cell constituents which show marked birefringence—chromatin threads, chloroplasts, cilia, sperm tails, etc.—consist chiefly of fibrillar proteins.[250] Similarly, glutenins, the proteins which are so important in baking, have a fibrillar structure, shown by the elastic properties of wheat protein dough after it has been freed from starch.

In the process known as denaturation, many globular proteins are converted into the fibrillar form (see section X6). Particularly striking and familiar to everyone are the rubberlike properties of melted cheese, which show that it must contain long-chain molecules, although the casein of milk is globular.

IV. GLOBULAR PROTEINS

1. General Introduction and Classification

In contrast to those of the proteins already considered, the molecules of the majority of soluble proteins do not exhibit the extreme disparity of dimensions which we meet in chain molecules; their molecules are particles of more compact shape. Following Astbury, we shall speak of them as globular proteins. Their shape may be spherical or they may depart to some extent from the spherical shape; the value of the ratio f/f_0 in Svedberg's table (see Table II) indicates the degree of departure from the spherical form. All the proteins included in this table are to be considered as globular proteins.

[248] G. Boehm, *Biochem. Z.*, **282**, 32 (1935).

[249] G. Boehm and R. Signer, *Helv. Chim. Acta*, **14**, 1370 (1931).

[250] See W. J. Schmidt, *Die Doppelbrechung von Karyoplasma, Cytoplasma und Metaplasma*. Borntraeger, Berlin, 1937.

In agreement with this conception of the shape of their particles is the observation that, in spite of their high molecular weight, these proteins do not exhibit in solution the properties which we find in solutions of chain polymers. Their viscosity is not particularly high nor do they diverge as widely from typical van't Hoff behavior as do chain polymers. In many cases the dissolved particles are themselves formed by the association of smaller particles, and under the influence of suitable solvents or salts they may be induced to dissociate into single molecules.

Many, if not all, globular proteins are transformed by treatment with certain reagents—for example, formamide or concentrated aqueous solutions of urea—into fibrillar proteins. This is revealed by x-ray analysis (if the solid product can be drawn out into fibers) or by the high viscosity of the solution. Since the reagents named are only capable of splitting secondary valences, one must assume that the compact structure is not held together by primary valences, but is built up from peptide chains folded in a definite way and maintained in the folded condition by secondary valences such as hydrogen bonds. Because of the great length of the chains, various types of folding are possible: from one and the same peptide chain many *configuration isomers* may arise and may be stable under ordinary conditions. As we have no idea as yet of the sequence of peptide residues in any protein, it is not surprising that we are entirely ignorant of the structure of the folded polypeptide chains.

Since an adequate constitutional basis for a rational classification of the proteins is still lacking, the non-conjugated proteins are classified as to their solubilities into: (*I*) albumins soluble in salt free water; (*II*) globulins, insoluble in water free from electrolytes but soluble in suitable salt solutions; and (*III*) prolamins, soluble in dilute alcohol. Molecules of the *proteids*, *i.e.*, compounds of proteins with other substances, for the most part have the same shape as those of the globular proteins. As it is still impossible to characterize a given protein by a constitutional formula, it is customary to determine as far as possible the units from which the protein is built up, and to collect information about solubility, optical rotation, isoelectric point, behavior in the ultracentrifuge and in the electrophoresis apparatus, and to determine the ratio f/f_0.

2. Albumins and Globulins

(*a*) *Albumins* (*Table XII*)

The albumins are heat-coagulable proteins, which are soluble in water at the isoelectric point and can be salted out by sulfates and other salts.

They occur in all animal cells, in milk, and in serum. Crystalline plasma albumins from man and ox have been isolated by Cohn,[251] those from horse by McMeekin.[252] Vegetable albumins have also been described. The molecular weight of the albumins ranges from 17,000 to 70,000. Ovalbumin crystallizes out as the sulfate (2 moles of albumin and 3 moles of sul-

TABLE XII

Amino Acids of Albumins (Per Cent)

Amino acid	Ovalbumin[265]	Lactalbumin[265]	Serum albumin (human[266])
Arginine	5.7	3.5	6.2
Histidine	1.8	2.0	3.5
Lysine	4.5	8.0	12.3
Tyrosine	4.2	5.3	4.7
Tryptophane	1.4	2.3	0.21
Phenylalanine	6	5.6	7.8
Cystine	1.7	3.0	6.3
Methionine	5.0	2.8	1.31
Serine	7.6	4 9	3.7
Threonine	3–4	5.3	5.0
Leucine	9.4	15	11.0
Isoleucine	--	—	1.7
Valine	6 8	4	7.7
Glutamic acid	16 3	—	17.4
Aspartic acid	8 2	—	10.4
Glycine	1.9	—	1.6
Alanine	7.4	0–1	0
Proline	4–5	—	5.1

furic acid). It contains small quantities of phosphorus and of a carbohydrate which has been shown to contain glucosamine and mannose (Langstein,[253] Steudel,[254] Levene,[255] Sørensen[256]). The molecular weight of the latter is 1200.[257] In urea solution ovalbumin dissociates and is denatured.[258] In this reaction two processes are superimposed: the unfolding of the chains, leading to an increase in viscosity and a lowering of solubility,

[251] J. T. Edsall, *Advances in Protein Chem.*, **3**, 383 (1947).
[252] T. L. McMeekin. *J. Am. Chem. Soc.*, **61**, 2884 (1939).
[253] L. Langstein, *Z. physiol. Chem.*, **31**, 49 (1900).
[254] H. Steudel, *ibid.*, **34**, 353 (1901).
[255] P. A. Levene and T. Mori, *J. Biol. Chem.*, **84**, 49 (1929).
[256] M. Sørensen, *Biochem. Z.*, **287**, 140 (1936).
[257] A. Neuberger, *Biochem. J.*, **32**, 1435 (1938).
[258] J. W. Williams and C. C. Watson, *Nature*, **139**, 506 (1937).

and molecular degradation associated with a reduction in viscosity and a rise in solubility.[259]

(*b*) *Globulins* (*Table XIII*)

The globulins are insoluble in pure water, but soluble in salt solutions of certain concentrations—for example, in solutions of common salt. On either side of the isoelectric point they are mostly soluble as salts in the pres-

TABLE XIII

Amino Acids of Globulins (Per Cent)

Amino acid	Serum globulin (human)[266]	β-Lacto-globulin[259a]	Edestin[265]	Cottonseed[265] globulin	Arachin[265]	Glycinin, soybean
Arginine	4.8	2.9	13.5	12.8	15.4	8.5
Histidine	2.5	1.6	2.3	3.0	1.9	3.3
Lysine	6.7	12 6	2.4	5.2	2.4	—
Tyrosine	6.8	3.6	3.3	3.1	4.5	1.8
Tryptophane	2.9	1.9	1.4	1.5	0.6	1.6
Phenylalanine	—	3.8	3.4	7.9	5.5	—
Cystine	3.1	2 9	1.1	1.0	1	1.1
Methionine	1.1	3 2	1.9	2.3	0.6	1.7
Serine	11.4	4.0	—	2.7	4.6	—
Leucine	9.3	15.5	7	8.0	—	—
Isoleucine	—	5.9	—	2.2	—	—
Threonine	8.4	4.9	—	2.7	2.3	—
Valine	—	5.6	5	6 1	—	—
Glutamic acid	—	19.1	17.8	17	17	18
Aspartic acid	—	11.5	10.3	—	5	9
Glycine	—	1.4	1.6	—	1.8	—
Alanine	—	7.1	4.8	—	—	—
Proline	—	5.1	5.4	—	—	—
Hydroxyproline	—	—	—	—	—	—

ence of bases or acids. They are readily denatured. Their molecular weights range from 100,000 to 600,000.

Among animal globulins may be mentioned: serum globulin, ovoglobulin, lactoglobulin (obtainable in the form of crystals), Bence-Jones protein, and numerous globulins from different organs. The vegetable globulins include those from oil seeds, such as edestin, excelsin, amandin, and others, of which some have been obtained in crystalline form and have been

[259] J. H. Clark, *J. Gen. Physiol.*, **28**, 539 (1945).

[259a] W. H. Stein and S. Moore, *J. Biol. Chem.*, **178**, 83 (1949).

found to be homodisperse in solution,[260] and the leguminous globulins: phaseolin, legumin, and vicilin.

Many pure enzymes as well as the proteins of the conjugated enzymes have the character of globulins.

The pseudoglobulins are proteins which are soluble in water-like albumins, but behave in the electrophoresis apparatus more like globulins than albumins.

(*c*) *Proteins of Blood Plasma*

The extensive studies of Pedersen,[260a] Cohn,[251,261] *et al.*, on the proteins of blood plasma afford insight into the manifold nature of the soluble proteins present in biological fluids. Cohn employed fractional precipitation of salt-containing serum with alcohol as a means of separation. In order to avoid denaturation, precipitations were performed at −5°C., and the

TABLE XIV

Proteins of Human Plasma[a]

Fraction	Contains	Amount, g./l. in. plasma	$[\eta]$	f/f_0	Mol. wt.	Approximate Length, Å.	Approximate Width, Å
Albumin . . .	—	32	0.042	1.28	69,000	150	38
Lipoglobulin . . .	Cholesterol Phosphatides Phosphatases	2	0.066	1 38	200,000	300	50
Globulin	Serum esterase Hypertensinogen	1	0.092	1.58	(300,000)	—	—
Globulin . .	Binds iron	2	0.055	1.37	90,000	190	37
Globulin	Complement Prothrombin	2	—	—	(150,000) 500,000	—	—
Globulin	—	1	—	—	1,000,000	—	—
Lipoglobulin . . .	Cholesterol Phosphatides, vitamin A Carotinoids Estrogen	2	0.041	1.7	1,300,000	185	185
Globulin	Isoagglutinins	2	—	—	(150,000)	—	—
Globulin	Antibodies, *e. g.*, measles	5	0.06	1.38	156,000	2.35	44
Globulin	—	1	—	—	300,000	—	—
Fibrinogen	Antihemophilic factor	2	0.25	1.98	400,000	700	38

[a] According to Oncley, Scatchard, and Brown in J. T. Edsall, *Advances in Protein Chem.*, **3**, 460 (1947).

[260] A. Polson, *Kolloid-Z.*, **87**, 149 (1939).

[260a] K. O. Pedersen, *Ultracentrifugal Studies on Serum.* Uppsala, 1925.

[261] E. J. Cohn *et al.*, *J. Clin. Invest.*, **23**, 417 (1944); *J. Am. Chem. Soc.*, **69**, 1753 (1947); **72**, 465 (1950).

alcohol concentration was not allowed to rise above 40%. By changing the *p*H with suitable buffers, as well as by changing the salt content and the temperature of precipitation, it was possible to obtain a very extensive separation. The first six fractions obtained, numbered I to VI according to solubility, were further fractionated. Table XIV gives a summary of the distribution of the proteins and their biological functions. The albumin fraction of human plasma was used during the war in the form of a 25% solution for transfusion as a means of replacing blood loss and so counteracting traumatic shock.

(*d*) *Plastics from Soybean Protein*

The protein of the soybean flour which remains after extraction of the oil consists for the greater part of a globulin known as glycinin. It is worked into thermosetting plastics by the addition of phenol and formaldehyde or furfural.

3. Prolamines

The prolamines are vegetable proteins soluble in 50–90% alcohol and (as salts) in acids and alkalis, but insoluble in water and in absolute alcohol.

TABLE XV

Analysis of Products of Hydrolysis of Prolamines[267]
(Figures denote grams of amino acid residue in 100 grams of protein)

Amino acid	Gliadin[267]	Zein[268]
Alanine	2.0	9.8
Valine	3.3	1.9
Leucine	6.6	25.0
Serine	—	1.0
Cystine	1.8	0.6
Phenylalanine	2.4	7.6
Tyrosine	1.2	5.2
Tryptophane	1.1	—
Aspartic acid	0.6	1.8
Glutamic acid	43.7	31.3
Hydroxyglutamic acid	7.7	2.5
Arginine	3.2	1.5
Lysine	0.9	—
Histidine	0.6	0.8
Proline	13.2	8.4
Ammonia	5.2	3.6
Total	94.5	101.3

To this group belongs gliadin (from wheat flour) which occurs associated with the alcohol-insoluble protein glutenin, also hordein from barley, and zein from corn. These proteins contain a large percentage of glutamic acid in the form of amide, much proline, and no glycine. The composition of zein is well established (see Table XV); it is probably a mixture of several closely related proteins.[262,263] Gliadin is also polydisperse, and glutenin is a mixture of molecules differing both in shape and size[264] (see Table XV).

V. CONJUGATED PROTEINS OR PROTEIDS

Compounds of proteins with other substances are classed as conjugated proteins or proteids; that part of the molecule which does not consist of peptide units is known as the prosthetic group. This may be simple, like the phosphoric acid units of phosphoproteins, or more complex, as in the prosthetic groups of enzymes. The linkage may be by primary valences (for example, ester formation) as in casein, or by complex or ionic linkages, as in hemoglobin.

1. Phosphoproteids

(a) *Casein and Vitellin (Table XVI)*

A number of proteins contain chemically bound phosphoric acid. The most important of these is casein, the chief constituent of milk protein. It is to be regarded as a complex mixture of proteins of molecular weights ranging from 75,000 to 375,000.[269] According to Posternak[270] the phosphoric acid is united with the hydroxyl group of serine. The isoelectric point of casein lies at *p*H 4.7. Casein is insoluble in water, but dissolves in

[262] J. W. Williams, S. Arrhenius, and C. C. Watson, *Nature*, **137**, 322 (1936).

[263] A. Polson, *Kolloid-Z.*, **87**, 149 (1939).

[264] A. G. McCalla and N. Gralén, *Nature*, **146**, 60 (1940).

[265] R. J. Block and D. Bolling, *The Amino Acid Composition of Proteins and Foods*. Springfield, 1945.

[266] E. Brand, B. Kassell, and L. J. Saidel, *J. Clin. Invest.*, **23**, 437 (1944).

[267] After T. B. Osborne and collaborators, *Am. J. Physiol.*, **13**, 35 (1905); **15**, 333 (1905–1906); **17**, 231 (1906–1907); **23**, 180 (1908–1909). D. B. Jones and R. Wilson, *Cereal Chem.*, **5**, 473 (1928).

[268] Analyses of Dakin, Osborne, and Liddle, from H. D. Dakin, *Z. physiol. Chem.*, **130**, 159 (1923).

[269] T. Svedberg, L. M. Carpenter, and D. C. Carpenter, *J. Am. Chem. Soc.*, **52**, 241, 701 (1930). E. Cherbuliez and J. Jeannerat, *Helv. Chim. Acta*, **22**, 952 (1939).

[270] S. Posternak, *Compt. rend.*, **184**, 306 (1927). See also P. A. Levene and D. W. Hill, *J. Biol. Chem.*, **101**, 711 (1933).

alkalis and strong acids. In milk it is present as the calcium salt. It is not coagulated by heat. Under the action of rennet, and during the early stages of peptic hydrolysis, casein is converted into so-called paracasein, the calcium salt of which is insoluble. The clotting of milk is due to the formation of paracasein. The low viscosity of milk and other aqueous solutions of casein shows that the casein molecules are compact and not thread-shaped. Nevertheless, under certain conditions, a transformation into the unraveled chain form may occur. The possibility of drawing out heated cheese into threads, and the elasticity of such threads, indicate without doubt the presence of chain molecules, as in rubber. Chain molecules are also present in the synthetic fibers discussed below. Casein has numerous commercial applications as an adhesive and cement, in the manufacture of synthetic resins for molding masses,[271] etc. Numerous other phosphoproteins have been described, such as vitellin from yolk, and proteins from the cell substance and from mucus.

(*b*) *Artificial Masses and Artificial Fibers from Casein*

Artificial horn (galalith) obtained from casein and formaldehyde was at one time of considerable commercial importance. The preparation of many other types of synthetic materials, however, has reduced its commercial importance. Artificial horn is prepared by kneading dry, fat-free paracasein with 20–35% of water, and squeezing in a screw press, in which the material is heated until it fuses together and becomes plastic. The strands, are brought to their final shape in the plastic condition and are then hardened in a dilute solution of formaldehyde. Hardening depends on the formation of cross linkages as a result of the coupling of amino groups by methylene bridges:

$$—NH_2 + CH_2O + NH_2— \longrightarrow —NHCH_2NH—$$

According to the thickness of the material, hardening may be a matter of days or months. Artificial horn is hard, tough, and elastic. A great disadvantage from many points of view is its ability to absorb water. It is used mainly for articles of every day use but not for any electrical equipment. One of the advantages of artificial horn is that it can be worked to give a fine polished surface. Artificial horn absorbs some water but is resistant to alcohols, ethers, and other organic solvents. Its specific weight is 1.3–1.4.

[271] See also E. Sutermeister and F. L. Browne, *Casein and Its Industrial Applications*. 2nd ed., Reinhold, New York, 1939. G. H. Brother, *Ind. Eng. Chem.*, **32**, 31 (1940).

Artificial fibers can also be prepared from casein (lanital). The procedure is derived from Todtenhaupt (1904) but has only recently been developed on a commercial scale, by Snia Viscosa (Ferretti, 1936) in Italy and subsequently in other countries. In Ferretti's method skim milk is precipitated with sulfuric acid and the resulting casein (*textile casein*) swells in water and is dissolved in alkali. After filtration and freeing from air, this solution undergoes a period of ripening, during which the viscosity passes through a maximum. The subsequent fall in viscosity is due to the destruction of the casein. Threads are obtained by spinning and stretching in a solution of sodium sulfate and sulfuric acid. The thread is then hardened by treatment at 30°C. and then at 70°C. with formaldehyde.

A more recent method makes use of rennet casein, the molecular weight of which is about twice that of textile casein. Sulfur bridges are introduced into the casein molecule by adding carbon disulfide to the alkaline spinning solution with resulting improvement in commercial properties, in particular in the tensile strength, both wet and dry.[272] Paracasein is also suitable for the manufacture of artificial fibers.[273]

The tensile strength—far below that of viscose—is about 6 kg. per sq. mm.; the wet strength is about 2.5 kg. per sq. mm.[274] The stress–strain curve of dry lanital differs considerably from that of cellulose rayon and resembles that of wool. The breaking extension for textile casein fibers is about 40%. The resistance of casein fibers,to water and alkali is improved by introducing acetyl groups (Aralac).

2. Chromoproteids[275]

(*a*) *Hemoglobin* (*Table XVI*)

The colored matter in the red blood corpuscles of vertebrates is known as hemoglobin. With the exception of the hemoglobin from the lowest class of *Vertebrata*, the *Cyclostomata*, all hemoglobins have the same molecular weight of 69,000. According to Fischer,[276] hemoglobin is a molecular compound of *hem*, the iron compound of protoporphyrin, and a particular protein component, *globin*, the nature of which varies in different species. A

[272] E. Franz, *Kunstseide u. Zellwolle*, **46**, 831 (1940). E. Franz, K. Riederle, F. Fleischmann, and W. Winkler, *Die Chemie*, Beiheft No. 45, 32 (1942).

[273] A. Schöberl and H. Wolff, *Die Chemie*, Beiheft No. 45, 48 (1942).

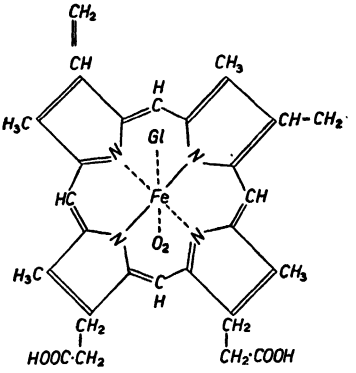
[274] From A. Braida, *Angew. Chem.*, **52**, 341 (1939).

[275] General account: J. Roche, *Essai sur la Biochimie Générale et Comparée des Pigments Respiratoires. Dissertation*, Marseille, 1935.

[276] H. Fischer, A. Treibs, and K. Zeile, *Z. physiol. Chem.*, **195**, 1 (1931). H. Fischer and H. Orth, *Die Chemie des Pyrrols*, Vol. 2, Akadem. Verlagsgesellschaft, Leipzig, 1937.

particle of hemoglobin of molecular weight 69,000 contains 4 molecules of hem.

Oxygen, carbon monoxide, and nitrous oxide enter into loose combination with hemoglobin; one molecule is bound by each iron atom, the iron remaining bivalent. It may be assumed that the hemoglobin fabric contains a suitable place in the neighborhood of the iron atom where oxygen, carbon monoxide, or nitrous oxide can enter to form a complex:



Oxyhemoglobin (diagrammatic): globin, protoporphyrin, and oxygen occupy the coordination positions of the bivalent iron atom

On oxidation, hemoglobin is converted into methemoglobin; this contains trivalent iron and is unable to bind oxygen.

On treatment in various ways, for example, by the addition of urea or salts to aqueous solutions, the hemoglobin molecule can be split reversibly into two equal fragments of molecular weight 34,500.[277] At extreme dilutions splitting of the molecule also occurs in pure water. As a result of the action of pyridine or dilute acid hemoglobin is broken down into hem and protein.

The protein component, globin, is basic in character, and this basicity depends on a considerable histidine and lysine content. The composition of the globin component depends on the species and, to a certain extent, on the age of the animal. Electrophoretic analysis in the Tiselius apparatus shows that globin consists of two components.[278]

[277] J. Steinhardt, *Nature*, **138**, 800 (1936); *J. Biol. Chem.*, **123**, 543 (1938). K. J. I. Anderisson, in T. Svedberg and K. O. Pedersen, *The Ultracentrifuge*, Oxford Univ. Press, London, 1940.

[278] L. Reiner, D. H. Moore, E. H. Lang, and M. Green, *J. Biol. Chem.*, **146**, 581 (1942).

TABLE XVI

Amino Acids of Some Conjugated Proteins[265] (Per Cent)

Amino acid	Casein	Vitellin	Hemoglobin	Thyroglobin	Yellow enzyme
Arginine	4 1	8.0	3.5	7.4	8.2
Histidine	2 5	1.4	7.6	3	2.8
Lysine	6 9	6	8 0	5–6	13.6
Tyrosine	6 4	5.3	3.0	3.0	7.7
Tryptophane	1.8	1.8	1–2	2.1	4.8
Phenylalanine	5 2	3	6.7	—	5 6
Cystine	0.36	1.3	1	2–4	0.5
Methionine	3.5	2.9	1	1.3	—
Serine	6–7	9.4	5.2	—	—
Threonine	3.9	4.9	6 8	—	—
Leucine	12.1	—	16.6	—	—
Isoleucine	6.5	—	1.5	—	—
Valine	7.0	—	8.2	—	—
Glutamic acid	22.8	12.7	5 7	—	6.8
Aspartic acid	6.3	—	8.1	—	—
Glycine	0 5	1	—	—	—
Alanine	5 6	--	7–8	—	8.1
Proline	8 2	—	2	—	6.4
Hydroxyproline	2	--	0	—	0

Globin is readily denatured by treatment with organic solvents, acids, bases, etc., and is then unable to unite with hem. In the course of denaturation the isoelectric point is shifted toward the alkaline side (Haurowitz).[279] From the denatured product a globin resembling native globin in its essential properties but more highly aggregated[280] can be recovered.

According to Hill and Holden[281] methemoglobin can be obtained from undenatured globin and hemin; in the presence of reducing agents hemoglobin is formed. In its essential properties this resynthesized hemoglobin is identical with native hemoglobin.

A chromoproteid which is probably a kind of hemoglobin has been found in the root nodules of *Leguminosae*.[282]

The proteid in the red blood corpuscles of the cyclostomes (*Lampetra*), has a molecular weight of 17,500. A proteid very similar to hemoglobin

[279] F. Haurowitz and H. Waelsch, *Z. physiol. Chem.*, **182**, 82 (1927).

[280] J. Roche, *Compt. rend. trav. lab. Carlsberg*, **22**, 465 (1937).

[281] R. Hill and H. Holden, *Biochem. J.*, **20**, 1326 (1926). See also J. Roche and R. Combette, *Bull. soc. chim. biol.*, **19**, 627 (1937).

[282] D. Keilin and Y. L. Young, *Nature*, **154**, 227 (1945).

myoglobin, occurs in muscle and contains hem as the prosthetic group. The molecular weight of carbonylmyoglobin is 17,500. Myoglobin may act as an oxygen reservoir for the organism.

X-ray study of hemoglobin has not as yet yielded complete information about its structure (sect. IX). On the other hand, from a spatial model of hem, such as one can construct on the basis of Hans Fischer's formula, it is clear that the hem molecule is a flat plate. The hem platelets presumably lie flat on a suitable facet of the globin molecule and are attached to the molecule at several points by secondary valences The small degree of dissociation of hemoglobin into globin and hem leads to the conclusion that the energy of association is high. This can be the case only if several groups take part in the association. It is probable that salt formation takes place between the acid hem and the basic globin; complex linkages with the ferrous ion may also occur. The latter possibility is suggested in particular by the ability of hem to form complex compounds with bases, such as hydrazine, ammonia, and pyridine. In this process 2 moles of base are united with 1 mole of iron. A complex compound of 1 hem, 1 carbon monoxide, and 1 pyridine has been described[283–285]; the existence of this compound suggests that oxygen or carbon monoxide and globin are all linked with hem in the same manner, that is, by addition to the iron complex. Of the six available positions, four are occupied by pyrrol nitrogen, one by globin, and the last by oxygen.

(*b*) *Hemocuprein and Hepatocuprein*

From the erythrocytes of mammals and from blood serum Mann and Keilin[286] were able to obtain a small amount of a copper proteid in the form of blue crystals, which they called hemocuprein. The crystals were double pyramids or hexagonal leaflets containing 0.34% of copper, 14.35% of nitrogen, and 1.12% of sulfur. The molecular weight amounted to about 35,000. The compound has no catalytic properties. Another copper proteid, the colorless hepatocuprein, was obtained from liver by the same authors.

(*c*) *Blood Pigments of Invertebrates*

The blood pigments of invertebrates fall into three classes: iron-containing erythro- and chlorocruorins, iron-containing hemerythrins, and copper-

[283] J. Roche, *Bull. soc. chim. biol.*, **8**, 362 (1926).
[284] R. Hill, *Proc. Roy. Soc. London*, **B100**, 419 (1926).
[285] O. Warburg and E. Negelein, *Biochem. Z.*, **200**, 414 (1928).
[286] T. Mann and D. Keilin, *Proc. Roy. Soc. London*, **B126**, 303 (1938).

containing hemocyanins. Erythrocruorins and chlorocruorins contain a hem as the prosthetic group, that is, an iron compound of porphyrin[287]; they absorb 1 mole of oxygen to 1 mole of iron. Hemerythrins, on the other hand, absorb only 1 mole of oxygen to 3 moles of iron.

The prosthetic group of the hemocyanins is cuprous ion, bound in a complex manner; according to the species this may amount to 0.17–0.26%. In spite of their high molecular weight (see Table II), it is possible to obtain hemocyanins in crystalline form (Dhéré,[288] Henze[289]). Like hemoglobin, hemocyanin forms a loose compound with oxygen, 1 mole of oxygen to 2 moles of copper. Oxyhemocyanin is blue, while the reduced compound is colorless. According to Kubowitz,[290] oxyhemocyanin is decomposed by hydrocyanic acid into oxygen, cuprous cyanide, and the protein component, from which it is possible to resynthesize hemocyanin by the addition of cuprous salts. Hemocyanin therefore lacks a colored component; the active group is the cuprous ion in complex linkage. The hemocyanin molecule is spherical, as is shown particularly clearly by electron micrographs.[291]

Svedberg and his collaborators[292] have studied the hemocyanins in detail, and find that a given blood frequently contains several molecular species. Svedberg summarizes his results as follows:

"The molecular weights of hemocyanin molecules, as they occur in the blood of certain species, are always simple multiples of the lowest well-defined components. Thus for *Malacostraca* the ratio is 1:2; for *Gastropoda*, 2*8*16*24. In addition, the weights of all well-defined hemocyanin molecules appear to be simple multiples of that of the hemocyanin of lowest molecular weight. In most cases the hemocyanin components of a given species are related to each other by reversible processes of dissociation dependent on *p*H. At certain *p*H values, a profound change in the number and quantitative proportions of the constituents occurs. The shift in *p*H necessary to induce this reaction may be not more than a tenth of a unit. The forces responsible for the cohesion of the separable parts of the molecules must therefore be very weak.

"In the case of *Helix pomatia* and *Helix nemoralis*, the protein at the isoelectric point contains one component only, while in *Helix arbustorum* and *Helix hortensis*, two components are present over the isoelectric range. If the *p*H is diminished or increased,

[287] Compare, for example, with *Spirographis* hemin: O. Warburg, A. Negelein, and E. Haas, *Biochem. Z.*, **227**, 171 (1930). O. Warburg and A. Negelein, *ibid.*, **244**, 9, 239 (1932). H. Fischer and C. von Seemann, *Z. physiol. Chem.*, **242**, 133 (1936). For other erythrocruorins, see J. Roche and R. Combette, *Bull. soc. chim. biol.*, **19**, 613 (1937).

[288] C. Dhéré, *Compt. rend.*, **157**, 309 (1913). C. Dhéré and A. Burdel, *ibid.*, **157**, 552 (1913).

[289] M. Henze, *Z. physiol. Chem.*, **33**, 370 (1901).

[290] F. Kubowitz, *Biochem. Z.*, **299**, 32 (1938).

[291] R. C. Williams and R. W. G. Wyckoff, *J. Applied Phys.*, **17**, 23 (1946).

[292] T. Svedberg, *Kolloid-Z.*, **85**, 122 (1938).

a point is reached at which a very slight shift in *p*H produces a profound change in the molecular condition. The original molecule of *Helix pomatia* of molecular weight 6,740,000 (s = 98.9[293]) breaks down, step by step, to units of one half (s = 62.0), one eighth (s = 16.0), and one sixteenth (s = 12.1) of this value. The products of *p*H dissociation are completely homogeneous components (see diagram, Fig. 15). If the *p*H is brought back to the isoelectric point, the dissociated fragments unite once more to form the original components."

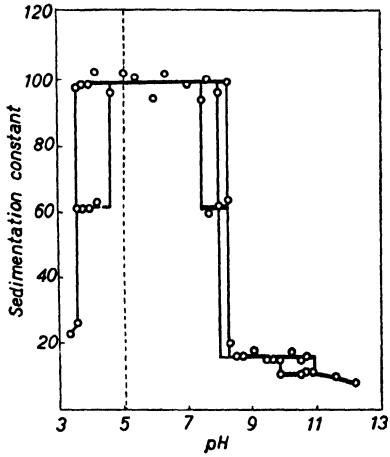


Fig. 15. Sedimentation constants of the particles of hemocyanin from *Helix pomatia* as a function of *p*H (Svedberg).

Osmotic measurements gave corresponding results.[294]

Reviewing the molecular weights of the various respiratory chromoproteids, it is seen that only those proteids present in corpuscles are of relatively low molecular weight, whereas pigments dissolved in the plasma, such as erythrocruorin, hemocyanins, and chlorocruorins, are characterized by very high molecular weights.

(*d*) *Pigments of Red Algae: Phycoerythrins and Phycocyanins*

The chromatophores of red algae contain blue and red water-soluble pigments, capable of crystallization, as shown by Kylin,[295] who was able to split up the pigment into a protein fraction and a colored component.

[293] s = sedimentation constant.

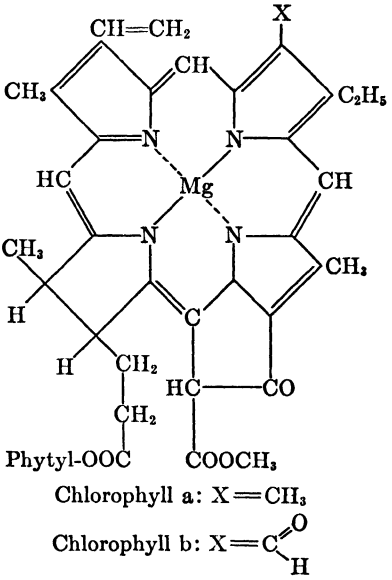
[294] J. Roche, A. Roche, G. S. Adair, and M. E. Adair, *Biochem. J.*, **29**, 2576 (1935).

[295] H. Kylin, *Z. physiol. Chem.*, **69**, 169 (1910); **76**, 396 (1912).

Lemberg[296] has identified the colored component as glaucobilin—a substance related to the bile pigments. The molecular weights of these pigments which, from their colors, are known as phycoerythrins and phycocyanins, are given in Table II.

(e) *Chlorophyll, Chloroplastin, and Phyllochlorin*

The suggestion that chlorophyll, which consists of two components a and b in the ratio of 3 to 1, is associated with protein in the living plant cell was put forward by many workers, and in particular by Tswett many years ago.



At first Tswett used the name *chloroplastin*, recommended by F. Schwarz, for the compound of chlorophyll and protein; later he used the name *chloroglobin*. This hypothetical chloroplastin was purified by Stoll[297]; in the electrophoresis apparatus of Tiselius it was homodisperse, and the molecular weight as determined in the ultracentrifuge was 5,000,000. Chloroplastin is a symplex of 69% of protein, 21% of lipoids, and 8% of pigment. A chlorophyll proteid of molecular weight greater than 70,000 was extracted from spinach by Smith,[298] who suggests the name phyllochlorin.

[296] R. Lemberg, *Ann.*, **461**, 46 (1928); **477**, 195 (1930).

[297] A. Stoll, *Naturwissenschaften*, **24**, 53 (1936). A. Stoll, E. Wiedemann, and A. Rüegger, *Verhandl. schweiz. naturforsch. Ges.*, **1941**, 125.

[298] E. L. Smith, *Science*, **88**, 170 (1938).

(f) *Ferritin and Apoferritin*

Laufberger[299] has obtained a relatively heat-resistant, dark brown, iron-containing proteid, forming beautiful crystals, from the spleen of horses, dogs, and cats, to which he has given the name of ferritin. It contains 54.5% of protein, 12.1% of nucleic acid, and 33.4% of ferric iron, probably in the form of colloidal iron hydroxide, HOFeO, built into the water-filled spaces of the open ferritin lattice. The iron can be removed by reduction,[300] and the remaining iron-free and colorless apoferritin has the same crystal structure as ferritin and is a homogeneous protein (mol. wt. 560,000). Crystals of apoferritin contain 45% of mother liquor in place of iron hydroxide. It is probable that ferritin constitutes the iron reserve of the organism, as it is only formed by the organism when it is needed for storage of iron. Ferritin is identical with the so-called vasodepressor material of liver.[300a]

3. Nucleoproteids[300b]

Nucleic acids of high molecular weight are present in combination with certain proteins known as histones, as nucleoproteids in the cell nucleus. By solution in alkali, followed by treatment with sulfuric acid, nucleic acid can be precipitated, while histone remains in solution. Of the histones, the one which has been most studied is thymus histone; the isoelectric point lies at *p*H 8.5, and the marked basic properties are due to the arginine and lysine content.

Nucleoproteids are probably to be regarded as salts of histones (proteins basic in character due to a high content of arginine and other basic amino acids) and nucleic acids. Astbury[301] points out that stretched nucleic acid fibers show a fiber period of 3.34 Å.; this period also occurs in proteins, so that the nucleic acid chain and the histone chain "fit together."

It would be interesting to investigate from this point of view thymus histone, the fiber period of which is unknown. Concerning the form of nucleoproteid particles nothing is known. It is possible that they belong to the fibrillar proteins. In fish sperm, nucleic acids are combined with basic proteins (protamines) of low molecular weight. There is also, how-

[299] V. Laufberger, *Bull. soc. chim. biol.*, **19**, 1575 (1937).

[300] S. Granick and L. Michaelis, *J. Biol. Chem.*, **147**, 91 (1943). L. Michaelis, *Advances in Protein Chem.*, **3**, 53 (1947).

[300a] A. Mazur and E. Shorr, *J. Biol. Chem.*, **176**, 771 (1948); **182**, 607 (1950).

[300b] Reviewed by J. P. Greenstein, *Advances in Protein Chem.*, **1**, 299 (1944).

[301] W. T. Astbury and F. O. Bell, *Nature*, **141**, 747 (1938).

ever, a protein of high molecular weight, chromosomin,[302] which is regarded by Stedmann as the carrier of inherited characters in the genes.

Proteids containing nucleic acids and lipoids have been extracted from animal cells by alkali or salt solutions with or without urea.[303] These solutions are highly viscous and exhibit flow birefringence; the proteids must therefore be of a fibrillar shape. They certainly belong to the structural elements of protoplasm.

Basic proteins unite with nucleic acids. According to experiments made by Przylecki[304] this association may also occur on the alkaline side of the isoelectric point in the case of proteins containing much arginine. Warburg and his collaborators[305] made use of precipitation with nucleic acid in the examination of mixtures of enzymes. Protein is set free from the precipitated nucleoproteid on adding protamine.

4. Glucoproteids

As mentioned previously, many proteins contain carbohydrates or carbohydrate residues in relatively small quantities. These include ovalbumin, which contains a prosthetic group consisting of three parts of hexasaccharide (4 mannose and 2 glucose residues) and one part of a nitrogen-containing compound.[306] Also to be mentioned here are lactalbumin with 1.82% of galactose, collagen, and many animal globulins, such as ovoglobulin, with 4% of mannose.[307] Other proteins may contain polysaccharide residues in larger amounts. To this group belong the bacterial glucoproteids (chapter F, sect. V4), and also the mucins and mucoids, in which the presence of mannose, galactose, glucose, and glucosamine has been demonstrated. In ovomucoid,[308] for example, acetyl glucosamine groups were shown to be the end groups of a branched polysaccharide fraction containing mannose and galactose. In the mucins, as in serum albumin and serum globulin, the carbohydrate fraction is probably united chemically with the protein fraction.

How the so-called "bound" sugar of the blood is united with other com-

[302] E. Stedmann and P. Stedmann, *Nature*, **152**, 267, 504, 586 (1943).

[303] R. R. Bensley, *Biol. Symposia*, **10**, 323 (1943). A. Szent-Györgyi, *Enzymologia*, **9**, 98 (1942). N. L. Hoess, *Biol. Symposia*, **10**, 185 (1943). A. Lawrence, J. Needham, and C. S. Shen, *Nature*, **146**, 104 (1940). J. Brachet and R. Jeener, *Biochim. et Biophys. Acta*, **1**, 13 (1947).

[304] St. J. von Przylecki, *Monatsh.*, **69**, 243 (1936).

[305] O. Warburg and W. Christian, *Biochem. Z.*, **303**, 40 (1939).

[306] A. Neuberger, *Biochem. J.*, **32**, 1435 (1938).

[307] M. Sørensen and G. Haugaard, *Biochem. Z.*, **260**, 247 (1933).

[308] M. Stacey and J. M. Wooley, *J. Chem. Soc.*, **1940**, 184.

ponents of the blood is unknown. It may perhaps be united with a blood protein. In many glucoproteids, carbohydrate may be united with the rest of the molecule by the secondary valences of tyrosine or arginine residues which, as Przylecki[309] has shown, can form addition compounds with sugar residues. As an example, the glucoproteid of egg white may be mentioned. This is a loose addition compound and can be split, according to Sevag,[310] by shaking the aqueous solution with chloroform and amyl alcohol, into 84% of protein and 16% of a nitrogen-containing carbohydrate.

5. Lipoproteids[311]

Although true chemical compounds between lipids (fatty acids and fats) and lipoids (for example, lecithin or cholesterol) and proteins have not been described, the lipids in cells and in plasma are very commonly associated with proteins and in this masked condition display special properties. They are, for example, water-soluble or are maintained by the protein in colloidal solution, in which case the lipophilic groups of the protein are associated with the hydrocarbon residues of the lipid (Meyer and Mark[312]).

It is not yet known which configuration of the protein molecule is responsible for the capacity of binding a large amount of lipids. The biological function of lipid-binding proteins consists in transporting fats, phosphatides, cholesterol, hormones of the sterin series, and carotinoids through the blood stream and the serum. Two lipoproteids or more correctly groups of lipoproteids have been found in human plasma: (*1*) lipoprotein α which precipitates together with α-globulin—it has a mol. wt. of 200,000 and contains 30–50% of lipoids including 16–18% of cholesterol[313–315]; and (*2*) lipoprotein β_1 (Pedersen's x protein) of mol. wt. 1,000,000 which precipitates and migrates electrophoretically together with β-globulin—it contains up to 75% of lipids among which 35% of cholesterol is found. The lipoid cannot be separated from the protein by solvents for the former.[314–316]

[309] St. J. von Przylecki and H. Ratalowska, *Biochem. Z.*, **277**, 416, 424 (1935); **280**, 92 (1936). St. J. von Przylecki and R. Majmin, *ibid.*, **277**, 420 (1935).

[310] M. G. Sevag, *Biochem. Z.*, **273**, 419 (1934).

[311] E. Chargaff, "Lipoproteins," in *Advances in Protein Chemistry*, **1**, 1 (1944).

[312] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, page 231.

[313] M. Macheboeuf, *Bull. soc. chim. biol.*, **11**, 268 (1929).

[314] G. Blix, A. Tiselius, and H. Svensson, *J. Biol. Chem.*, **137**, 485 (1941).

[315] J. T. Edsall, *Advances in Protein Chem.*, **3**, 383 (1947).

[316] K. O. Pedersen, *Ultracentrifugal Studies on Serum.* Uppsala, 1925.

A protein has been obtained from a saline extract of ox lung containing 18% of bound phosphatide and behaving as a homogeneous material in the Tiselius apparatus.[317] The material had a thromboplastic action, that is to say, it induced the formation of thrombin from prothrombin, both of which play a part in the clotting of blood. The lipid could be separated from the protein, with loss of activity, by treatment with a mixture of alcohol and ether, not, however, by treatment with acetone alone. The lipoprotein which has been longest known (since Hoppe-Seyler, 1867) is undoubtedly the vitellin–lipid complex from egg yolk. The purified product contains 23% of lipoids, of which 17.9% are phosphatides.[318]

Addition compounds of the carotinoids with protein are of particular biological interest. The pigment present in the eggs of the crustacean *Maia squinado*, a carotene, is water-soluble as long as it is not separated from the protein with which it is associated. The light absorption of this solution is considerably displaced toward the red end of the spectrum as compared with the absorption of an etheric solution of pure carotene (Dhéré and Vegezzi[319]). It is probable that the carotene is united with protein in multimolecular aggregates, so forming a colloidal solution. The compound of ordinary carrot carotene and protein is ill-defined. The relatively loose compound of the two components is broken down on the addition of protein precipitants, such as benzyltrimethylammonium chloride.[320] Ovoverdin,[321] on the other hand, is relatively well-defined according to Kuhn; this is a green chromoproteid which can be split into protein and the red pigment astaxanthin, an oxygen-containing carotinoid. On this splitting depends the development of the characteristic red coloration of a boiled crab.

According to Brunner, Baroni, and Kleinau[322] an addition compound of a protein and a carotinoid pigment is present in visual purple; Wald and Morton[323,324] showed that retinin, the prosthetic group of visual purple, is the aldehyde of vitamin A:

[317] E. Chargaff, D. H. Moore, and A. Bendich, *J. Biol. Chem.*, **145**, 593 (1943).

[318] E. Chargaff, *J. Biol. Chem.*, **142**, 491, 505 (1942).

[319] G. Vegezzi, *Dissertation*, Fribourg, 1916. See also W. Strauss, *Helv. chim. Acta*, **25**, 179 (1942).

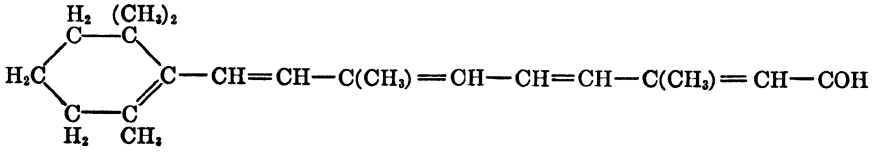
[320] R. Kuhn and H. Bielig, *Ber.*, **73**, 1080 (1940).

[321] R. Kuhn, I. Stone, and N. A. Soerensen, *Ber.*, **72**, 1688 (1939).

[322] O. Brunner, E. Baroni, and W. Kleinau, *Z. physiol. Chem.*, **236**, 257 (1935); *Sitzber. Akad. Wiss. Wien, Math. naturw. Klasse, Abt. IIb*, **146**, 464 (1936).

[323] G. Wald, *J. Gen. Physiol.*, **19**, 351, 781 (1935); **20**, 45 (1936); *Nature*, **139**, 587, 1017 (1937). *Biochem et Biophys. Acta*, **4**, 215 (1950).

[324] R. A. Morton, *Biochem. J.*, **40**, LIX (1946).



Furthermore, phospholipids are associated with the protein.[325]

VI. PROTEINS OF MARKED BIOLOGICAL ACTIVITY[326]

A number of proteins exert specific biological and biochemical effects at very low concentrations. These may be enzymes or hormones essential for the normal course of vital processes. They also include the toxins and the various protective or defensive substances which the organism manufactures.

1. Proteohormones

Proteins which have the properties of hormones will be referred to as proteohormones. Their efficacy is due to the intact protein alone and is lost if this is digested. In this respect the proteohormones differ from those proteins which serve as food and are hydrolyzed in the alimentary canal, but which fulfil their biological purpose precisely in this form.

(*a*) *Secretin*

As has been found by Bayliss and Starling duodenal mucosa contains an extractable substance which stimulates the secretion of enzymes from the pancreas. These authors concluded that this substance acts as a chemical messenger and proposed the term *hormones* for substances of this character. Secretin is a crystallizable polypeptide of mol. wt. 5000; it contains lysine, arginine, proline, histidine, glutamic acid, and probably also methionine.[326a]

(*b*) *Insulin*

Insulin, which regulates carbohydrate metabolism, is present as a zinc compound in Langerhans' Islets of the pancreas.[327] It has been ob-

[325] E. E. Broda, *Biochem. J.*, **35**, 960 (1941).

[326] For a short review, see W. Dirscherl, in *Chemie und Physiologie des Eiweisses*. Frankfurter Vorträge, Leipzig, 1938.

[326a] G. Ågren, *J. Physiol.*, **94**, 553 (1938).

[327] Industrial preparation of crystalline insulin, see R. G. Romans, D. A. Scott, and A. M. Fisher, *Ind. Eng. Chem.*, **32**, 908 (1940).

tained in a crystalline condition by Abel. Its particle weight in solution amounts to 46,000; four submolecules of molecular weight 12,000 are present. According to Sanger and Tiselius,[327a,b] the submolecule consists of four open peptide chains, connected by the dithio bridges of cystine. Two of these chains end in glycyl residues, the two others having phenylalanine as terminal groups. On the other hand, the terminal amino acids with free carboxyl groups consist of alanine. Insulin also contains lysine, arginine, histidine, proline, cystine, tyrosine, glutamic acid, and leucine. Since all of the amino acids are well known, and since activity is lost by denaturation to a fibrous form, but may be restored by treating with alkalis, the action of the substance can depend only on the spatial structure of the entire molecule.

According to Freudenberg and Dirscherl,[328] certain of the groups present in insulin are of particular importance, since, if these are blocked, the hormonal activity disappears. These groups are, above all, the hydroxyl groups of tyrosine, and, in addition, carboxyl, amino, and amide groups and perhaps the S—S linkages.

Insulin is present in the pancreas in the form of an insoluble compound from which so-called *native insulin* can be separated by enzymatic activity. Native insulin contains ordinary crystallizable insulin bound to a basic component of low molecular weight; this can be removed by hydrolysis.[329] An addition compound of insulin with protamine remains active after injection for a longer period than does insulin alone, presumably because it forms larger associates in solution and hence penetrates, and is excreted, more slowly than pure insulin.

According to Cori[330] the first product in the storage of glucose as well as in its breakdown is glucose 6-phosphate, which is formed from glucose and adenosine triphosphoric acid under the influence of the enzyme hexokinase. *In vitro* the action of hexokinase is inhibited by a hormone from the anterior lobe of the hypophysis; this inhibition is abolished (*in vitro*) by insulin.

[327a] F. Sanger, *Biochem. J.*, **39**, 507 (1945); *Nature*, **160**, 295 (1947).

[327b] A. Tiselius and F. Sanger, *Nature*, **160**, 433 (1947).

[328] K. Freudenberg and W. Dirscherl, *Z. physiol. Chem.*, **157**, 64 (1926); **175**, 1 (1928); **180**, 212 (1929); **202**, 192 (1931). W. Dirscherl, *ibid.*, **180**, 217 (1929); **202**, 116 (1931). K. Freudenberg, W. Dirscherl, and H. Eyer, *ibid.*, **187**, 89 (1930); **202**, 128 (1931). W. Kuhn, H. Eyer, and K. Freudenberg, *ibid.*, **202**, 97 (1931). K. Freudenberg, W. Dirscherl, H. Eichel, and E. Weiss, *ibid.*, **202**, 159 (1931). K. Freudenberg and H. Eyer, *ibid.*, **213**, 226 (1932). K. Freudenberg, E. Weiss, and H. Eichel, *ibid.*, **213**, 248 (1932).

[329] C. L. Lautenschläger, *Medizin und Chemie Abhandlungen aus den medizinisch-chemischen Forschungshäften der I. G. Farbenindustrie*, **4**, 21 (1942).

[330] C. F. Cori, *Chem. Eng. News*, **24**, 895 (1946).

(*c*) *Hormones of the Hypophysis (Pituitary Gland)*

Several other hormones are also protein-like in character, in particular the numerous hormones of the hypophysis. They control the activity and the growth of other endocrine glands. The following hormones have been found in the anterior lobe: (*a*) a lactation hormone the crystallization of which has been reported by White.[331] (*b*) Two gonadotropic hormones of glucoproteidic character have been isolated by Li and Evans[332]: metakentrin or ICSH (interstitial-cell-stimulating hormone) which stimulates these cells in ovary and testes and thylakentrin or FSH (follicle-stimulating hormone) which stimulates the sperm-forming tissues of the testes and produces the formation of the corpora lutea. (*c*) A hormone of mol. wt. 20,000 stimulating the adrenal cortex and a thyreotropic hormone stimulating the growth of the thyroid gland have been prepared. (*d*) A growth hormone obtained in a crystalline form by Fishman, Wilhelmi, and Russell.[332a] The following hormones have only been identified by their hormonal activity: (*a*) the diabetogenic hormone influencing the metabolism of carbohydrates; and (*b*) another one acting on fat metabolism.

A homogeneous protein has been obtained from the posterior lobe (Abel,[333] van Dyke[334]). This apparently consists of a complex of mol. wt. 30,000 containing various hormones which can be separated from each other. These include: (*a*) the oxytocic hormone, stimulating smooth muscle, such as that of the uterus; (*b*) the vasopressor hormone which raises the blood pressure and diminishes the secretion of urine. The so-called melanophore hormone, which controls the stage of the pigment cells of amphibia and fish, but whose physiological significance in man is unknown, is located in the pars intermedia of the hypophysis.

(*d*) *Other Proteohormones*

The placenta secretes a gonadotropic hormone known as chorionic gonadotropin, which stimulates the growth of the interstitial cells.[335] It is a

[331] A. White, H. R. Catchpole, and C. N. Long, *Science*, **86**, 82 (1937).

[332] C. H. Li and H. M. Evans, *Science*, **99**, 183 (1944). C. H. Li, L. E. Simpson, and H. M. Evans, *J. Biol.*, **149**, 413 (1943). Reviewed by B. F. Chow, *Advances in Protein Chem.*, **1**, 153 (1944).

[332a] A. E. Wilhelmi, J. B. Fishman, and J. A. Russell, *J. Biol. Chem.*, **176**, 735 (1948)

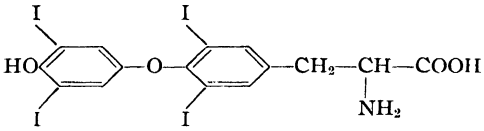
[333] J. J. Abel, *J. Pharmacol. Exptl. Therap.*, **40**, 139 (1930).

[334] H. B. van Dyke, B. F. Chow, R. O. Greep, and A. Rothen, *J. Pharmacol. Exptl Therap.*, **74**, 190 (1942).

[335] S. Aschheim and B. Zondek, *Klin. Wochschr.*, **6**, 1322 (1927); L. Claesson *et al.*, *Acta Endocrinologica*, **1**, 1 (1948).

glucoproteid containing glucosamine[336] and has a molecular weight of 60,000 to 80,000. According to Guyénot[337] it can be split into two components of different hormonal activity. Another gonadotropic hormone has been extracted from the serum of pregnant mares.[338]

The parathyroid hormone, essential for calcium metabolism, is also a protein. Thyroglobulin (mol. wt. 650,000[339]) the active protein of the thyroid gland, contains thyroxine in its protein chains. Since thyroxine alone also exerts hormonal action, however, even when taken by the mouth, thyroglobulin does not belong, strictly speaking, to the proteohormones. The activity of this hormone is presumably associated with the presence of thyroxine, which is set free by the digestion of thyroglobulin in the alimentary tract:



Thyroxine

2. Toxins

(*a*) *Toxalbumins*

Proteins exhibiting markedly poisonous properties are known as toxins. Several plant toxins, albuminous in character, such as ricin, abrin, and crotin, have been studied in some detail. These are blood poisons, inducing the agglutination of red blood corpuscles. Ricin may be deprived of its poisonous properties by formaldehyde without, however, losing its antigenic powers (see later). It appears then that the ability to stimulate the formation of a suitable antibody and the ability to produce toxic effects are due to different parts of the molecule. Worthy of note is the fact that pure urease (sect. VIII1*a*), intravenously administered, has marked toxic effects, probably due to its enzymatic action. If urease is injected, an antibody forming an insoluble precipitate with urease is produced. Ricin has recently been obtained in a crystalline form[339a]; M. W. 36,000.

[336] S. Gurin, C. Bachmann, and D. W. Wilson, *J. Biol. Chem.*, **133**, 467, 477 (1940); *J. Am. Chem. Soc.*, **61**, 2251 (1939).

[337] E. Guyénot and A. Ruffoni, *Compt. rend. soc. phys. hist. nat. Genève*, **58**, 171 (1941).

[338] C. H. Li, H. M. Evans, and D. H. Wonder, *J. Gen. Physiol.*, **23**, 733 (1940).

[339] M. Heidelberger and K. O. Pedersen, *J. Gen. Physiol.*, **19**, 95 (1936).

[339a] M. Kunitz, *J. Gen. Physiol.*, **32**, 25 (1948).

(*b*) *Snake Venoms*

Slotta[340] has obtained from rattlesnake venom a highly toxic protein, crotoxin, in crystalline form. The molecular weight is 33,000. It contains 4% of sulfur—most as cystine, to a slight extent as methionine. Hydrolysis of the S—S linkages abolishes toxic activity. Very small quantities of crotoxin are able to split off fatty acid from lecithin, and it is probable that the neurotoxic effect of crotoxin depends on such an enzyme-like, lecithinase action on the lecithins of the nervous system.

(*c*) *Bacterial Toxins*[341]

In spite of repeated attempts, only one bacterial toxin, that of *Clostridium botulinum* has as yet been obtained in crystalline form. As we have seen, glucoproteids appear to be present in this group. Seibert, Pedersen, and Tiselius[342] have isolated a highly toxic protein, of molecular weight 32,000, from tubercle bacilli, by electrophoresis. The endotoxin (as toxins are called which occur only in the bacteria themselves, not in the substrate) of the Shiga-Kruse dysentery bacillus is a glucopolypeptide which can be separated into nontoxic components and resynthesized from these (Morgan[343]). The polysaccharide contains galactose, L-rhamnose, and hexosamine residues; the presence of tyrosine and arginine in the polypeptide has been demonstrated.

In the purification of these substances, the greatest advance has been made by Eaton[344] and Pappenheimer,[345] who have obtained pure diphtheria toxin. According to Eaton, the aqueous solution of the crude toxin is treated with kaolin, calcium phosphate, or magnesium hydroxide to remove impurities, submitted to fractional ammonium sulfate precipitation, and finally precipitated with alum at *p*H 6, or with cadmium chloride. From 100 liters of culture medium, 1–2 g. of very pure and extremely active toxin can be obtained.

The molecular weight of this toxin is about 72,000; the isoelectric point lies at *p*H 4.1; $[\alpha]_D = -40°$ to $-47°$. It is albuminoid in character,

[340] K. H. Slotta and G. Szyska, *Ber.*, **71**, 258 (1938). K. H. Slotta and H. L. Fraenkel-Conrat, *ibid.*, **71**, 264, 1076 (1938); K. H. Slotta and W. Forster, *ibid.*, **71**, 1082 (1938).

[341] For a review see T. Wagner-Jauregg, *Angew. Chem.*, **52**, 389 (1939); **53**, 319 (1940).

[342] F. B. Seibert, K. O. Pedersen, and A. Tiselius, *J. Exptl. Med.*, **68**, 413 (1938).

[343] W. T. J. Morgan, *Helv. Chim. Acta*, **21**, 469 (1938). See the review by T. Wagner-Jauregg and E. Helmert, *Die Chemie*, **55**, 21 (1942).

[344] M. D. Eaton, *J. Bact.*, **31**, 347, 367 (1936); **34**, 139 (1937); *Bact. Revs.*, **2**, No. 1 (1938).

[345] A. M. Pappenheimer, Jr., *J. Biol. Chem.*, **120**, 543 (1937); **125**, 200 (1938).

contains 16% of nitrogen and displays no special chemical properties. The products of hydrolysis include 1.4% of tryptophane, 2.3% of histidine, 3.8% of arginine, 5.3% of lysine, and 9% of tyrosine. Activity is destroyed by proteolytic enzymes and even by shaking the solution. The toxicity is very high; 0.0001 mg. kills a guinea pig within five days.

Tetanus toxin has also been obtained by Eaton[346] in highly purified form. It is a polypeptide of medium molecular weight. In contrast to this the molecular weight of the toxin of *Clostridium botulinum* type A is very high and amounts to 900,000.[347] The toxin has been obtained in crystalline form by Lamanna[348] and Abrams.[349]

Many bacterial toxins display enzymatic action. Maschmann,[350] for example, has shown that the bacilli of the gas edema group produce a proteolytic enzyme, which attacks collagen in particular. Toxins of several species of *Clostridium* have been found to possess lecithinase activities.[350a] It is possible that the toxic action of many, if not all, toxins depends on enzymatic activity, the nature of which is still unknown.

3. Antibodies[351]

If certain definite substances, antigens—most proteins, certain polysaccharides and lipoids, and even foreign red blood corpuscles or whole bacteria—are injected into the circulation of a vertebrate, an antibody is set free in the serum which has the power of reacting with the antigen in one or another of several ways. If the antigen is a chemical substance, an insoluble compound is formed with the antibody. Such an antibody is known as a precipitin, and the reaction occurs both *in vitro*—if the antigen is added to serum containing the antibody—and in the living organism: the antigen is converted into an insoluble compound. If the antigens are red blood corpuscles or bacteria, or, more correctly, if it is the sum of the cell proteins or bacterial proteins which evokes antibody formation, the action of the antibody results in the agglutination or cytolysis of the blood cells or bacteria. The action of the antibodies—precipitins, agglutinins,

[346] M. D. Eaton, *J. Bact.*, **36**, 423 (1938).
[347] F. Putnam, C. Lamanna, and D. G. Sharp, *J. Biol. Chem.*, **165**, 735 (1946).
[348] C. Lamanna, O. E. McElroy, and H. W. Eklund, *Science*, **103**, 613 (1946).
[349] A. Abrams, G. Kegeles, and G. A. Hottle, *J. Biol. Chem.*, **164**, 63 (1946).
[350] E. Maschmann, *Angew. Chem.*, **51**, 403, 770 (1938).
[350a] M. G. Macfarlane, *Biochem. J.*, **42**, 590 (1948).
[351] J. R. Marrack, *The Chemistry of Antigens and Antibodies*, London, 1938. K. Landsteiner, *The Specificity of Serological Reactions*, Thomas, Springfield, 1936; *Harvey Lectures*, **36**, 251 (1941).

lysins—is directed, therefore, against the foreign substance; the organism adapts itself to the situation by the choice of a suitable antibody.

The properties of antibodies are, within certain limits, strictly specific for the corresponding antigens, but an antibody also reacts with proteins closely related to the antigen. Thus, the antibody evoked by crystallized ovalbumin from hen eggs also precipitates crystallized ovalbumin from duck eggs. As soon as proteins of less closely related animals are tested, however, specificity is marked; rabbit serum antibody does not react, for example, with horse serum.

Great progress has been made as a result of the observation of Obermayer and Pick[352] that new antigens can be prepared from natural proteins by chemical treatment (addition of iodine, for example) and that these stimulate the formation of specific antibodies. Particularly informative is Landsteiner's[353] technique of introducing azo groups into the protein. In this way, colored antigens are obtained, while the antibodies are colorless—indicating that the antigen itself does not enter into the formation of the antibody molecule. Such antibodies precipitate not only the antigen but also other proteins coupled with the same diazo compound. Thus, for example, by coupling diazotized arsanilic acid, $H_2O_3AsC_6H_4NNCl$, with horse serum, a substance is obtained which evokes an antibody when injected into a rabbit. The serum of the rabbit treated in this way reacts, not only with horse serum coupled with arsanilic acid, but also with hen serum coupled with arsanilic acid, although uncoupled hen serum does not react with the horse serum antibody. Specificity is therefore localized in the arsanilic group. Following Landsteiner, such groups are known as haptenes.

Certain polysaccharides—those of pneumococci, for example—are able in combination with proteins to act as complete antigens, that is, to induce the formation of antibodies; even when deprived of protein, however, they are precipitated by the antibody (Chap. F, sect. V 4). The action of the antibody must, therefore, be directed solely toward the polysaccharide. When the antibody reacts with the complete antigen (glucoproteid), the reaction occurs in the polysaccharide fraction; the protein fraction is passively carried down.

A polysaccharide displaying the properties of such a haptene can be split off from the natural glucoproteid of *Pneumococci*. This contains aldobionic acid, which can also be prepared synthetically. Such substances of

[352] D. Obermayer and E. P. Pick, *Wien. klin Wochschr.*, **19**, 327 (1936).

[353] K. Landsteiner, *Biochem. Z.*, **93**, 106 (1919); **104**, 280 (1920). See also K. Landsteiner, *The Specificity of Serological Reactions*, Thomas, Springfield, 1936.

low molecular weight responsible for the characteristic haptene and antigen properties are known as *determinant groups*. In many cases it has been possible, by progressive breakdown of complete antigens, to obtain a series of fragments of diminishing molecular weight, recognizable by their various serological reactions (inhibition test, anaphylactic shock, etc.)[354] and known as haptene, half-haptene, and determinant group.

Many natural antigens of different origin obviously have the same determinant group. For example, *Bacterium proteus* X 19 is agglutinated by serum from typhus patients. The typhus virus has therefore induced the formation of antibody which also acts with the determinant group of *Bacterium proteus*. This similarity in serological behavior is to be referred to a common constituent of both antigens which contains both lipid and carbohydrate.[354]

In recent years the determinant groups of natural antigens of very different origin have been studied, and it has been shown that they belong to definite classes of substance. In most cases they are polysaccharides, often containing an amino sugar; in other cases polysaccharide–lipid compounds, so-called glucolipids, are the determinant groups.[355] The so-called Forssmann antigen, present in many different cells, which if injected intravenously into a rabbit evokes the formation of a hemolysin active against sheep red blood corpuscles, is a protein-free complex of a hexosamine-containing polysaccharide and a higher fatty acid.[356]

It has been possible to obtain antibodies in concentrated form from the serum of injected animals, and in this way to establish that these substances are proteins which do not occur in the normal animal. Felton[357] was able to isolate the antibody to the polysaccharides from *Pneumococcus* I and II, by hydrolyzing the precipitate of polysaccharide and antibody with weak alkali, and removing the polysaccharide by selective adsorption

The antibodies hitherto investigated are globulin-like in character; chemical analyses reveal no difference in composition between normal globulins and antibodies. Studies with the ultracentrifuge showed that purified antibodies have the same molecular weights as the globulin components present in normal serum (Heidelberger, Kabat, and Pedersen[358,359]). An antibody to *Pneumococcus*-polysaccharide III, from rabbit serum, had

[354] O. Westphal, *Die Chemie*, **56**, 325 (1943).

[355] For a review see M. W. Chase and K. Landsteiner, *Ann. Rev. Biochem.*, **8**, 579 (1938); see also Chapter F, V5, page 446 on bacterial polysaccharides.

[356] W. F. Goebel, H. Hedlowski, G. I. Lawin, and M. H. Adams, *J. Biol. Chem.*, **148**, 1 (1943).

[357] L. D. Felton, *J. Immunol.*, **22**, 453 (1932).

[358] M. Heidelberger and K. O. Pedersen, *J. Expil. Med.*, **65**, 393 (1937).

[359] E. A. Kabat and K. O. Pedersen, *Science*, **87**, 372 (1938).

the molecular weight of normal serum globulin (150,000). The carefully purified antibody to *Pneumococcus*-carbohydrate I, from horse or cow serum, had a molecular weight of 920,000; small quantities of a globulin of this molecular weight occur in normal serum.

The antibody formed against diphtheria toxin, that is, diphtheria antitoxin, has been obtained in crystalline condition (Northrop[360]) by digesting the diphtheria toxin–antitoxin complex with trypsin, followed by fractional precipitation with ammonium sulfate. In this form it is very soluble and unstable, and it changes readily into a less soluble, noncrystalline form. Its molecular weight is 90,500 and the isoelectric point is at *p*H 7. The sedimentation constant is 5.7×10^{-13}. The molecular weight is the same as the globulin fraction prepared from normal horse serum.[361]

In spite of identity in particle size, antibodies and normal globulins differ, as the specific reaction of the antibody with the antigen shows. According to Tiselius[362] antibodies can be separated electrophoretically from the normal proteins. By comparative electrophoresis of (*1*) normal horse serum, (*2*) serum containing *Pneumococcus* antibody from a horse treated with *Pneumococcus*, and (*3*) serum from which the antibody had been removed by precipitation with bacterial polysaccharide, he was able to show that serum (*2*) contained a protein component absent from (*1*) and (*3*).

Beard, Finkelstein, Sealy, and Wyckoff[363] have isolated in highly purified form an antibody to horse encephalomyelitis; the molecular weight is approximately three million.

No explanation of the specificity of antibodies is as yet available. It is clear that, in the precipitins at least, molecular regions must be developed which fit exactly on to certain regions of the antigen so that a complex compound is formed. The question, formerly much discussed, whether their association is due to "chemical" linkage or to adsorption, no longer has any meaning. All types of linkages may take part, including ionic linkages.

Heidelberger[364] has studied in detail the relations prevailing in the reac-

[360] J. H. Northrop, *J. Gen. Physiol.*, **25**, 465 (1941).

[361] A. Rothen, *J. Gen. Physiol.*, **25**, 487 (1941).

[362] A. Tiselius, *Biochem. J.*, **31**, 1464 (1937); *Kolloid-Z.*, **85**, 129 (1938). A. Tiselius and E. A. Kabat, *Science*, **87**, 416 (1938).

[363] J. W. Beard, H. Finkelstein, W. C. Sealy, and R. W. G. Wyckoff, *Science*, **87**, 89 (1938).

[364] M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, **50**, 809 (1929); **55**, 555 (1932); **59**, 519 (1934); **61**, 563 (1935). M. Heidelberger, R. H. P. Sice, and F. E. Kendall, *ibid.*, **52**, 477 (1930). H. E. Stokinger and M. Heidelberger, *ibid.*, **66**, 251 (1937).

tion between haptene polysaccharide from *Pneumococcus* III (S) and the purified antibody (A), a globulin. Optimal precipitation occurs when a definite proportion of haptene, S, to antibody, A, is reached; in the presence of a considerable excess of S, no flocculation occurs. Presumably a soluble product, SAS, is formed from the first addition product, SA. Heidelberger concludes that the antibody is multivalent with respect to S; he gives an equation, based on this assumption, from which the quantity of precipitate formed can be calculated, knowing the concentration of both components. The precipitate, SA, can be hydrolyzed by weak alkali into the unchanged components.

In principle, the process of agglutination is a precipitin reaction taking place at the surface of bacteria or blood cells. The mass of agglutinated bacteria, etc., contains agglutinin; as a consequence of its *multivalence*, the antibody units with the surface proteins of several bacteria and thus causes them to adhere.

As already mentioned the antibodies hitherto isolated have the same molecular weight as the globulin fraction in normal blood serum, from which they differ only in their electrochemical properties and in their reaction with the antigen. It seems reasonable to suppose that antibody is formed from this globulin, or that globulin and antibody arise from the same precursor of equal molecular weight. Pauling[365] has developed the following theory of the formation of normal globulins and of antibodies. Globulin is first synthesized as an open polypeptide chain (by a mechanism as yet unknown) from amino acids or still smaller units. This chain may then be folded in a great variety of ways. It is supposed that the residues in the chain are arranged in such a manner that a certain internal arrangement of the folded molecule is more stable than all other arrangements, while for the two ends of the chain many configurations of approximately equal energy are possible (configuration isomers). Under normal conditions the primary chain is folded to give rise to normal globulin (Fig. 16, page 602); in the presence of an antigen, the primary chain is adsorbed on the latter and the 2 ends of the chain are folded in such a way that impressions of the haptene groups of the antigen are formed on the surface of the molecule (Fig. 16 Ia–Va). Finally, the center also becomes folded and part of the compound formed in this way dissociates into free antibody and free antigen; the latter is able to form new antibody. Antigen thus catalyzes the formation of antibody.

Antibodies, therefore, are normally bivalent, while the antigen may be

[365] L. Pauling, *J. Am. Chem. Soc.*, **62**, 2643 (1940).

multivalent. The agglutination of foreign cells is shown in the drawing; the formation of the compound antigen–antibody is shown in figure 16. Although this theory is at the present without proof, Pauling's idea, that a modifiable protein present in the serum is the precursor of the antibody unquestionably has much to be said for it.

The reaction between toxins and their antibodies, leading to the detoxication of the poison, is of great biological importance. The formation of a compound between toxin and antitoxin is indicated not only by detoxication, but, frequently, by flocculation, in particular at the isoelectric point. Concerning the composition of the toxin–antitoxin complex, little is known.

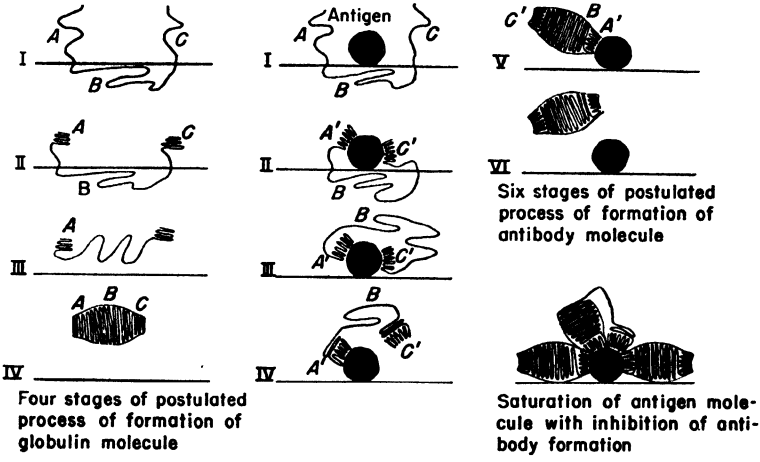


Fig. 16. Scheme illustrating Pauling's theory.

According to Pappenheimer and Robinson,[366] the precipitate formed in the detoxication of diphtheria toxin by its antitoxin consists of 1 part by weight of toxin to 3.5 parts by weight of antitoxin.

We have already seen that the poisonous action of toxins is associated with definite regions of the molecule. These regions are not, however, identical with those responsible for antigenic action. Many toxins, for example, diphtheria toxin, are detoxicated by the action of formaldehyde, but the resulting nontoxic toxoid retains the antigenic properties of the original toxin. Such detoxicated preparations are used for active immunization (prophylactic inoculation).

[366] A. M. Pappenheimer, Jr., and E. S. Robinson, *J. Immunol.*, **32**, 291 (1937).

4. Natural Blood Agglutinins; Blood Groups

The blood of most human beings contains genetically determined, *natural* agglutinins (not evoked by the introduction of foreign protein into the blood stream) directed against the red corpuscles of other human beings, agglutinating these and so making indiscriminate blood transfusion for therapeutic purposes impracticable; on the other hand, the blood of certain human beings can be injected intravenously into any of their fellows without harm. Investigation of these, at first sight, complex relationships by Landsteiner, showed that there are two different agglutinins in blood serum, α and β, and two different agglutinable substances, A and B, in the red corpuscles. Red cells containing A are agglutinated by α, and conversely. If the corpuscles contain A (blood group A) the serum is free from α but contains β; if the corpuscles contain B (blood group B) the serum contains α only. When the red cells contain both A and B (blood group AB) the serum is free from agglutinins; while if both A and B are absent from the corpuscles (blood group O), the serum contains α and β.

Agglutination only occurs if the agglutinin (α or β) is present in a certain concentration. Since during transfusion the serum of the recipient is present in excess and is practically undiluted, while that of the donor is considerably diluted, only the agglutinins of the recipient and the red cells of the donor are important in deciding whether agglutination will occur and, therefore, whether transfusion is practicable. The scheme below indicates by plus signs under what conditions agglutination occurs.

		Recipient			
		O	A	B	AB
Donor	O	−	−	−	−
	A	+	−	+	−
	B	+	+	−	−
	AB	+	+	+	−

The agglutinin from blood group A has been investigated chemically. Freudenberg[367] was able to concentrate this agglutinin by fractionation with lead acetate. On hydrolysis it gave 20–25% of galactose, 30% of acetylglucosamine, and 28–30% of amino acids, among which threonine was identified.

It seems that in addition to the groups mentioned above other groups may occur much less frequently, *e.g.*, group Rh (*Rhesus*).

[367] K. Freudenberg, H. Walch, and H. Molter, *Naturwissenschaften*, **30**, 87 (1942). See also W. T. J. Morgan, *Ann. Repts. Progress Chem. Chem. Soc. London*, **42** (1946).

VII. PROTEINS WITH ENZYMATIC PROPERTIES[368,369]

The ferments or enzymes are usually defined as organic catalysts of colloidal nature produced by living organisms. Hence, catalysts of low molecular weight, such as glutathione, adenosine triphosphoric acid, and the quinoid redox systems are not regarded as enzymes. All enzymes obtained so far in a pure or highly purified condition have proved to be proteins. In the following brief review, enzymes will be considered only insofar as they fall within the sphere of protein chemistry. Enzymes known only by their effects will not be included, and the account will be limited to those which have been obtained in a pure condition or whose constitution is known to some extent.

From now on, therefore, the enzymes will not be classified according to their action, but in accordance with their chemical composition. They can be classified as: (*1*) pure proteins, composed only of amino acids, and (*2*) combined or *conjugated* proteins. The latter are composed of a pure protein, forming the bulk of the weight, called the apoenzyme, and a nonprotein prosthetic group, called the coenzyme. Apoenzyme and coenzyme may be bound together by primary valencies, *e.g.*, in cytochrome c and in the muscle phosphorylase. They may act together as biologically active holoenzyme, even without such a linkage, perhaps bound in the form of a salt; an example is provided by the yellow enzyme. Neither apoenzyme nor coenzyme separately possess any enzymatic action. Whereas the apoenzyme is responsible for the affinity to the substratum, the coenzyme is the carrier of the actual activity. In many well-known cases (flavin enzyme, pyridine-adenine dinucleotide, peroxidases, etc.) the coenzyme takes part in the catalytic reaction as an intermediate.

The prosthetic groups of coenzymes may be organic groups or compounds of greater or lesser complexity, especially phosphorus derivatives of the B vitamins. The supply of vitamins presumably enables the organism to synthesize enzymes essential to life.

Many enzymes contain a complex including a metallic ion—zinc, magnesium, manganese, iron, or copper. The presence of the metallic ion is indispensable if the enzyme is to retain its catalytic activity. It has long been known that traces of certain elements are of great importance for the normal working of the cell. They are probably necessary for the synthesis

[368] Contributed by P. Bernfeld.

[369] General accounts: J. B. Sumner and G. F. Somers, *Chemistry and Methods of Enzymes*, New York, 1943. E. Bamann and K. Myrbäck, *Die Methoden der Fermentforschung*, Leipzig, 1941. F. F. Nord and R. Weidenhagen, *Handbuch der Enzymologie*, Leipzig, 1940.

of particular enzymes. Several enzymes considered up to now as nonconjugated proteins have been found to require metallic or other ions for full activity.

1. Nonconjugated Enzymes

(*a*) *Urease*

Crystalline urease, the enzyme hydrolyzing urea to ammonia and carbon dioxide, was obtained in 1926 by Sumner[370] from jackbean flour (*Canavalia ensiformis*) by rubbing up with 31.6% of aqueous acetone solution. On cooling, the enzyme crystallizes out, the yield (referred to the flour) amounts to as much as 0.12%. Urease was the first enzyme to be obtained in a crystalline form; it has the solubility of a globulin; its molecular weight is 483,000[371] and its isoelectric point is at *p*H 5.0. Free SH groups are essential for the catalytic activity; if these are blocked by oxidizing agents (Ur—SH + HS—Ur $\rightleftharpoons$ Ur—S—S—Ur + H_2) or by suitable metallic salts, activity is reversibly removed. Hellerman[372] has found that urease contains two different kinds of SH groups. One of these groups can be combined with *p*-chloromercuric benzoate, without loss of activity. The second SH group reacts only if sufficient quantities of the mercury compound are added; the enzyme is then inactivated.

With regard to antiurease, see section VI 2*a*.

(*b*) *Carboxypeptidase*

An enzyme capable of breaking down peptides with a free terminal carboxyl group was prepared in a concentrated form by Waldschmidt-Leitz and Purr,[373] and later obtained crystalline by Anson[374] from pancreatic juice. It is formed from an inactive precursor which is activated by trypsin. It is insoluble in water, but soluble in dilute alkali. Its isoelectric point is at *p*H 4.4. Magnesium ions are indispensable for its action.[374a]

(*c*) *Pepsin and Pepsinogen*

Northrop prepared crystalline pepsin (the proteolytic enzyme, acting in an acid medium, which is secreted by the gastric mucosa) by repeatedly

[370] J. B. Sumner, *J. Biol. Chem.*, **69**,435 (1926); **76**, 149 (1928); **79**, 489 (1929).
[371] J. B. Sumner, N. Gralén, and I. B. Eriksson-Quensel, *ibid.*, **125**, 37 (1938).
[372] L. Hellerman, F. P. Chinard, and V R. Deitz, *J. Biol Chem*, **147**, 443 (1943).
[373] E. Waldschmidt-Leitz and A. Purr, *Ber.*, **62**, 2217 (1929).
[374] M. L. Anson, *Science*, **81**, 467 (1935); *J. Gen. Physiol.*, **20**, 663, 777 (1937).
[374a] E. L. Smith and H. Th. Hanson, *J. Biol. Chem.*, **179**, 803 (1949)

dissolving commercial pepsin in water and precipitating it with acid or magnesium sulfate. By further fractionation crystallized pepsin can be separated into fractions differing in activity.[375] It has the solubility of an albumin. The molecular weight of crystalline pepsin has been found to be 35,500[376]; its isoelectric point is situated in a strongly acid region (below *p*H 3). The acid reaction of pepsin is the result of its high content of acid amino acids: 18.6% of glutamic acid, 6.8% of aspartic acid, and 10.3% of tyrosine. The last unit or its phenolic hydroxyl is of particular importance for the catalytic activity of the enzyme. If this is blocked by acetylation, activity disappears. Saponification leads to the reappearance of catalytic activity.

Pepsin is formed from an inactive precursor, pepsinogen, which has also been obtained in crystalline form by Northrop.[377] Its molecular weight is 42,000, *i.e.*, somewhat higher than that of pepsin. Immunological tests have shown that pepsin and pepsinogen are different protein products. In the presence of traces of pepsin, pepsinogen is converted autocatalytically into pepsin when in an acid medium.

(*d*) *Trypsin, Trypsinogen, and Trypsin Inhibitor*

Pancreatic trypsin, the protease from the digestive tract which acts in an alkaline medium, has also been obtained in crystalline form by Northrop and Kunitz.[378] Its molecular weight is 34,000, its isoelectric point is situated between *p*H 7 and 8. Trypsin is very stable in an acid medium. If warmed for a short period in 0.05 *N* hydrochloric acid, it may be denatured and inactivated, but on cooling, the activity is restored.

Trypsin is formed from trypsinogen, a crystalline proenzyme, by the splitting off of a small molecule which appears to mask the active group in trypsinogen. This process is catalyzed by enterokinase or by trypsin itself, so that the formation of trypsin from trypsinogen proceeds autocatalytically.

Northrop has isolated from pancreatic juice a trypsin inhibitor. It is a crystallizable polypeptide with a molecular weight of approximately 8000; at *p*H below 7 it combines with trypsin to given an inactive compound

[375] R. M. Herriott, V. Desreux, and J. H. Northrop, *J. Gen. Physiol.*, **24**, 213 (1940).

[376] J. St. L. Philpot and I. B. Eriksson-Quensel, *Nature*, **132**, 932 (1933).

[377] R. M. Herriott and J. H. Northrop, *Science*, **83**, 469 (1936). See also J. H. Northrop, *Crystalline Enzymes. The Chemistry of Pepsin, Trypsin and Bacteriophage*, Columbia Univ. Press, New York, 1939.

[378] J. H. Northrop and M. Kunitz, *J. Gen. Physiol.*, **16**, 267 (1932); *Ergebn. Enzymforsch.*, **2**, 104 (1933).

which is again split into active trypsin and inhibitor at *p*H 8. Another kind of trypsin inhibitor has been obtained from soybeans in a crystalline form by Kunitz.[379] It is a protein with globulin properties and a molecular weight of 24,000. It forms an inactive compound with trypsin which is very stable and can be crystallized.

(*e*) *Chymotrypsin and Chymotrypsinogen*

According to Kunitz and Northrop[380] the pancreas contains an inactive, crystallizable protein, chymotrypsinogen, converted by trypsin into a protease, chymotrypsin. This enzyme differs in solubility, crystal form, and serological properties from trypsin. In contrast to trypsin, chymotrypsin may provoke the clotting of milk but does not coagulate blood. Chymotrypsin and its inactive precursors have the same molecular weight of about 40,000. According to Northrop, the process of activation by trypsin involves the opening of a ring structure, whereby amino groups of lysine are set free. These are essential for proteolytic activity. Another protease (chymotrypsin B) has been crystallized.[380a]

(*f*) *Rennin* (*Rennet Enzyme*)

Rennin, the enzyme which appears in the mucosa of the calf's stomach and causes milk to clot, is also considered to be a protease. Hankinson[381] and Berridge[382] obtained rennin in a crystalline form. Rennin proceeds from an inactive precursor, prorennin, through mild acid reaction.

It can be seen that the proteolytic enzymes of animal origin often pos-

TABLE XVII

Substratum (proenzyme)	Active enzyme from activated precursor	Catalyst of conversion
Pepsinogen	Pepsin	Pepsin
Prorennin	Rennin	?
Trypsinogen	Trypsin	Enterokinase
Trypsinogen	Trypsin	Trypsin
Chymotrypsinogen	Chymotrypsin	Trypsin
Procarboxypeptidase	Carboxypeptidase	Trypsin

[379] M. Kunitz, *J. Gen. Physiol.*, **29**, 149 (1946); **30**, 291, 311 (1947).
[380] M. Kunitz and J. H. Northrop, *J. Gen. Physiol.*, **18**, 433 (1935).
[380a] K. D. Brown, R. E. Shupe, and M. Laskowski, *J. Biol. Chem.*, **173**, 99 (1948).
[381] C. L. Hankinson, *J. Dairy Sci.*, **26**, 53 (1943).
[382] J. Berridge, *Biochem. J.*, **38**, 179 (1943); *Nature*, **151**, 473 (1943).

sess an inactive precursor, the proenzyme, from which they originate by means of a protease, frequently in an autocatalytical reaction. The reactions are indicated schematically in Table XVII. As can be seen, these proteolytic enzymes partly activate themselves and in part mutually activate each other.

(g) *Papain and Ficin, Mexicain, Asclepain*

Green pawpaws contain a vegetable protease, papain, active in acid media. The enzyme has been obtained in the form of fine needles by Balls and Lineweaver.[383] Its molecular weight is 27,000, its isoelectric point situated at *p*H 9, and its optimum activity between *p*H 5 and 7. It is inactivated by oxydizing agents, like hydrogen peroxide, and the activity may be restored by addition of reducing agents, such as hydrogen sulfide, cysteine, hydrogen cyanide, etc. A sulfhydryl group is probably necessary for the activity and this group is reversibly inactivated by oxidation to the disulfide group.

An enzyme related to papain has been obtained from fig latex and isolated in crystalline form as ficin by Walti.[384] It contains 1.6% of sulfur as sulfhydryl sulfur and, like papain, can be inactivated and reactivated.

Another protease, called mexicain, has been isolated from the latex of *Pileus mexicanus* in a crystalline form.[385] A further enzyme, similar to papain, is the asclepain, occurring in *Asclepias syriana* and crystallized by Carpenter.[386] Its isoelectric point is at *p*H 3.1.

(h) *Ribonuclease and Desoxyribonuclease*

A crystalline ribonuclease, capable of splitting ribonucleic acid, has been obtained from ox pancreas.[387] The molecular weight is 15,000 and its isoelectric point is at *p*H 8. The enzyme is active as an antigen. The stability of this substance in the presence of hot, dilute acids is remarkable; almost all other proteins are denatured under these conditions and enzymes are mostly deactivated. In contrast thereto, ribonuclease is completely deactivated by only short warming at *p*H 7.

Desoxyribonuclease has also been obtained in a crystalline form.[387a]

[383] A. K. Balls and H. Lineweaver, *J. Biol. Chem.*, **130**, 669 (1939).
[384] A. Walti, *J. Am. Chem. Soc.*, **60**, 493 (1938).
[385] M. Castañeda-Agulló, *J. Biol. Chem.*, **159**, 751 (1945).
[386] D. C. Carpenter and F. E. Lovelan, *J. Am. Chem. Soc.*, **65**, 2364 (1943).
[387] M. Kunitz, *J. Gen. Physiol.*, **24**, 15 (1940).
[387a] M. Kunitz, *Science*, **108**, 19 (1948); *J. Gen. Physiol.*, **33**, 349, 363, (1950).

(*i*) *α-Amylase*

α-Amylases are enzymes which split up starch in the middle of the molecule and set free the maltose in the upward mutarotating form. Several amylases have been obtained in a pure and crystalline form. α-Amylase from pig pancreas has been crystallized by Meyer, Fischer, and Bernfeld.[388] It has a molecular weight of 45,000 and is free from phosphorus. In the pancreas extract and during purification, it is accompanied by a low molecular phosphorus compound which stabilizes the amylase but has no influence on the activity. α-Amylase from human saliva[389] and α-amylase from human pancreas have also been crystallized. Both are identical compounds but differ in solubility, crystal form, and degree of activity from pig pancreas amylase.[390] It has thus been proved that enzymes with the same action, occurring in the same organs but of different species (man and pig) are chemically different. In contrast, different organs of the same species (pancreas and saliva glands) produce enzymes of the same chemical composition. α-Amylase from saliva is sparingly soluble in water from *p*H 7 to 8.5; it is soluble in 0.01 *N* alkali.

Bacterial α-amylase of *Bacillus subtilis* has also been obtained in a crystallized form.[391] Its solubility and crystal form show that it is not identical with any of the other amylases.

Malt α-amylase which has been obtained in a crystalline condition by Schwimmer and Balls[391a] can be inactivated by salts of heavy metals. Ca ions are indispensable for its enzymatic action, while chlorine ions are essential for animal, mold, and bacterial α-amylases.

(*j*) *β-Amylase*

β-Amylases are enzymes which attack starch from the nonaldehydic end. β-Amylase of sweet potatoes[392] and β-amylase from barley malt[392a] have been crystallized.

[388] K. H. Meyer, E. H. Fischer, and P. Bernfeld, *Helv. Chim. Acta*, **30**, 64 (1947).
[389] K. H. Meyer, E. H. Fischer, P. Bernfeld, and A. Staub, *Experientia*, **3**, 455 (1947).
[390] K. H. Meyer, E. H. Fischer, P. Bernfeld, and F. Duckert, *Arch. Biochem.*, **18**, 203 (1948); E. H. Fischer, E. Duckert, and P. Bernfeld, *Helv. Chim. Acta*, **33**, 1060 (1950).
[391] K. H. Meyer, M. Fuld, and P. Bernfeld, *Experientia*, **3**, 411 (1947).
[391a] S. Schwimmer and J. Balls, *J. Biol. Chem.*, **179**, 1063 (1949).
[392] A. K. Balls, R. R. Thompson, and M. K. Walden, *J. Biol. Chem.*, **163**, (1946).
[392a] K. H. Meyer, E. H. Fischer, G. Noelting and A. Piguet, *Arch. Biochem.*, in press.

(*k*) *Aldolase*

Warburg and Christian[393] crystallized aldolase from rat muscle. This enzyme splits fructose diphosphate reversibly into phosphodioxyacetone and phosphoglyceraldehyde. Crystallized aldolase is free from ash. Its molecular weight amounts to 100,000.

(*l*) *Lipoxidase*

Theorell[394] obtained from soybeans a crystalline enzyme which brings about the oxidation of unsaturated aliphatic acids by oxygen. Lipoxidase has a molecular weight of 100,000.

All the enzymes discussed so far are pure, nonconjugated proteins. At all events, it has not been possible to trace in any of the enzymes mentioned above a nonprotein, prosthetic group. They therefore contain no coenzyme. In some of these enzymes, however, for instance, in urease, it can be proved by means of kinetic measurements that the enzymatic action is composed of two-part reactions: (*1*) formation of an intermediate addition compound of enzyme and substratum; and (*2*) the true catalytic reaction, in the case of urease the hydrolysis to ammonia and carbon dioxide. The next step which suggests itself is to look for the carrier of this true catalytic reaction, which is attributed to the coenzyme in the case of conjugated enzymes. As a matter of fact, the enzymes mentioned above contain active groups, for instance, sulfhydryl or phenolic hydroxyl groups, which are indispensable for the catalytic activity. These active groups are in this case, however, integral parts of the protein. Whether they are necessary for the formation of the intermediate addition compound enzyme substratum or whether they act as carriers of the catalytic activity, has not yet been ascertained.

2. Conjugated Enzymes with Organic Compounds as Coenzymes

In recent years, as a result of the work of Warburg, Christian, Negelein, Theorell, and von Euler, a number of conjugated enzymes have been obtained, partly in a pure form. The Table XVIII gives a survey of the organic compounds known as coenzymes, their holoenzymes, and their relation to the vitamins. In most of the cases, these active proteids may be separated into apoenzyme and coenzyme. In many cases it has also been possible to resynthesize the active proteid from the components.

[393] O. Warburg and W. Christian, *Biochem. Z.*, **314**, 149 (1943).

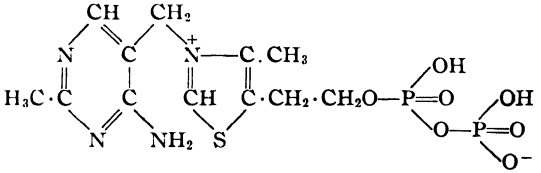
[394] H. Theorell, R. T. Holman, and Akeson, *Arch. Biochem.*, **14**, 250 (1947). R. T. Holman, *ibid.*, **15**, 403 (1947).

TABLE XVIII
Relation between Coenzymes and Vitamins

Vitamin	Coenzyme	Holoenzyme
B_1 Thiamin	Diphosphothiamin	α-Ketocarboxylase
B_2 Riboflavin	Riboflavin phosphate	Alloxazin proteids, yellow enzyme, aerodehydrogenases
	Riboflavin-adenine dinucleotide	Same as above
B_6 Pyridoxine	Pyridoxal phosphate	α-Aminodecarboxylases, transaminases
B_8 Adenylic acid	Adenylic acid	Muscle phosphorylase
Nicotinamide	Codehydrases I and II	Pyridine proteids, anaerodehydrogenase
Pantothenic acid	Pantothenic acid derivative	Acetylating enzymes
Biotin	Biotin	Lysozyme
---	Creatine	Adenosine triphosphatase
· ·	Guanine	Muscle hexokinase
- -	Glutathione	Glyoxalase
—	Iron protoporphyrin	Iron porphyrin proteids, peroxidases, catalase

(*a*) *Ketocarboxylase*

Carboxylase, which converts α-ketonic acids into aldehyde and carbon dioxide, was discovered by Neuberg and identified by Auhagen[395] as a conjugated ferment. Lohmann[396] has identified the coenzyme as the pyrophosphoric ester of aneurine, also called thiamin pyrophosphate:



Cocarboxylase (diphosphoric ester of aneurin)

The actual carboxylase which splits up pyruvic acid into acetaldehyde and carbon dioxide contains 1 atom of magnesium per mole of thiamin. The molecular weight of the enzyme is 75,000.[397] The amino group of thiamin is essential for the action of the coenzyme. It possibly gives a ketinine with the keto acid. According to Langenbeck,[398] even simple amines have the action of a carboxylase to some extent.

[395] E. Auhagen, *Z. physiol. Chem.*, **204**, 149 (1932).

[396] K. Lohmann and P. Schuster, *Biochem. Z.*, **294**, 183, 188 (1937). K. Lohmann, *Angew. Chem.*, **50**, 221 (1937).

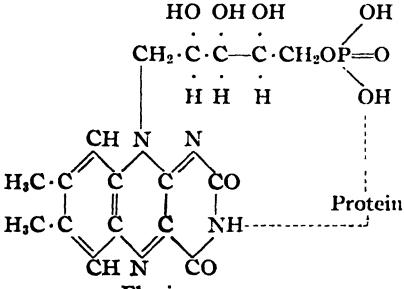
[397] F. Kubowitz and W. Lüttgens, *Biochem. Z.*, **307**, 170 (1941).

[398] W. Langenbeck, *Ergebn., Enzymforsch.*, **2**, 314 (1933).

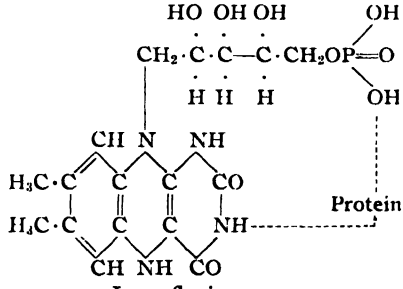
(*b*) *Alloxazine Proteid* (*Flavin Enzyme*)

An oxidative enzyme occurring in many if not in all cells is the *yellow enzyme* discovered by Warburg and Christian[399] and later obtained in a pure condition by Theorell[400]—alloxazine proteid or the flavin enzyme. It consists of a protein of molecular weight 80,000 (Kekwick and Pedersen[401]) and an alloxazine derivative, lactoflavin phosphoric acid, in the molecular proportions 1:1. The constitutions of the latter and of the leuco compound have been established by Kuhn and are shown in the formulas which also indicate the nature of the linkage with the apoferment.

By treatment of the aqueous solution with methanol, the proteid may be separated into the protein, which is precipitated, and the coferment, the alloxazine derivative, which remains in solution. Conversely, as Theorell[402] has shown, the coferment unites with the protein in aqueous solution to form the fully active enzyme, and, moreover, the synthesis of the enzyme is possible only with this entirely specific protein and no other:



Flavin-enzyme



Leucoflavin-enzyme

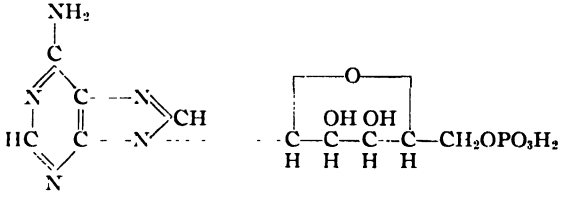
The activity of the enzyme depends on the ability of the alloxazine residue to take up hydrogen from compounds rich in hydrogen, while, on the other hand, hydrogen bound in this way is readily surrendered to atmospheric oxygen. In the living cell the compounds rich in hydrogen which are dehydrogenated by the yellow enzyme are themselves enzymes of the pyridine proteid series in the hydrogenated state (these will be discussed in § f). The coferment is capable of participating in this reaction only when in combination with the protein. As a result of this linkage, the velocity of reaction of the alloxazine residue with hydrogen donors is considerably increased.

[399] O. Warburg and W. Christian, *Biochem. Z.*, **258**, 496 (1933).
[400] H. Theorell, *ibid.*, **272**, 155 (1934); **275**, 37, 344 (1935); **278**, 263 (1935).
[401] R. A. Kekwick and K. O. Pedersen, *Biochem. J.*, **30**, 2201 (1936).
[402] H. Theorell, *Ergebn. Enzymforsch.*, **6**, 111 (1937).

Another enzyme containing lactoflavin phosphoric acid is the cytochrome c reductase of yeast; this enzyme receives the hydrogen from a pyridine nucleotide group and transmits it to an iron porphyrin enzyme.[403] The proteid has a molecular weight of 75,000 and can easily be split into apoenzyme and coenzyme. The latter can combine itself with the apoenzyme of the yellow enzyme to form the active yellow enzyme.

(*c*) *Alloxazine-Adenine Proteids*

Several other yellow enzymes contain a compound of flavin phosphoric acid with adenosine-5-phosphoric acid (flavin-adenine diphosphoric acid, also known as alloxazine-adenine dinucleotide) as coenzyme:



Adenosine phosphoric acid (muscle adenylic acid)

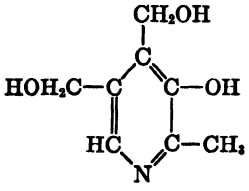
The protein fractions of these enzymes are different and they catalyze different reactions; the coenzymes, however, are the same. One of these enzymes is capable of oxidizing the optical antipodes of the natural amino acids to keto acids, for example, D-alanine to pyruvic acid; it is unable, however, to dehydrogenate pyridine proteid. Krebs[404] recognized it as a distinct enzyme. It was prepared in a pure condition from sheep kidneys by Warburg and Christian,[405] to whom our ideas on the constitution of the prosthetic group are due. The associated apoferment (a protein) was isolated by Negelein and Brömel.[406] On the other hand, an enzyme isolated by Haas,[407] having the same coferment but a different apoferment, is able to dehydrogenate pyridine proteids but cannot oxidize amino acids. A third enzyme to be mentioned here is xanthinoxydase (Ball[408]) and a fourth has been obtained from milk.[408a] Notatin from *Penicillium notatum* catalyzes the oxidation of glucose by air.[408b]

[403] E. Haas, C. J. Harrer, and T. R. Hogness, *J. Biol. Chem.*, **143**, 344 (1942).
[404] H. A. Krebs, *Z. physiol. Chem.*, **217**, 191 (1933); **218**, 157 (1933).
[405] O. Warburg and W. Christian, *Biochem. Z.*, **298**, 150, 368 (1938).
[406] E. Negelein and H. Brömel, *Biochem. Z.*, **300**, 225 (1939).
[407] E. Haas, *Biochem. Z.*, **298**, 378 (1938).
[408] G. G. Ball, *Science*, **88**, 131 (1938).
[408a] H. S. Corran and D. E. Green, *Biochem. J.*, **32**, 2231 (1938).
[408b] D. Keilin and E. F. Hartree, *Biochem. J.*, **42**, 221 (1948).
[409] O. Warburg and W. Christian, *Biochem. Z.*, **298**, 368 (1938).

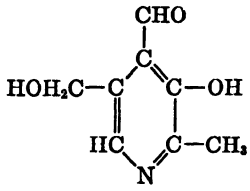
Warburg and Christian[409] succeeded in preparing a *synthetic* yellow enzyme by addition of alloxazine-adenine dinucleotide to the apoenzyme of the old yellow enzyme. The synthetic enzyme possesses the same enzymatic activity as the old yellow enzyme.

(*d*) *Pyridoxal Proteids*

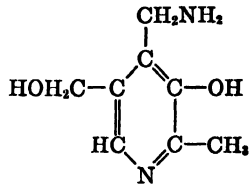
Two derivatives of vitamin B_6 or pyridoxine, pyridoxal and pyridoxamine, have been found by Snell to be natural growth factors[410] for microorganisms. Gunsalus[411] has shown that a phosphoric acid ester of pyridoxal acts as coenzyme of amino acid decarboxylases:



Pyridoxine



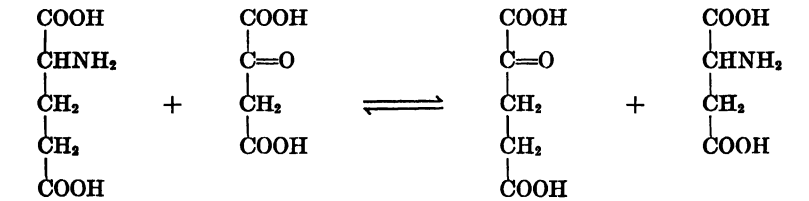
Pyridoxal



Pyridoxamine

the latter transform natural amino acids like tyrosine, dopa,[412] lysine, ornithine, arginine, and glutamic acids into the corresponding amines.

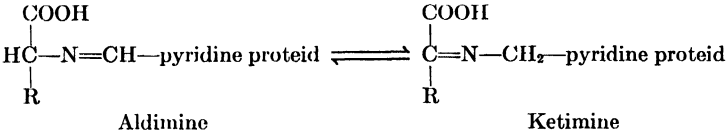
Whereas the coenzyme of α-keto acid decarboxylase contains an amino group which is essential for the enzymatic activity, and which probably forms a ketimine with the substratum, the coenzyme of the α-amino acid decarboxylases contains an aldehyde group. Obviously here, the formation of an aldimine in a similar way is highly probable. Moreover, pyridoxal has also been found to be the coenzyme[413] of several other enzymes, the transaminases, which catalyze the following and similar reactions:



L(+)Glutamic acid + Oxalacetic acid $\rightleftharpoons$ α-Ketoglutaric acid + L(−)Aspartic acid

[410] E. E. Snell, B. M. Guirard, and R. J. Williams, *J. Biol. Chem.*, **143**, 519 (1942).
[411] I. C. Gunsalus and A. W. Bellamy, *J. Biol. Chem.*, **155**, 357, 557, 685 (1944).
[412] Dopa-3,4-dihydroxyphenylalanine.
[413] F. Schlenk and A. Fischer, *Arch. Biochem.*, **12**, 69 (1947).

The presumed role of the coenzyme in the transamination is as follows: the pyridoxal proteid forms, with the amino acid, an aldimine and the pyridoxamine proteid forms, with the keto acid, a ketimine; an equilibrium is then set up between aldimine I + ketimine II and ketimine I + aldimine II, therefore:



The course of the reaction may be represented schematically as follows: Amino acid I + pyridoxal proteid ⇌ aldimine ⇌ ketimine ⇌ keto acid I + pyridoxamine proteid; keto acid II + pyridoxamine proteid ⇌ ketimine ⇌ aldimine ⇌ amino acid II + pyridoxal proteid.

(*e*) *Adenosine Proteids*

A series of enzymes, which contain adenylic acid together with riboflavin phosphoric acid, have already been described above. A further group of enzymes, containing adenylic acid in conjunction with nicotinamide will be dealt with next.

An enzyme containing only adenylic acid as coenzyme is muscle phosphorylase obtained by Cori in a crystallized form.[414] It phosphorolyzes the 1,4-α glucosidic linkages of glycogen, amylopectin, and amylose with the formation of glucose 1-phosphate and conversely builds up from the latter a polysaccharide with exclusive 1,4-α glucosidic linkages, *i.e.*, of the amylose type. In muscle phosphorylase, the adenylic acid is bound by primary valence to the protein. According to Cori[415] it can, however, be split off by crystallized trypsin or by another specific enzyme occurring in muscle. The natural concentration of adenylic acid so produced is no longer sufficient to produce enzymatic action; the scission provokes inactivation. By addition of adenylic acid, however, the activity is restored but may then be suppressed reversibly by dialysis.

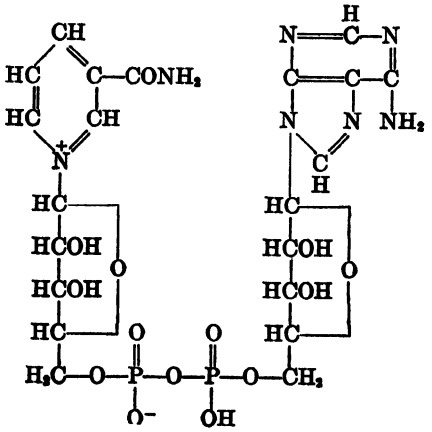
(*f*) *Pyridine Proteids*

According to Warburg, several enzymes contain nicotinic acid as a constituent of their prosthetic group and are therefore grouped together as

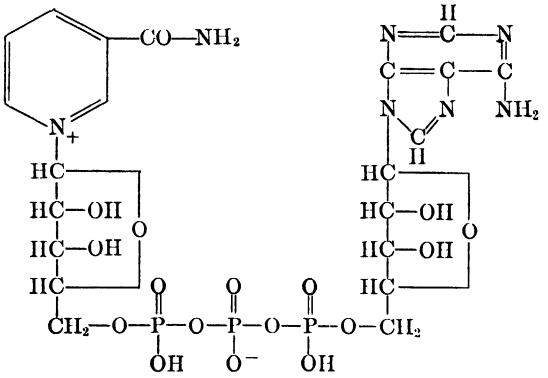
[414] A. A. Green and G. T. Cori, *J. Biol. Chem.*, **151**, 21 (1943). G. T. Cori and C. F. Cori, *ibid.*, **151**, 57 (1943).

[415] G. T. Cori and A. A. Green, *J. Biol. Chem.*, **151**, 31 (1943).

pyridine proteids. They have been studied in detail by Warburg[416] and von Euler.[417] The pyridine proteids are dehydrogenases capable of taking



Codehydrogenase I (Cozymase)



Codehydrogenase II

up hydrogen, thereby passing into the hydrogenated condition, and in this state are able to surrender hydrogen to a suitable acceptor.

[416] O. Warburg and W. Christian, *Biochem. Z.*, **275**, 112, 464 (1935). O. Warburg, *Ergebn. Enzymforsch.*, **7**, 210 (1938).

[417] H. von Euler, E. Adler, and H. Hellström, *Svensk Kem. Tidskr.*, **47**, 290 (1935); *Z. physiol. Chem.*, **241**, 239 (1936). R. Vesin and H. von Euler, *ibid.*, **247**, 43 (1937). H. von Euler and E. Adler, *ibid.*, **246**, 83 (1937). H. von Euler, E. Adler, and G. Günther, *ibid.*, **249**, 1 (1937).

Whereas the flavin proteids discussed above, as well as the iron porphyrin proteids, which will be dealt with later, are able to transmit the accepted hydrogen direct to the oxygen of the air, and are therefore called aerodehydrogenases, the pyridine proteids can only transmit hydrogen to other enzymatic systems or other substrata, as well as to methylene blue. They are therefore called anaerodehydrogenases.

If this hydrogen is handed on to the flavin enzyme just considered, which finally surrenders hydrogen to atmospheric oxygen, the reaction is part of an oxidation process, a stage in respiration. The enzyme is then a component in the sequence of enzyme reactions involved in respiration. The hydrogen may, however, be used for the hydrogenation of other compounds formed in the course of biological reactions, for example, acetaldehyde formed during fermentation. In this case, the pyridine proteid (a compound of protein and cozymase) is a component enzyme of alcoholic fermentation.

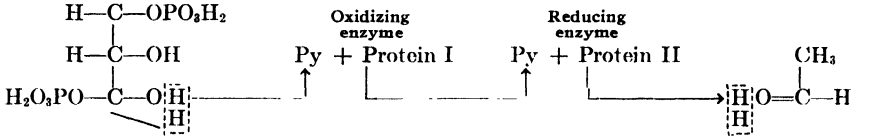
Pyridine proteids contain as prosthetic group a compound which may be hydrolyzed to give 1 mole of nicotinamide, 1 mole of adenine, 2 moles of D-ribose, and phosphoric acid. The respiratory enzyme contains 3, and the yeast ferment, cozymase, 2, phosphoric acid residues in the form of polyphosphoric acid. Both codehydrases are also called pyridine nucleotides. The constitutional formulas shown on the opposite page apply.

Pyridine proteids dissociate in aqueous solution into pyridine nucleotide and protein; the nucleotide can be separated by dialysis. It appears that many kinds of protein can combine with the nucleotide to form enzymes. According to Warburg and Negelein,[418] yeast contains several proteins, each of which unites with pyridine nucleotide to form a dehydrogenase. Two such proteins, acting in association with pyridine coenzyme as dehydrogenases and serving as hydrogen carriers in coupled reactions during fermentation, have been crystallized. They are: (*1*) the protein of the *oxidizing fermentation enzymes*,[419] which, in combination with codehydrogenase, withdraws hydrogen from its substrate, diphosphoglyceraldehyde (an intermediate product of fermentation), and oxidizes it to diphosphoglyceric acid; and (*2*) the protein of the *reducing fermentation enzyme*,[420] which accepts hydrogen from the oxidizing enzyme and transfers it to acetaldehyde:

[418] E. Negelein and H. J. Wulff, *Biochem. Z.*, **293**, 351 (1937).

[419] O. Warburg and W. Christian, *Biochem. Z.*, **303**, 40 (1939).

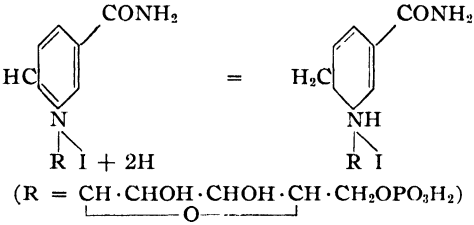
[420] E. Negelein and H. J. Wulff, *Biochem. Z.*, **289**, 436 (1937); **293**, 351 (1937).



Similarly in muscle, a system consisting of two pyridine proteins effects the dehydrogenation of diphosphoglyceraldehyde in the course of glycogenolysis, whereby hydrogen is finally transferred to pyruvic acid, which is reduced to lactic acid.

The dissociation constant of the enzyme into apoferment and nucleotide has been measured by Negelein[421]; half of the proteid is dissociated when the total concentration is 2.2×10^{-5} mole per liter. The dissociation constant is therefore 1.1×10^{-5}. From this, according to Nernst's theorem, the heat of dissociation can be calculated and amounts to about 18,000 cal. per mole. From this high value for the heat of dissociation, it follows that coenzyme and apoferment must be linked by secondary valences at several points.

The mode of reaction of pyridine dehydrogenases has been explained by Karrer[422,423]; the formulas given here show the two stages of nicotinamide and dihydroamide:



(g) *Various Enzyme Proteids*

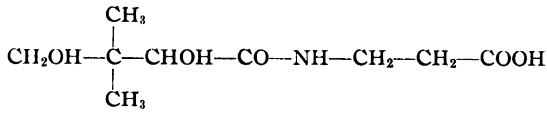
According to Lipmann[424] a derivative of pantothenic acid acts as coenzyme in the acetylation of aromatic amines, as well as the oxidation of pyruvic acid. The nature of this derivative is not yet known.

[421] E. Negelein and E. Haas, *Biochem. Z.*, **282**, 206 (1935).

[422] P. Karrer, G. Schwarzenbach, F. Benz, and U. Solmssen, *Helv. Chim. Acta*, **19**, 811 (1936).

[423] P. Karrer, B. H. Rignier, J. Büchi, H. Fritzsche, and U. Solmssen, *ibid.*, **20**, 55 (1937).

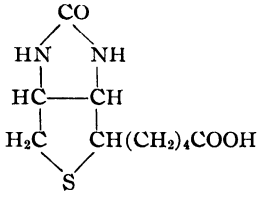
[424] F. Lipmann, M. O. Kaplan, G. D. Novelli, L. C. Tuttle, and B. M. Guirard, *J. Biol. Chem.*, **167**, 869 (1947). G. D. Novelli and F. Lipmann, *Arch. Biochem.*, **14**, 23 (1947); *J. Biol. Chem.*, **171**, 833 (1947).



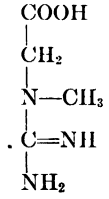
Pantothenic acid

Laurence[425] contends that biotin is the coenzyme of lysozyme. This assertion has, however, been contested by Alderton.[426] Fleming[427] discovered lysozyme in the nasal secretion, in tears, and in the saliva. It has been obtained in a crystalline form from the white of hen's egg by Abraham and Robinson.[428] It has the capacity of dissolving bacteria, especially the *Micrococcus lysodeikticus*, probably by hydrolysis of glucosidic linkages in glycoproteids.[429] Its molecular weight amounts to 18,000.

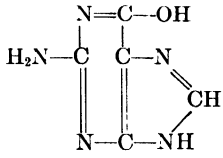
Cori found[430] that the activity of adenosine triphosphatase which catalyzes the reaction: adenosine triphosphoric acid → adenosine diphosphoric acid + orthophosphoric acid, is increased by creatine, but that no phosphocreatine is formed in the course of that reaction.



Biotin



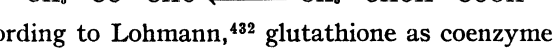
Creatine



Guanine

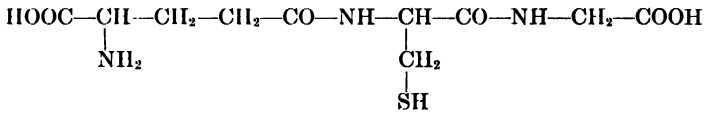
Muscle hexokinase, but not yeast hexokinase, needs guanine as coenzyme, according to Colowick.[431] Hexokinase catalyzes the following reaction: glucose + adenosine triphosphoric acid → glucose 6-phosphoric acid + adenosine diphosphoric acid.

The glyoxalase, the enzyme which transforms methylglyoxal into lactic acid:

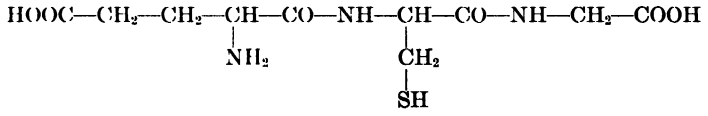


contains, according to Lohmann,[432] glutathione as coenzyme. Behrens[433] has found that glutathione can be replaced by isoglutathione or asparthione:

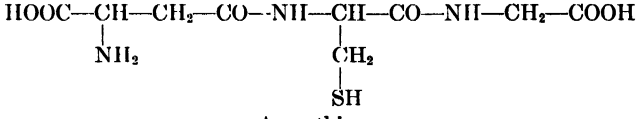
[425] W. Laurence, *Science*, **99**, 392 (1944).
[426] G. Alderton, W. H. Hard, and H. L. Fenold, *J. Biol. Chem.*, **157**, 43 (1945).
[427] A. Fleming, *Proc. Roy. Soc. London*, **B93**, 306 (1922).
[428] E. P. Abraham and R. Robinson, *Nature*, **140**, 24 (1937).
[429] C. Hallauer, *Zentr. Bakt. Parasitenk. Abt. I Orig.*, **114**, 519 (1929).
[430] W. H. Price and C. F. Cori, *J. Biol. Chem.*, **162**, 393 (1946).
[431] S. P. Colowick and W. H. Price, *J. Biol. Chem.*, **159**, 563 (1945).
[432] K. Lohmann, *Biochem. Z.*, **254**, 332 (1932).
[433] O. K. Behrens, *J. Biol. Chem.*, **141**, 503 (1941).



Glutathione



Isoglutathione



Asparthione

(*h*) *Iron Porphyrin Proteids*[434]

A series of enzymes which participate in the transfer of oxygen contain, as prosthetic group, an iron protoporphyrin complex. In this complex, the iron may change from the ferric into the ferrous form and *vice versa*. This is the case with the cytochromes, the cytochromoxidases, and Warburg's respiratory enzyme.[435] In contradiction to this, iron always remains in the trivalent form in the peroxidases and catalase, while in hemoglobin and myoglobin, which also contain iron protoporphyrin as prosthetic group, iron always appears in the bivalent form. All these linkages possess very characteristic absorption spectra. Knowledge of this class of enzymes and their functions is due to the work of Hans Fischer, Keilin and Hartree, Sumner and Dounce, Theorell, Agner and Akeson, Warburg and Negelein, and Willstätter.

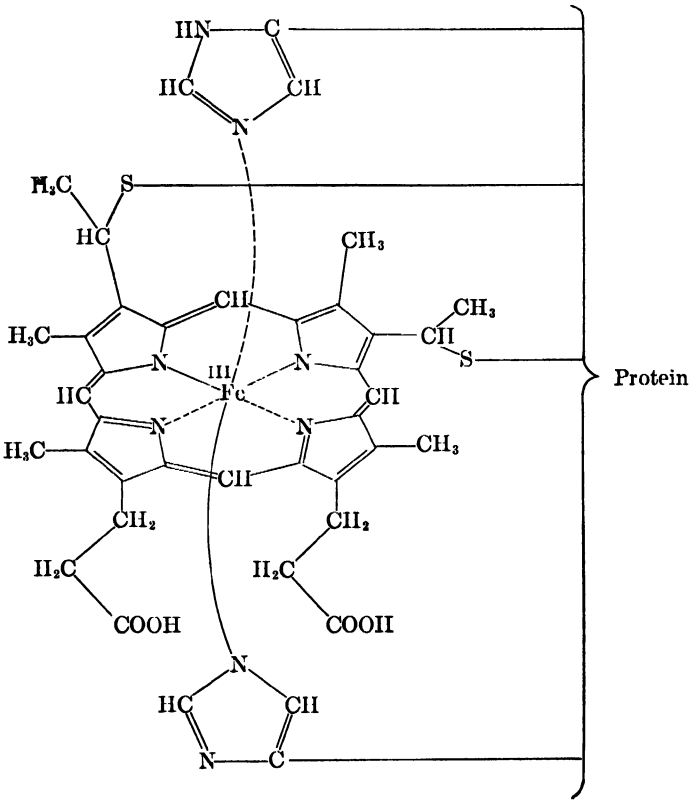
Three different cytochromes, a, b, and c, are known; their biological function is to remove hydrogen from the pyridine adenine proteids (anaerodehydrogenases) and to pass it on to atmospheric oxygen with the cooperation of cytochromoxidase. Cytochrome c has been prepared in a pure form by Theorell.[436] It has a molecular weight of 13,000; its isoelectric point is at *p*H 9.9, and it contains 0.43% of iron, *i.e.*, one iron protoporphyrin group per mole. The protoporphyrin part is bound to the protein by cysteine, involving sulfur linkages, and in addition to these, secondary

[434] Survey: see A. Stoll, *Experientia*, **4**, 6 (1948).

[435] O. Warburg, *Biochem. Z.*, **152**, 479 (1924); *Naturwissenschaften*, **22**, 441 (1934).

[436] H. Theorell and A. Akeson, *Science*, **90**, 67 (1939).

valences are involved with the imidazol cycles of histidine, which is found in amounts which correspond to 3.3% of the apoenzyme:



Ferricytochrome c

A peroxidase with an iron content of 0.127% has been obtained by Theorell[437] from horseradish. Its molecular weight amounts to 44,000 and it contains one iron protoporphyrin group (hematin) per mole. The apoenzyme is a glucoprotein with approximately 20% of carbohydrate. Peroxidase could be hydrolyzed into apoenzyme and hematin by hydrochloric acid in acetone at $-15°$.[437] Both components recombine slowly to form the active holoenzyme. Hemin may be replaced for this operation by

[437] H. Theorell, *Arkiv. Kemi Mineral. Geol.*, **B14**, No. 20 (1940).

similar porphyrin derivatives, such as deuterohemin and mesohemin,[438] but in each case the peroxidase activity obtained is somewhat smaller than that obtained with hematin. In combining apoperoxidase with a metal protoporphyrin containing, instead of iron, manganese, Sumner[439] has obtained an enzyme, the activity of which amounts to approximately one fourth of the original peroxidase activity; peroxidase catalyzes the reaction of hydrogen peroxide with many phenols and aromatic amines. The enzyme takes part in this reaction in the following way:

$$\text{Enzyme—Fe}^{III}\text{—OH} + H_2O_2 \longrightarrow \text{enzyme—Fe}^{III}\text{—O—OH} + H_2O$$

$$\text{Enzyme—Fe}^{III}\text{—O—OH} + \text{substratum} \longrightarrow \text{enzyme—Fe}^{III}\text{—OH} + \text{oxidized substratum}$$

The enzyme catalyzing the decomposition of hydrogen peroxide into water and oxygen, is widespread and occurs in nearly all animal organs. It was obtained for the first time in a crystallized form by Sumner,[440] from ox liver: it has a molecular weight of 225,000 to 250,000 and contains four iron protoporphyrin groups per mole. As Bonnichsen[441] has shown by comparing crystallized catalases of different origin (horse liver, horse blood, horse kidneys, human blood, human liver), catalase preparations from different organs of the same species are identical.

A bacterial catalase has been obtained in a crystalline form by Herbert[442] from *Micrococcus lysodeikticus*. This likewise has a molecular weight between 225,000 and 250,000 and, like the animal catalases, contains four hematin groups per molecule.

Theorell[443] has elucidated the reaction mechanism of catalase. In the presence of small amounts of hydrogen peroxide and an oxygen acceptor such as methanol catalase behaves like a peroxidase:

$$\text{Porphyrin proteid}\begin{cases}=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\end{cases} + H_2O_2 \longrightarrow \text{porphyrin proteid}\begin{cases}=\text{Fe—O—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\end{cases} + H_2O$$

$$\text{Porphyrin proteid}\begin{cases}=\text{Fe—O—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\end{cases} + CH_3OH \longrightarrow \text{porphyrin proteid}\begin{cases}=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\end{cases} + CH_2O + H_2O$$

[438] H. Theorell, *Arkiv. Kemi Mineral Geol.*, **A16**, No. 13 (1942).
[439] E. C. Gjessing and J. B. Sumner, *Arch. Biochem.*, **1**, 1 (1942).
[440] J. B. Sumner and A. L. Dounce, *J. Biol. Chem.*, **121**, 417 (1937); **127**, 439 (1939).
[441] R. K. Bonnichsen, *Arch. Biochem.*, **12**, 83 (1947); *Acta Chem. Scand.*, **1**, 114 (1947).
[442] D. Herbert and A. J. Pinsent, *Nature*, **160**, 125 (1947).
[443] H. Theorell, *Experientia*, **4**, 100 (1948). B. Chance, *Acta Chem. Scand.*, **1**, 235 (1947); *Rev. Sci. Instruments*, **18**, 601 (1947).

In the presence of an excess of hydrogen peroxide oxygen evolution occurs according to one or other of the two following reactions:

$$\text{Porphyrin proteid}\begin{cases}=\text{Fe—O—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\end{cases} + H_2O_2 \longrightarrow \text{porphyrin proteid}\begin{cases}=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\end{cases} + H_2O + O_2$$

$$\text{Porphyrin proteid}\begin{cases}=\text{Fe—O—OH}\\=\text{Fe—O—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\end{cases} \longrightarrow \text{porphyrin proteid}\begin{cases}=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\\=\text{Fe—OH}\end{cases} + O_2$$

The latter mode of reaction explains why peroxidase, which has only one hematin group per molecule, has no catalase effect.

(*i*) *Enzyme Activity Resulting from Combined Action of Several Component Enzymes*

The various oxidases and dehydrogenases of the flavin, pyridine, or hemin types already mentioned, together with the copper proteids (to be considered later), all of which Wieland classes together as hydrokinases, are ineffective by themselves and do not operate alone in biological processes. In practice it is found that several proteids act together: several component enzymes are associated to form a complete enzyme system. The most widespread system of this kind is undoubtedly that known as the cytochrome system:

Substrate ——→ pyridine dehydrogenase–cytochrome complex

(3 cytochromes)-respiratory enzyme–oxygen

Let us consider as an example (following Warburg) the dehydrogenation of hexose by molecular oxygen. This reaction can be evoked by adding a pyridine proteid together with an alloxazine proteid or copper proteid plus pyrocatechol to a solution of phosphorylated hexose. The course of the hydrogen through the successive intermediary reactions is then as follows:

Chain I: Hexose—pyridine/dihydropyridine—alloxazine/dihydroalloxazine—oxygen

Chain II: Hexose—pyridine/dihydropyridine—orthoquinone/pyrocatechol—cupric/cuprous—oxygen

In chain I, pyridine dehydrogenates hexose, alloxazine dehydrogenates the hydrogenated pyridine, molecular oxygen dehydrogenates the hydrogenated alloxazine and is itself hydrogenated to form hydrogen peroxide.

At the end of the reaction, the substrates, oxygen and hexose, have disappeared, while, with respect to the proteids, the initial condition prevails once more. This hexose–dehydrogenase system consists, therefore, of the nonautoxidizable pyridine proteid and the autoxidizable alloxazine proteid.

In chain II, pyridine dehydrogenates hexose as in the first case, but now orthoquinone dehydrogenates the hydrogenated pyridine, copper dehydrogenates pyrocatechol, oxygen oxidizes univalent copper and is itself hydrogenated to form water.

Both dehydrogenases lead to the formation of the same oxidation product of hexose, since the proteid reacting with hexose is common to both. But, since the proteid which reacts with oxygen is different in both cases, different hydrogenation products of oxygen arise—in chain I, hydrogen peroxide, in chain II, water.

3. Metal Proteids with Enzyme Properties

The metal proteids now to be described can be separated into two groups. (*1*) This group consists of the copper proteids in which the valence of the metal changes reversibly from the bivalent to the monovalent state, and so confers upon the proteid oxidizing and reducing properties analogous to the function of iron in cytochrome. (*2*) This group consists of the proteids containing bivalent metals—zinc, magnesium, manganese, etc. The metal ion is usually very readily removed by dialysis with loss in activity. It is characteristic for this group of enzymes that the apoferment is not only reactivated by the metal ion removed by dialysis, but also a whole series of other divalent metallic ions can combine to give active holoferments. The following metallic ions, consistently bivalent, are interchangeable coenzymes: magnesium, calcium, zinc, cadmium, manganese, iron, cobalt, and nickel. The enzymatic effect is in all cases considerably lower than that of the natural enzyme.

(*a*) *Copper Proteids*

According to Nelson[444] the name tyrosinase is applied to two very similar enzymes, polyphenoloxidase and monophenoloxidase.

Polyphenoloxidase has been prepared from potatoes by Kubowitz[445] and from the *Psalliota campestris* by Mann.[446] It can bring about atmospheric oxidation of catechol to *o*-benzoquinone, and likewise of other poly-

[444] J. M. Nelson and C. R. Dawson, *Advances in Enzymol.*, **4**, 99 (1946).
[445] F. Kubowitz, *Biochem. Z.*, **292**, 221 (1937); **299**, 32 (1938).
[446] D. Keilin and T. Mann, *Proc. Roy. Soc. London*, **B125**, 187 (1938).

and monohydroxy phenols. It contains 0.2 to 0.3% of copper, which effects the oxygen transfer by cupric–cuprous interchange. The enzyme can be split up into protein and copper salt by means of hydrocyanic acid under neutral conditions and can be resynthesized from copper ion and the protein purified by dialysis. Monophenoloxidase has been prepared from the mold *Lactarius piperatus* by Dalton and Nelson.[447] It contains 0.23% of copper and is able to oxidize *p*-cresol ten times faster than catechol.

Ascorbic acid oxidase has been obtained in a crystalline form from pumpkin seeds by Tadokoro and Takasugi.[448] This enzyme contains 0.25% of copper bound in the complex.

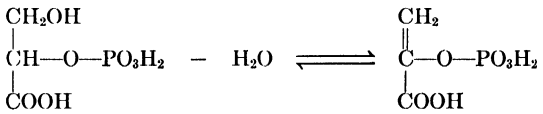
Laccase, an enzyme containing 0.24% of copper, has been isolated from an East Asiatic lac tree by Keilin and Mann.[449] It oxidizes various phenols, guaiacol, catechol, hydroquinone, pyrogallol, and others, but not tyrosine, *p*-cresol, or resorcinol.

(*b*) *Zinc Proteids*

Carbonic anhydrase catalyzes the reaction: $H_2CO_3 \rightleftharpoons H_2O + CO_2$. This enzyme, which contains 0.2% of zinc has been isolated by Scott and Fisher[450] in a crystalline form from mammalian blood cells. It is soluble in 99% alcohol. It is extraordinarily sensitive toward sulfanilamide which has an inhibiting effect even at a concentration as low as 2.10^{-6} molar.

(*c*) *Magnesium Proteids*

Enolase is the effective catalyst for the removal of water from 2-phosphoglyceric acid to give the enol ester of phosphopyruvic acid:



It was discovered by Meyerhof and Lohmann,[451] prepared in a pure state by Warburg and Christian,[452] and crystallized as the mercury salt. By dialysis of the enzymatically inactive mercury salt against hydrocyanic acid, an equally inactive apoenzyme is obtained. By the addition of magnesium

[447] H. R. Dalton and J. M. Nelson, *J. Am. Chem. Soc.*, **61**, 2946 (1939).

[448] T. Tadokoro and H. Takasugi, *J. Chem. Soc. Japan*, **60**, 188 (1939).

[449] D. Keilin and T. Mann, *Nature*, **143**, 23 (1939); **145**, 304 (1940).

[450] D. A. Scott and A. M. Fisher, *J. Biol. Chem.*, **144**, 371 (1942). D. A. Scott, *ibid.*, **142**, 959 (1942).

[451] K. Lohmann and O. Meyerhof, *Biochem. Z.*, **273**, 60 (1934).

[452] O. Warburg and W. Christian, *Biochem. Z.*, **310**, 394 (1942).

salts, but also of manganese or zinc salts, the active holoenzyme is obtained. The natural enzyme is the magnesium compound. Enolase is inhibited by fluorine ions in the presence of inorganic phosphate, this being due to the fixing of magnesium fluorophosphate.

Hexokinase catalyzes the reaction:

Glucose + adenosine triphosphate ⟶ glucose 6-phosphate + adenosine diphosphate

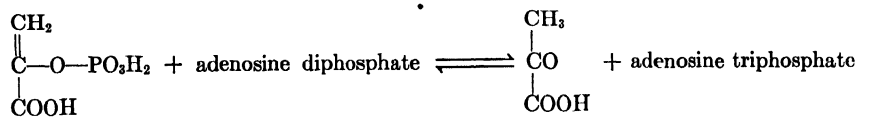
It was obtained in a crystalline form simultaneously by Cori[453] and by Kunitz,[454] starting from bakers' yeast. The molecular weight amounts to 96,600; the isoelectric point lies between *p*H 4.5 and 4.8. Hexokinase is only effective in the presence of magnesium or manganese ions. According to Colowick[455] muscle hexokinase, but not yeast hexokinase, requires in addition both guanine and dihydrocozymase. Furthermore, the muscle enzyme, according to Cori,[456] is inhibited by a hormone present in the adrenal cortex and the hypophysis. Insulin suppresses this inhibition.

Phosphohexokinase catalyzes the reaction:

Fructose 6-phosphate + adenosine triphosphate → fructose 1,6-diphosphate + adenosine diphosphate

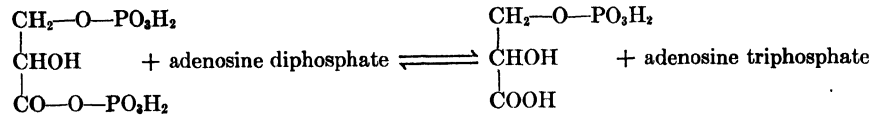
This enzyme likewise is only effective in the presence of magnesium ions.

Phosphopyruvic acid phosphopherase has been obtained in a crystalline form from human muscle by Kubowitz and Ott.[457] It catalyzes:



and here the presence of magnesium and potassium ions is necessary.

Diphosphoglyceric acid phosphopherase has been obtained in a crystalline form from yeast by Bücher. It catalyzes the reaction:



[453] L. Berger, M. W. Slein, S. P. Colowick, and C. F. Cori, *J. Gen. Physiol.*, **29**, 379 (1946).

[454] M. Kunitz and M. R. McDonald, *J. Gen. Physiol.*, **29**, 393 (1946).

[455] S. P. Colowick and W. H. Price, *J. Biol. Chem.*, **159**, 563 (1945).

[456] S. P. Colowick, G. T. Cori, and M. W. Slein, *J. Biol. Chem.*, **168**, 583 (1947).

[457] F. Kubowitz and P. Ott, *Biochem. Z.*, **317**, 193 (1944); H. A. Lardy and J. A. Ziegler, *J. Biol. Chem.*, **159**, 343 (1945).

Again magnesium ions are necessary. Bücher[458] has measured the dissociation constant of the compound formed with the enzyme protein and each of the four substrates participating in the reaction and with the coferment (Table XIX).

TABLE XIX

Substrate or coferment	Dissociation constant, moles/cc.
Adenosine diphosphate	2×10^{-7}
Adenosine triphosphate	1.1×10^{-7}
1,3-Diphosphoglyceric acid	1.8×10^{-9}
3-Phosphoglyceric acid	2×10^{-7}
Magnesium	2.5×10^{-7}

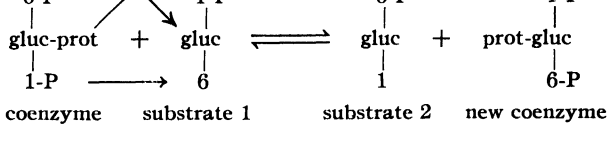
Thus the linkage between apo- and coenzyme is considerably weaker than that between the active enzyme and one of the four substrates, 1,3-diphosphoglyceric acid.

Phosphoglucomutase,[459] which has been crystallized by Najjar[459a] and phosphoglyceromutase[460] are activated by Mg ions. The two enzymes catalyze, respectively, the reactions:

$$\text{Glucose-1-phosphate} \rightleftharpoons \text{glucose-6-phosphate}$$

$$\text{3-Phosphoglyceric acid} \rightleftharpoons \text{2-phosphoglyceric acid}$$

Leloir[460a] has found that the coenzyme of phosphoglucomutase is glucose 1,6-diphosphate. According to Cori[460b] phosphomutation proceeds in the following way:



[458] T. Bücher, *Biochim. et Biophys. Acta*, **1**, 292 (1947).

[459] G. T. Cori, S. P. Colowick, and C. F. Cori, *J. Biol. Chem.*, **121**, 465 (1937); **123**, 375 (1938).

[459a] V. A. Najjar, *J. Biol. Chem.*, **175**, 281 (1948).

[460] O. Meyerhof and W. Kiessling, *Naturwissenschaften*, **22**, 838 (1934).

[460a] C. E. Cardini, A. C. Paladini, R. Caputto, L. F. Leloir, and R. E. Trucco, *Arch. Biochem.*, **22**, 87 (1949).

[460b] E. W. Sutherland, Th. Posternak, and C. F. Cori, *J. Biol. Chem.*, **179**, 501 (1949).

in which the substrate, glucose 1-phosphate, is phosphorylated to glucose 1,6-diphosphate (thus becoming the new coenzyme) while the former coenzyme is liberated as glucose-6-phosphate. The same reaction occurs with phosphoglyceromutase in the presence of 1,3-diphosphoglyceric acid which also acts as coenzyme.

Phosphatases are enzymes which bring about the fission of phosphates. Numerous different phosphatases are known, distinguished by their ability to break down mono-, di-, or pyrophosphate esters and by their effectiveness in acid or alkaline media. Most of these are activated by magnesium ions in larger or smaller amounts; others are activated by manganese, iron (II), cobalt (II), nickel (II), calcium, barium, or cadmium ions.[461]

As mentioned previously (sect. VII2*a*), carboxylase is also a magnesium proteid. It is a remarkable fact that almost all enzymes requiring magnesium or other bivalent metals as activators are active on phosphorus-containing substrates and one of these (carboxylase) has a phosphate-containing coferment.

(*d*) *Manganese Proteids*

Arginase, which brings about the breakdown of arginine to ornithine and urea, contains complex-bound manganese (Edlbacher[462]). After deactivation by dialysis, arginase can be reactived by cobalt (II), nickel (II), iron (II), cadmium, or vanadium (II). Liver arginase has been obtained in a crystalline form by Bach.[463]

4. Mechanism of Enzyme Catalysis

The results of experiments made on pure enzymes suggest that enzyme action may be pictured in the following way. The enzyme contains a particular group suitable for the catalysis of one reaction only: that characteristic of the enzyme in question. Thus, the transfer of oxygen by the flavin enzyme depends on the presence of flavin, while oxygen transfer by catalase is dependent on the ionic iron present. As a result of the influence of certain so-called activating protein groups in the neighborhood of the active group, however, the reaction velocity is extraordinarily increased. How the heat of activation is lowered by close association with a particular configura-

[461] See E. Bamann and M. Meisenheimer, in Bamann and Myrbäck, *Methoden der Fermentforschung*, Leipzig, 1941, page 1620. H. Albers, in *Handbuch der Enzymologie*, Leipzig, 1940.

[462] S. Edlbacher and H. Baur, *Z. physiol. Chem.*, **259**, 275 (1938).

[463] S. J. Bach, *Nature*, **158**, 376 (1946).

tion of active groups is still not clear. An excellent example of such "activation" is provided by catalase (the enzyme destroying hydrogen peroxide), studied by Kuhn, as compared with hemin and ionic iron. At 0°C. in aqueous solution the following quantities of hydrogen peroxide are decomposed per second:

1 mole of	H_2O_2 decomposed, moles
Fe^{+++}	10^{-5}
Hemin	10^{-2}
Catalase	10^{5}

The change in the absorption spectrum shows that the electronic structure of the prosthetic group is in some way modified by linkage with the protein. The absorption band of flavin phosphoric acid, for example, shifts toward the red by 20 mμ on linkage with protein (Warburg and Christian[464]). This indicates that the energy of activation of alloxazine is lowered by combination with protein.

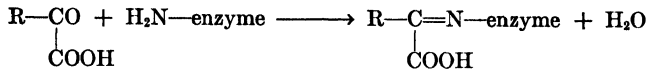
Yet another function of the apoferment may be that of establishing a link with the reacting partners of the coferment. Indications that such intermediate compounds are formed are afforded by the system flavin enzyme–pyridine proteid. The latter is dehydrogenated by the former, and the reacting system takes on a distinct color which has been ascribed to the formation of an addition compound (Haas[465]).

Even in the case of those enzymes of which the active group is still unknown, catalytic activity is localized in a particular group, which becomes activated as a result of its environment in the molecule. In this way the barely detectable catalytic activity of the group in compounds of low molecular weight is considerably increased, or else activity, which in practice escapes detection in compounds of the group of low molecular weight, makes its appearance. An example of the augmentation of activity in this way is provided by carboxylase. Simple amines also act as carboxylases and, as Langenbeck[466] has shown, this activity depends on the constitution of the molecule; thus alanine is thirty times as active in the decarboxylation of phenylglyoxylic acid as is methylamine. Carboxylase itself is an amine and, hence, the amino group may be regarded as the active group. According to Langenbeck, the reaction can be represented in the following way:

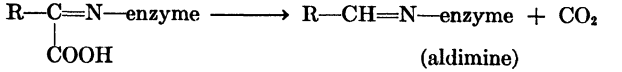
[464] O. Warburg and W. Christian, *Naturwissenschaften*, **20**, 980 (1932).

[465] E. Haas, *Biochem. Z.*, **290**, 291 (1937).

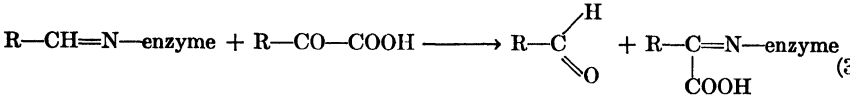
[466] W. Langenbeck, *Ergebn. Enzymforsch.*, **2**, 314 (1933); *Die organischen Katalysatoren*, Springer, Berlin, 1935.



(1)



(2)



(3)

VIII. VIRUSES AND VIRUS PROTEINS[467]

It has been known for a long time that certain infectious diseases of animals, plants, and bacteria are caused by agents which cannot be cultivated on a culture medium but can only multiply inside living cells. These agents are called viruses, and are known to be very small particles, frequently compared with protein molecules. It is accordingly legitimate to discuss the virus problem from the chemical and physicochemical points of view in these pages.

1. Size of Virus Particles

Virus particles are neither retained by bacterial filters nor visible under the microscope. Their dimensions must therefore be considerably smaller than the mean wave length of visible light. Barnard,[468] using ultraviolet light down to a wave length of 100 mμ, has been able to detect the agents of certain diseases, such as smallpox, while others (presumably smaller) could not be detected. He was also able to estimate the size of certain viruses.

Particularly successful systematic studies by Bechhold[469] have led to the determination of the size of even smaller viruses. He used the method of filtration through graduated ultrafilters and also estimated the particle weight from the velocity of sedimentation in a high-speed laboratory centrifuge. Here, as elsewhere, the quantitative estimation of the virus was made

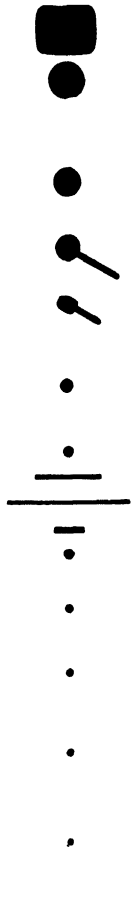
[467] Summaries: R. Doerr and C. Hallauer, *Handbuch der Virusforschung*, Wien, 1931; G. Seiffert, *Virus und Viruskrankheiten*, Leipzig, 1938. F. C. Bawden, *Plant Viruses and Plant Diseases*, Leiden, 1939. W. M. Stanley, *Ergebn. Physiol.*, **39**, 294 (1937); *J. Phys. Chem.*, **42**, 55 (1938); *J. Applied Physics*, **9**, 148 (1938). R. W. G. Wyckoff, *Naturwissenschaften*, **25**, 481 (1937). F. Lynen, *Kolloid-Z.*, **85**, 222 (1938).

[468] E. Barnard, *Proc. Roy. Soc. London*, **B109**, 360 (1931); *J. Roy. Microscop. Soc.*, **59**, 1 (1939).

[469] H. Bechhold, *Kolloid-Z.*, **66**, 329 (1934); **67**, 66 (1934).

TABLE XX

	Diameter or width × length in mμ
Psittacosis	450
Canary pox	260 × 310
Myxoma	230 × 290
Vaccinia	210 × 260
Pleuropneumonia organism	150
Pseudo rabies	150
Herpes simplex	150
Rabies fixe	125
Influenza	115
Newcastle disease	115
Vesicular stomatitis	100
Staphylococcus bacteriophage	100
Fowl plague	90
T_2 *coli* bacteriophage	60 × 80
Chicken tumor I	70
Equine encephalomyelitis	50
T_3 *coli* bacteriophage	45
Rabbit papilloma (Shope)	44
Pneumonia virus of mice	40
Tobacco mosaic and strains	15 × 280
Latent mosaic of potato	10 × 525
Gene (Muller's est. of max. size)	20 × 125
Southern bean mosaic	31
Rift valley fever	30
Tomato bushy stunt	26
Poliomyelitis (Lansing)	25
Hemocyanin molecule (*Busycon*)	22
Yellow fever	22
Hemocyanin molecule (*Octopus*)	20
Louping ill	19
Tobacco ring spot	19
Japanese B encephalitis	18
Alfalfa mosaic	17
Tobacco necrosis	16
Foot-and-mouth disease	10



All viruses down to and including T_2 *coli* bacteriophage seem to have an internal structure. T_2 has a membrane. The dimensions of the smallest viruses are probably greater than indicated in the table.

by means of its infectivity; special methods of testing are necessary for each virus.

The method of determining size by ultrafiltration has been perfected by Elford[470] and applied to many other viruses. The values obtained by these authors have been proven to be correct by more recent researches, in particular electron micrographs. Table XX, taken from Stanley,[471] gives the dimensions of a number of viruses according to our actual knowledge.

Before 1936, therefore, the size of virus particles was known with some certainty, while nothing was known of the chemical substances occurring in them. For this purpose large quantities of pure virus were required. The task of preparing and analyzing pure viruses was undertaken by Stanley and accomplished in masterly fashion.

2. Tobacco Mosaic Virus

In the preparation of the virus, infected tobacco plants are frozen, three weeks after infection, and pressed on thawing. By various and somewhat laborious methods of preliminary purification, and precipitation with ammonium sulfate, Stanley[472] obtained from the expressed sap a substance soluble in water at *p*H 7, giving an opalescent solution which, on the addition of ammonium sulfate and acetic acid, yielded needle-like crystals.

A second method of concentration has been developed by Bauer, Pickels, and Wyckoff[473] using the preparative ultracentrifuge. The expressed sap is centrifuged in a field of 50,000 g. and the active substance is thrown down. The deposit is dissolved in water and centrifuged in a weaker field to get rid of coarse particles. The solution can then be recentrifuged at high speed and the virus thrown down. The virus is soon obtained in so pure a condition that it crystallizes in the centrifuge.

From the velocity of sedimentation in the ultracentrifuge a "molecular weight" of $\sim 17{,}000{,}000$ was determined[474]; this means that the weight of a single particle is 3×10^{-17} g. (30 billion particles weigh 1 γ). The density of the crystals is 1.35; they contain 50.7% C, 7.6% H, 16.6% N, 0.5% P, 0–0.2% S, and 0.5% ash. The phosphorus is combined in a nucleic acid, which may be separated from the protein in various ways.[475] Bawden and Pirie as well as Stanley therefore speak of the agent as a nucleoproteid.

[470] W. J. Elford, *Trans. Faraday Soc.*, **33**, 1094 (1937).

[471] W. M. Stanley, *Chem. Eng. News*, **25**, 3786 (1947).

[472] W. M. Stanley, *Science*, **81**, 644 (1935).

[473] R. W. G. Wyckoff, *Naturwissenschaften*, **25**, 481 (1937).

[474] I. B. Eriksson-Quensel and T. Svedberg, *J. Am. Chem. Soc.*, **58**, 1863 (1936). R. W. G. Wyckoff, *J. Biol. Chem.*, **121**, 219 (1937).

[475] F. C. Bawden and N. W. Pirie, *Proc. Roy. Soc. London*, **B123**, 274 (1937).

The isoelectric point is at *p*H 3.5. From *p*H 2 to *p*H 8 the protein is stable, but if the reaction is more acid or more alkaline, it breaks up into smaller fragments, and activity is lost. Under certain conditions a crystallizing but inactive high molecular protein may be formed from these fragments.[476] Formaldehyde and hydrogen peroxide abolish activity without influencing the power of crystallization. The S—H groups of the virus can be oxidized to S—S groups in the presence of iodine without the virus losing its activity.[477]

3. Shape of Particles of Tobacco Mosaic Virus

The aqueous solution of the crystals shows very marked birefringence of flow. On stirring the solution, illuminated from the side, marked opalescent turbidity is seen—as in other solutions, such as those of benzopurpurine or mercurosulfosalicylate, which exhibit birefringence of flow. From this we may conclude that submicroscopic rod-shaped particles are present, which, on stirring, are brought to lie parallel to each other.

The birefringence of flow of a virus-containing plant extract is considerably less than that of a solution of pure virus of the same concentration. Apparently, more virus particles are associated to form elongated aggregates in the pure solution than in the sap, where association is hindered by the presence of other substances. This view is supported by the behavior on ultrafiltration. Particles from crude sap are able to pass through pores 53 mμ in diameter (Smith and Doncaster[478]), while pure virus suspended in water is held back by coarse filters with pores 450 mμ in diameter. Pure virus also settles spontaneously on standing.

The crystals have been subjected to x-ray analysis by Bernal and Fankuchen.[479] According to them, the virus particles composing the crystals are minute rods, the length of which is more than ten times the diameter, which amounts to $\sim$150 Å. Within the needle-like crystal, these rods are not arranged in a regular three-dimensional lattice but are packed hexagonally in two dimensions at right angles to the long axis. That is to say, they are arranged as a bundle of rods of equal thickness becomes arranged when grasped in the hand. Such imperfect crystals are known as paracrystals. This two-dimensional, paracrystalline, hexagonal arrangement also

[476] E. Pfankuch and E. Piepenbrock, *Naturwissenschaften*, **31**, 94 (1943). G. Schramm, *ibid.*, **31**, 94 (1943).

[477] M. L. Anson and W. M. Stanley, *J. Gen. Physiol.*, **24**, 679 (1941).

[478] K. M. Smith and J. P. Doncaster, quoted from Lynen, *Kolloid-Z.*, **85**, 222 (1938).

[479] F. C. Bawden, N. W. Pirie, J. D. Bernal, and I. Fankuchen, *Nature*, **138**, 1051 (1936). J. D. Bernal and I. Fankuchen, *ibid.*, **139**, 923 (1937).

arises spontaneously in concentrated aqueous solutions; but, whereas the spacing of the rods in the crystal is 152 Å., that in a 13% solution is about 450 Å. In the latter case, therefore, the reflections of the x-ray beam lie nearer the primary beam as is seen in figure 17.

In addition to the interferences indicating the arrangement described above, a number of spots corresponding to much smaller spacings are visible; these indicate a regular arrangement of material within the virus particle. According to Bernal the dimensions of these subparticles of which the virus particle is composed are ~22 × 20 × 20 Å.

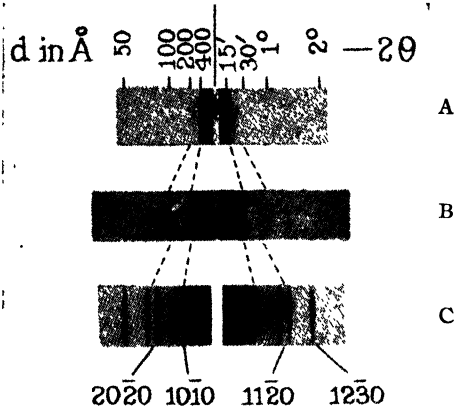


Fig. 17. Equatorial spots from oriented gels of tobacco mosaic virus. A = dried gel; B = moist gel; and C = gel containing 87% H_2O.

The conclusions concerning the shape of the particles drawn from birefringence of flow and crystal structure have been confirmed by direct observation with the electron microscope.[480] As figure 37, Chapter A shows, the particles are seen as threads of 0.3–0.5 μ length and 0.015 μ width.

4. Other Viruses

Using the ultracentrifuge, other viruses have been concentrated and obtained as paracrystals. Of these may be mentioned the tobacco ring spot virus (Stanley and Wyckoff[481]), the potato virus (Stanley and Wyckoff[481];

[480] G. A. Kausche, E. Pfankuch, and H. Ruska, *Naturwissenschaften*, **27**, 292 (1939).
[481] W. M. Stanley and R. W. G. Wyckoff, *Science*, **85**, 181 (1937).

Bawden and Pirie,[482] the severe etch virus,[483] and the virus of the polyhedral disease of caterpillars. Others, such as Shope's rabbit papilloma virus[443] and equine encephalomyelitis,[484] are amorphous.

Bawden and Pirie[485,486] have obtained the virus of Bushy stunt in tomatoes in the form of dodecahedra (Fig. 18). The x-ray analysis by Bernal, Fankuchen, and Riley[487] showed two reflections, corresponding to spacings of 279 Å. and 160 Å. The dodecahedral form suggests a body-centered lattice; from this and the indexing (110) and (112), the particles should

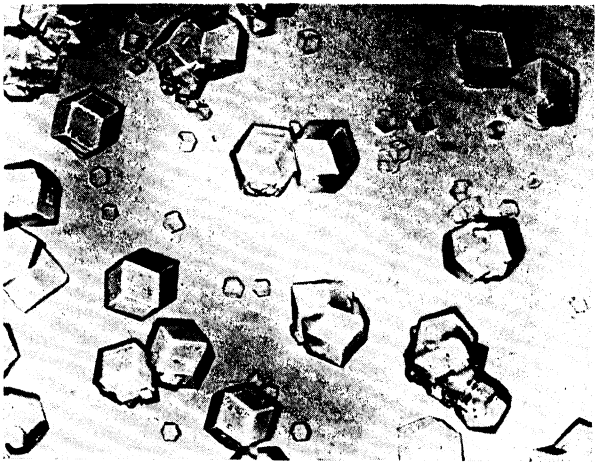


Fig. 18. Crystals of bushy stunt virus.

have a radius of 170 Å. Since the density is 1.29, this corresponds to a molecular weight of 24,000,000. On drying, the crystals shrink, and the molecular weight of the dry particles amounts to 12,800,000 as compared with the value of 8,800,000 obtained with the ultracentrifuge.

Stanley proposed to classify the viruses as proteins on the grounds of the newly discovered crystallizability of tobacco mosaic virus and of their small size which supposedly indicates a simple chemical constitution. He also ascribed to them a specific method of multiplication. While living cells multiply by division: "*Omnis cellula ex cellula*," the virus proteins already

[482] F. C. Bawden and N. W. Pirie, *Nature*, **141**, 1105 (1938).
[483] J. W. Beard and R. W. G. Wyckoff, *J. Biol. Chem.*, **123**, 461 (1938).
[484] R. W. G. Wyckoff, *Proc. Soc. Exptl. Biol. Med.*, **36**, 771 (1937).
[485] F. C. Bawden and N. W. Pirie, *Nature*, **141**, 513 (1938).
[486] *Brit. J. Exptl. Path.*, **19**, 251 (1938).
[487] J. D. Bernal, I. Fankuchen, and D. P. Riley, *Nature*, **142**, 1075 (1938).

existing in the host cell stimulate catalytically the formation of new virus molecules. The following considerations ought to be borne in mind. Organisms of identical shape and size could, in the same way as molecules, arrange themselves in a lattice and crystallize provided that their form is not too irregular and that they are sufficiently small to undergo a vigorous Brownian movement. Their crystallization is therefore no reason for classifying the viruses as proteins rather than living organisms. Furthermore, earlier data, indicating that several viruses are extremely small in size, has been shown to be incorrect. Even the smaller viruses have molecular weights of several million so that many different molecules may be present, and, in fact, the composition of the viruses is now known to be much more complicated than at first appeared.

Multiplication of virus has been studied extensively with bacteriophage of *Escherichia coli*. Although the details of the mechanism are not yet known there is now definite proof that it does not multiply by division as do complete cells. Spontaneous mutants of phage are known and new types of phage have been obtained by hybridization of bacterial cells infected with two phages. In view of all this evidence the terms "molecules" or "proteins" should not be applied to the virus particles which behave truly as organisms.

IX. CRYSTAL STRUCTURE OF GLOBULAR PROTEINS

The first attempts to obtain x-ray diagrams from crystalline proteins revealed an entire absence of crystal reflections. It is now known that this result was due either to the inadequacy of the methods used, or to the condition of the material. Subsequently, powder diagrams were obtained from crystalline proteins; but from these no conclusions could be drawn. It was therefore a great advance when Bernal and Crowfoot[489,490] determined the unit cell of single crystals of several proteins by x-ray analysis. Since then a number of other proteins have been investigated. Table XXI[491] summarizes the results of these studies.

The observed interferences indicate in the clearest cases (*e.g.*, wet hemoglobin) lattice plane spacings down to 2.4 Å.; generally, however, only larger spacings are measurable, *e.g.*, upward of 13 Å. in dried hemoglobin. Only the larger groups can be located therefore. Nevertheless, certain

[489] J. D. Bernal and D. Crowfoot, *Nature*, **133**, 794 (1933).
[490] D. Crowfoot, *Proc. Roy. Soc. London*, **A164**, 580 (1938).
[491] Compiled by M. F. Perutz, *Research*, **2**, 53 (1949).

conclusions concerning the form and arrangement of the molecules have been drawn although some contradictions remain.

Crystallized proteins contain important quantities of water of crystallization; they shrink upon loss of water, whereby the outer interferences indicating the smaller spacings disappear, and other interferences are altered (Fig. 19). The following results have been obtained from Patterson diagrams of protein crystals in various states of hydration and of crystals in which water was replaced by other liquids or solutions.[492,493]

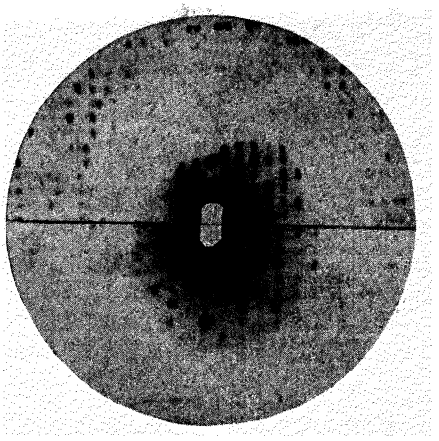


Fig. 19. X-ray diagrams of chymotrypsin (Bernal, Fankuchen, and Perutz). Upper diagram, hydrated; lower, dried chymotrypsin.

A cell weight of 133,400 follows from the density and dimensions of the cell of horse hemoglobin; of this, 52% is water, so that the value for the dry protein is 63,300. Each of the two molecules (M.W. 33,500 in urea solution) contains four Fe atoms and is a flattened cylinder of diameter 57 Å. and height 34 Å.; the molecules form layers with water in the interspaces in a similar manner to graphite oxide. The protein molecule does not change its form or size during contraction of the crystal; only the water layer becomes thinner. The four hem groups lie on the surface of the molecule; according to Perutz, this consists of 4 parallel layers, 9 Å. thick, which are formed of folded polypeptide chains in the α-keratin form.

The molecule of horse myoglobin forms flat disks of 57 Å. diameter and

[492] M. F. Perutz, *Proc. Roy. Soc.*, **A195**, 474 (1949); *Research*, **2**, 53 (1949).

[493] J. Boyes Watson, E. Davidson, and M. F. Perutz, *Proc. Roy. Soc. London*, **A191**, 83 (1947).

TABLE XXI
X-Ray Data of Protein Crystals[1]

	References	Wet or dry	Space group	Unit cell a	b	c	β	Volume Å.³ × 10⁻³	Number of moles*	Best value for M.W. × 10⁻³: In soln.	In crystal*
Ribonuclease I	1*a*	W	$P2_1$	30.8	38.5	53.5	107°	60	2	13 to 15	
Ribonuclease II	1*a*	D	$P2_12_12_1$	28.7	29.3	45.2	100°	37.4	2		13.7
		D		36.6	40.5	52.3	—	77.3	4		
Insulin	2, 3	W	R3	49.4	—	—	114°16′	68	3	*n* × 12	12
		D		44.4	—	—	114°28′	50			
Metmyoglobin											
horse	4	W	$P2_1$	57.3	30.8	57.0	112°	93	2	17	(17)
		D		51.5	28.0	37.0	98°	53.3			
whale	4	D	$P2_12_12$	97.4	39.8	42.5	—	165	4?		(17)?
γ-Chymotrypsin	5	W	$P4_22_1$	69.5	—	97.5	—	471	8		
		D		63.0	—	74.5	—	298	8	27	30.1
Chymotrypsin	6	W	$P2_1$	49.6	67.8	66.5	102°	219	2	41	
Lysozyme	7	D	$P4_12_1$	71.1	—	31.3	—	158	8	—	14
Pepsin	8	W	$C6_12$	67.9	—	292	—	1,160	12	35.5 to 39.2	(50)
		D		60.5	—	268	—	848			
Lactoglobulin I (tabular)	9, 10	W	$P2_12_12_1$	67.5	67.5	154	—	702	8	36	(40)
		D		60	63	110	—	416			
Lactoglobulin II (needles)	9, 10	W	$P4_22_1$	67.5	67.5	133.5	—	608	8	36	(40)
		D		56	56	130	—	408			
Met-, oxy-, and carboxy-hemoglobin											
horse I	11	W	C2	109	63.2	54.4	111°	349	2	66.7	33.4
		D		102	51.4	47	130°	189			
horse II	11	W	$P2_12_12_1$	122	82.4	63.7	—	640	4	66.7	66.7
		D		95	72	61	—	416			
horse III	8	W	P2	64.5	101	104	102°	662	4	66.7	133.4
Reduced hemoglobin horse	8	W	$C3_12$	56.1	—	354	—	965	6	66.7	66.7
		D		47.4	—	308	—	600	6		
Methemoglobin											
sheep adult	12	W	C2	164	70	66	94.5°	755	4	68	(68)
sheep foetus I	12	W	$B22_12$	112	108	56	—	677	4	68	(34)
		D		78	99	54	—	417			
sheep foetus II	12	W	$P2_1$	55	83	64	102°	286	2	68	(68)
		D		48	71	58	101°	194			

Met-, oxy-, and carboxy-hemoglobin										
human I.....13	W	$P2_12_12_1$	85.2	77.0	86.0	—	564	4	68	(68)
	D		70.4	73.3	77.0	—	393			
human II.....8	D	$P2_12_12_1$?	50	55	123	—	338	4	68	(68)
Reduced hemoglobin human.....8	W	$P2_1$	66	98	110	98°	700	4	68	(136)
Serum albumin horse.....5	W	H	96.7	—	145	—	1,170	6	70 to 73	(82 8)
	D		74.5	—	130	—	610			
Tobacco seed globulin.....14	D	F (cub.)	123	—	—	—	1,860	4	(300)	(322)
Excelsin.....15	D	R3	85.5	—	—	60.5°	445	1	294	(100)?
Ferritin.....16	W	F (cub.)	186	—	—	—	6,220	8?	650	—
Apoferritin.....16	W	F (cub.)	186	—	—	—	6,220	8?	500	—
Bushy stunt virus.....17	W	I (cub.)	386	—	—	—	57,600	2	7,600 to 10,600	(13,000)
	D		314	—	—	—	31,000			
Tobacco necrosis virus derivative.....18	W	Pl (tricl.)	179	219	243	$\alpha = 87.5°$ $\beta = 97.5°$ $\gamma = 97.5°$	9,200	1	1,850	(1,600)
Turnip mosaic virus.....19, 20	W	F (cub.)	706	—	—	—	35,100	8	—	—
	D		528	—	—	—	14,700			

* Values in parentheses are approximate; those with question marks (?) are uncertain.

[1] Compiled by M. F. Perutz, *Research*, **2**, 53 (1949).
[1a] I. Fankuchen, *J. Gen. Physiol.*, **24**, 315 (1941).
[2] D. Crowfoot and D. P. Riley, *Nature*, **144**, 1011 (1939).
[3] D. Crowfoot, *Proc. Roy. Soc. London*, **A164**, 580 (1938).
[4] J. C. Kendrew, *Acta Cryst.*, 1, 336 (1948).
[5] I. Fankuchen, reported in E. J. Cohn and J. T. Edsall, *Proteins, Amino Acids, and Peptides as Ions and Dipolar Ions*. Reinhold, New York, 1943.
[6] J. D. Bernal, I. Fankuchen, and M. F. Perutz, *Nature*, **141**, 523 (1938).
[7] K. J. Palmer, M. Ballantyne, and J. A. Galvin, *J. Am. Chem Soc.*, **70**, 906 (1948).
[8] M. F. Perutz, *unpublished work*.
[9] D. P. Riley, *The Crystal Structure of Some Proteins*. Thesis, Oxford, 1942.
[10] D. Crowfoot, *Chem. Revs.*, **28**, 215 (1941).
[11] J. Boyes-Watson, E. Davidson, and M. F. Perutz, *Proc. Roy. Soc. London*, **A191**, 83 (1947).
[12] J. C. Kendrew and M. F. Perutz, *ibid.*, **A194**, 375 (1948).
[13] M. F. Perutz and O. Weisz, *Nature*, **160**, 786 (1947).
[14] D. Crowfoot and I. Fankuchen, *ibid.*, **141**, 522 (1938).
[15] W. T. Astbury and F. O. Bell, *Tab. Biol.*, **17**, 90 (1939).
[16] I. Fankuchen, *J. Biol. Chem.*, **150**, 57 (1943).
[17] C. H. Carlisle and K. Domberger, *Acta Cryst.*, **1**, 194 (1948).
[18] D. Crowfoot and G. M. J. Schmidt, *Nature*, **155**, 504 (1945).
[19] J. D. Bernal and C. H. Carlisle, *ibid.*, **162**, 139 (1948).
[20] R. Markham, R. E. F. Matthews, K. M. Smith, *ibid.*, **162**, 88 (1948); V. E. Cosslett, R. Markham, *ibid.*, **161**, 250 (1948).

9–10 Å. thickness. In contrast to methemoglobin these disks are not grouped in fours but are rather arranged in long piles of disks.[494]

The insulin cell contains three asymmetric units of M.W. 12,000. In solution, however, one finds particles of M.W. 46,000 containing four submolecules of M.W. 12,000. Comparison of the Patterson diagrams of wet and dry crystals indicates that the molecule is rigid and probably cylindrical (40 Å. in diameter and 30 Å. in height). X-ray evidence shows that it is composed of densely packed granules, so that a flat folding of the chains which occurs for hemoglobin and myoglobin is excluded in this case.[495] The cell of β-lactoglobulin contains eight large molecules of M.W. 35,300 and is 64 Å. thick and 125–145 Å. high. On drying, the form remains unchanged, the particles drawing closer together. In the crystal of ribonuclease, molecules occur in layers separated by a distance of 9 Å.[496]

X. BEHAVIOR OF PROTEINS IN SOLUTION: SOLUBILITY, ASSOCIATION, FLOCCULATION, AND DENATURATION

The solubility of proteins[497,498] and the changes in solubility induced by the addition of various substances to the solution offer a study of great variety and complexity. The following phenomena are particularly striking and occur especially with proteins: the action of many salts, for example, sodium chloride, in raising the solubility of globulin; the effect of slight changes in *p*H on solubility; the changes in solubility which result from denaturation, and, finally, precipitation by specific substances, such as we have encountered in the reaction of antibody and antigen.

1. Significance of Individual Groups for Solubility

Solubility is determined by the interaction of groups at the surface of the protein molecule, with water on the one hand, and with the groups of other protein molecules on the other. Competition between these two effects, which themselves result from numerous individual group effects, determines the concentration of dissolved protein at equilibrium. Only the superficial groups are of importance, since those in the interior are unaffected by the process of solution.

[494] J. C. Kendrew, *Acta cryst.*, **1**, 336 (1948).

[495] D. Crowfoot, *Chem. Revs.*, **28**, 215 (1941).

[496] C. H. Carlisle, *unpublished data*.

[497] See W. Pauli and E. Valkó, *Kolloidchemie der Eiweisskorper*, 2nd ed., Steinkopff, Dresden, 1933.

[498] E. J. Cohn and J. T. Edsall, *Proteins, Amino Acids, and Peptides as Ions and Dipolar Ions*, Reinhold, New York, 1943.

Since the analysis of the structural units present gives no indication of the spatial structure and distribution of groups at the surface, it is impossible to estimate solubility from composition, as is possible, to some extent, in compounds of low molecular weight. Changes in three-dimensional structure, such as the unfolding of folded chains, may bring new groups to the surface and thus change the solubility. Only in the case of fibrillar proteins, the chain molecules of which are completely surrounded by solvent, do all groups interact mutually.

It is, nevertheless, desirable to consider the influence of the individual groups; it will be seen that certain general conclusions can be drawn from such consideration.

(*a*) *Peptide Group:* —*CO*—*NH*—*CH*$_2$—

Polypeptides of glycine and its homologues of low molecular weight associate in aqueous solution (sect. III1); the higher polypeptides are insoluble. It follows from this that the forces of attraction between peptide groups, due to particularly strong hydrogen bonds, are stronger than those between peptide groups and water. In this behavior the peptide group resembles the amide group. Even the first member of the amide series, formamide, absorbs heat on mixing with water ($\Delta H > 0$). Correspondingly, during the association of amide molecules in water, heat is liberated. The peptide group alone, therefore, cannot confer water solubility on a high polymeric substance; still less can it render the substance soluble in the usual organic solvents.

Higher polypeptides such as polyglycylglycine or silk fibroin dissolve, however, in concentrated mineral acids or aqueous lithium bromide and in certain other salt solutions. This process presumably involves the formation of specific complexes as in the case of cellulose, where the hydroxyl groups unite with lithium ions.

In order to study the influence of salts on the peptide group in a simple case, Klemm[499] made determinations of the solubility of glycine anhydride (diketopiperazine) which consists of two peptide groups and no others. It was found that, at low concentrations, most salts produce a salting-out effect, while sodium and lithium iodides and bromides at higher concentrations produce a considerable increase in solubility. Figure 20 shows the solubility of diketopiperazine in solutions of certain lithium and potassium salts as a function of the molar concentrations.

Acids and alkalis increase the solubility, even at low concentrations.

[499] K. H. Meyer and O. Klemm, *Helv. Chim. Acta*, **23**, 25 (1940).

There can be no doubt that the peptide groups of the proteins are in part responsible for similar solubility changes; this is clear from the swelling action of lithium salts on silk, collagen, and elastin. Nevertheless, the effect of salts on the peptide groups is of considerably less importance than the interaction of salts with the free carboxyl and amino groups, to the hydrophilic character of which the hydration and water solubility of proteins is due.

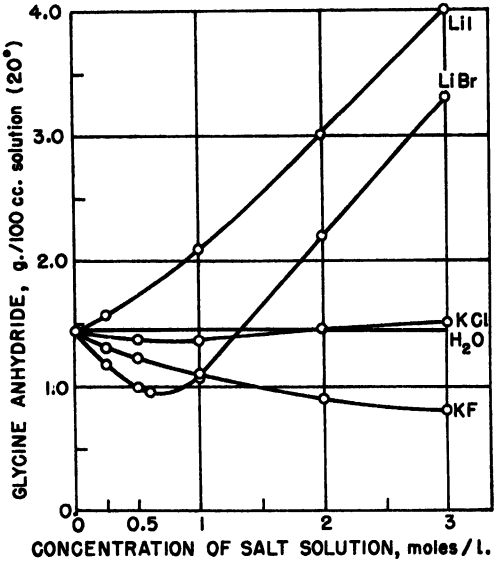


Fig. 20. Solubility of glycine anhydride in aqueous salt solutions.

(*b*) *Lateral Groups*[500,501]

The lateral groups of protein molecules can be divided roughly into apolar hydrocarbon residues and polar carboxyl, hydroxyl, and amino groups. The former—hydrophobic, hydrofuge, or lipophilic groups—hinder solubility in water and favor solubility in alcohol, while the hydrophilic, polar groups increase both swelling and solubility in water and aqueous solutions of electrolytes. These latter groups are also responsible for the considerable evolution of heat which takes place when many proteins unite with a

[500] For discussions of the significance of lipophilic and hydrophilic groups for solubility, see K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930, page 229.

[501] D. Jordan-Lloyd and H. Phillips, *Trans. Faraday Soc.*, **29**, 132 (1933).

certain amount of water. A rough indication of the apolar or polar character of a protein is given by the value of the ratio (O + N):C. For proteins soluble in water and salt solutions, this is 0.6–0.7, but it falls to 0.53 in alcohol-soluble, vegetable proteins, such as zein.

Experiments by Cohn[502] take us somewhat further. Cohn studied the influence of methylene groups on the ratio of the solubility in alcohol to solubility in water for amino acids. If a methylene group is introduced into a side chain terminating in a carboxyl or amino, the quotient remains unchanged, as is clear, for example, from a comparison of glutamic with aspartic acid. If, however, the methylene group is introduced into a side chain with a methyl end group, the quotient increases by a factor of 3. This means that a methylene group can be shielded by a carboxyl or amino group.

(*c*) *Ionizable Groups*

Of decisive importance for the solubility of proteins in water are the ionizable groups: the carboxyl groups of dicarboxylic acids and the amino groups of histone bases and arginine. The solubility is least at the isoelectric point, that is, at a *p*H at which the protein particles possess no excess charge, either positive or negative, and at which, therefore, the number of dissociated COO^- groups attached to the protein is equal to the number of cations NH_3^+ attached. The particle then forms a dipolar ion (*Zwitterion*), in which the ionized groups form ions and neutralize each other in pairs.

If a dipolar ion is brought into an acid or alkaline medium, it reacts with evolution of heat: in an acid medium, the dissociation of the carboxylic groups is suppressed; in an alkaline medium, the association to form charged ammonium ions is suppressed. According to Weber:[503]

$$NH_3^+\text{—R—}COO^- + H^+ \longrightarrow NH_3^+\text{—R—}COOH + \text{several hundred calories}$$

in an acid medium, while in an alkaline medium:

$$NH_3^+\text{—R—}COO^- + OH^- \longrightarrow H_2O + NH_2\text{—R—}COO^- + \textit{ca.}\ 1000\ \text{calories}$$

If n hydrogen atoms are attached to a protein molecule, the evolution of heat is n times as great; n may be quite large—in the case of hemoglobin n is 13. Hence the evolution of heat in salt formation may be considerable, and solubility changes very great (see the calculation in Chap. H, sect.V2*c*). In general, solubility increases as the *p*H of the solution shifts from the iso-

[502] T. L. McMeekin, E. J. Cohn, and J. H. Weare, *J. Am. Chem. Soc.*, **57**, 626 (1935).

[503] Quoted from W. Pauli and E. Valkó, *Kolloidchemie der Eiweisskorper*, 2nd ed., Steinkopff, Dresden, page 63. H. H. Weber, *Biochem. Z.*, **189**, 381, 407 (1927); **218**, 1 (1930).

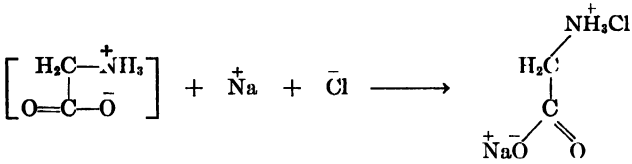
electric point. When all the groups are charged, a further addition of acid or alkali may lead to precipitation of the protein as a salt. This has been observed by Sørensen[504] in the system carboxyhemoglobin + sulfuric acid; at *p*H 5.4 a second solubility minimum occurs, in addition to that at the isoelectric point, *p*H 6.6.

2. Influence of Salts

(*a*) *Inorganic Salts*

Up to the present, it has not proved possible to express the influence of salts on solubility by a quantitative relationship of general validity nor even to systematize the observed phenomena.

The solubility of ions (including dipolar ions) increases as the dielectric constant of the solvent increases, and electrolytes have the effect of increasing the dielectric constant of water (Fig. 21). Conversely, the solubility of apolar regions is diminished by the addition of electrolytes. Moreover, as the calculations of Scatchard and Kirkwood[505] show, the solubility of dipolar ions is also increased by interaction with free ions. This ionic effect, which is independent of the nature of the ion, has not as yet been observed in pure form in protein solutions, but only in solutions of amino acids in dilute alcohol. No difference could be detected between the action of sodium chloride and that of lithium chloride on glycine in 80% alcohol.[506] The effect of salts on dipolar ions may be represented schematically as follows:



The solubility of albumins and globulins in accordance with their nature as dipolar ions is increased by the addition of small quantities of most salts. This increase is proportional to the ionic strength μ, which is defined by the equation:

$$\mu = 0.5 \sum_i m_i z_i^2$$

where m_i is the molar concentration of individual ions and z is the valence.

[504] S. P. L. Sørensen and M. Sørensen, *Biochem. Z.*, **258**, 16 (1933).
[505] G. Scatchard and J. G. Kirkwood, *Physik. Z.*, **33**, 297 (1932).
[506] E. J. Cohn, *Naturwissenschaften*, **20**, 663 (1932).

There are, however, marked differences observable between the effects of various salts on the solubility of proteins (compare Fig. 21). Dipolar ions like glycine also increase the solubility of proteins.

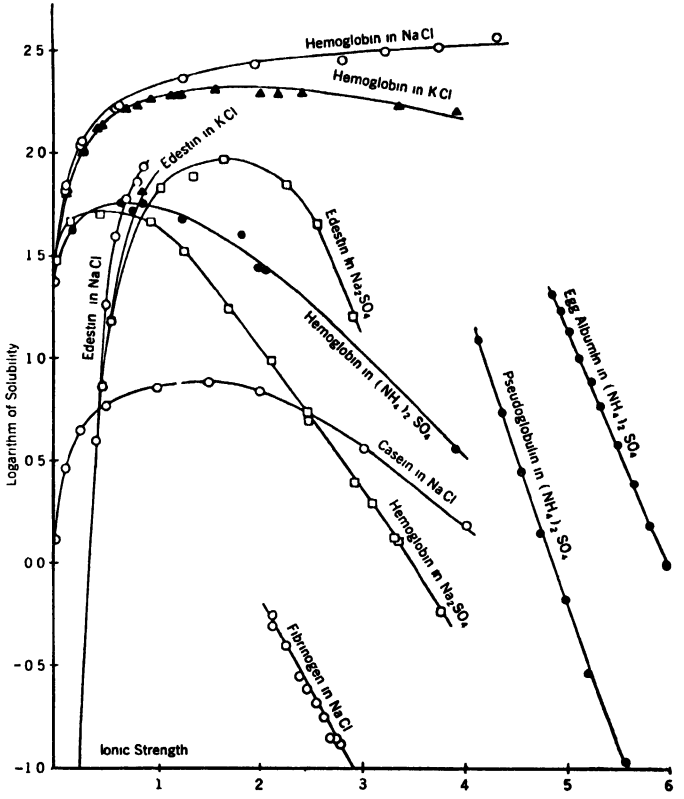


Fig. 21. Solubility of various proteins as a function of the ionic strength (E. J. Cohn).

Over and above these effects are the specific effects of ions, which lead to salting-in or salting-out, depending on whether the affinity of the ion for particular groups of the protein is greater or less than its affinity for water. The existence of such specific forces between ions and peptide groups has been shown above in the case of glycine anhydride. Amino, hydroxyl, and ether groups also react with salts, however, as is shown by the occurrence of addition compounds of salts with alcohols, ethers, etc. Pfeiffer[507] has

[507] P. Pfeiffer, *Organische Molekulverbindungen*. 2nd ed., Enke, Stuttgart, 1922.

described compounds of this kind between amino acids and salts, and has pointed out that such complex compounds are also formed by proteins.

According to Meyer and Dunkel,[508] the affinity of the lithium ion for amino, alcoholic hydroxyl, ether, and peptide groups is greater than its affinity for water, while that for phenolic hydroxyl as well as for carboxyl is less than for water. In the first case the lithium ion has a salting-*in* effect, in the latter, a salting-*out* effect. The formation of complex ions:

$$RNH_2 + Li^+ \longrightarrow RNH_2Li^+$$

may be compared with the formation of ammonium ion from hydrogen ion and ammonia. Of the univalent anions, the fluoride ion has the greatest energy of hydration; but the affinity of the carboxyl group and, apparently, of phenolic hydroxyl for fluoride ion is greater than that of water, while all other groups have a lesser affinity than that of water.

The effect of a salt is the sum of the effects of both ions, so that we encounter now salting-in, now salting-out. As the concentration changes, the two effects may increase at different rates, so that in many cases a reversal of the total effect results. Examples are provided by glycine anhydride, butyl alcohol, and ethyl acetate, which are salted out by dilute lithium bromide, but which dissolve in concentrated lithium bromide, thus exhibiting a solubility minimum. On the other hand, a maximum is observed in aqueous solutions of cystine if increasing quantities of ammonium sulfate are added. This superposition of salting-in and salting-out effects is also observed with proteins.

Albumins and globulins when dissolved in solution of salts with small influence on solubility exhibit a maximum of the latter at a certain salt concentration. At still higher concentrations the protein is salted out. The solubility, L, of a homogeneous protein follows in this case the equation:[509]

$$\log L = \beta - K\mu$$

where μ is the ionic strength and K and β are constants.

In contrast to this behavior a minimum of solubility has been found by Szent-Györgyi for the system myosin–potassium chloride at 0.025 mole per liter of potassium chloride.[510]

(*b*) *Salts of Organic Acids*

Concentrated solutions of certain organic alkali salts—for example, sodium benzenesulfonate, alkali salicylates, benzoates, and also alkali salts of

[508] K. H. Meyer and M. Dunkel, *Z. physik. Chem., Bodensteinfestband*, **1931**, 553.
[509] E. J. Cohn, *Chem. Revs.*, **28**, 395 (1941).
[510] A. Szent-Györgyi, *J. Colloid Sci.*, **1**, 1 (1946)

fatty acids, tannic acids, and paraffin sulfonates—are able to dissolve many water-insoluble proteins. The solubility of casein, for example, in sodium benzenesulfonate and sodium cymenesulfonate (McKee and Gould[511]), is made use of in the fractionation of casein. All these solutions are able to increase the water solubility of hydrocarbons or of substances with apolar groups; this phenomenon has received the name *hydrotropy* (Neuberg).

These organic salts form aggregates in solution, the hydrophobic residues uniting in bundles; it is probable that hydrocarbons and such, as well as proteins, are built into these packets.

3. Hydration of Proteins

All proteins are sensitive to water; they either absorb a limit quantity of water—limited swelling—or they dissolve in water. The question of the mechanism of this reaction between protein and water and of the water-binding power of proteins has often been discussed; nevertheless, the answers which have been given are mostly incomplete.

A simple but not complete satisfactory method of determining the water-binding power is the freezing method.[512] This method merely indicates, however, that the vapor pressure of protein containing water is the same as that of ice at the temperature of freezing; from this the free energy of the water-binding process can be calculated. The same result is obtained by determining the swelling pressure or vapor pressure of water in swollen proteins. If now the free energy of swelling is measured as a function of the water content, it is found in general that the quantity of water first absorbed is very tightly bound, and that, as the concentration diminishes, the free energy of swelling continually diminishes. Using this method, no evidence of the formation of stoichiometrically defined compounds has been obtained.

Knowledge of the free energy of swelling does not, however, afford any insight into the mechanism by which water is bound, nor does it offer an answer to the question of how far forces of attraction between polar or ionized protein groups and water molecules determine the binding of water, and how far the diffusion tendency (entropy of mixing) is responsible for the mixing of protein and water, and hence for the binding of water. This question can be answered only by the experimental separation of the free energy into the entropy and energy fractions. Fricke and Lüke[513] find that dry casein absorbs water at first with evolution of heat, and that the partial

[511] R. H. McKee and S. P. Gould, *J. Agr. Research*, **57**, 125 (1938).

[512] Survey by W. Pauli and E. Valkó, *Kolloidchemie der Eiweisskörper*. Steinkopff, Dresden, 1933, page 160.

[513] R. Fricke and J. Lüke, *Z. Elektrochem.*, **36**, 309 (1930).

heat evolved $\Delta\bar{H}_1$, is greater than the free energy, $\Delta\bar{F}_1$; the entropy therefore diminishes. This indicates that the water absorbed initially is bound by forces of attraction at definite points on the protein fabric. When 20% of water has been absorbed, $\Delta\bar{F}_1$ and $\Delta\bar{H}_1$ become equal; on further dilution, the free energy change $\Delta\bar{F}$, is chiefly supplied by the entropy of mixing. The system water–gelatin (see Chap. H, sect. III3*e*) behaves in a similar way.

The picture is presumably similar for most proteins; both swelling and the binding of water are influenced by *p*H and are least at the isoelectric point. It may be assumed that the ionizable groups become surrounded by a shell of water, energy being liberated in the process, and that the outer layers of the water atmosphere are held more and more loosely. A stoichiometrical formula cannot be given for a hydrated protein in solution, any more than for a hydrated metallic ion.

In our opinion, measurements of the *nondissolving space* are entirely useless as a measure of the degree of hydration. The method consists in determining the distribution of glucose or other water-soluble substance between water and a protein solution separated therefrom by a semipermeable membrane, or else in determining the diminution in solubility of a water-soluble substance in the presence of protein. A water-soluble substance will always interact in some way or other with those groups of the protein which are responsible for the binding of water, and will, therefore, displace a certain amount of the bound water. By this method, one measures only the relative affinity of protein for water as compared with that, for example, for glucose.

4. Deviation from van't Hoff's Law. Viscosity

(*a*) *Osmotic Anomalies*

In aqueous solution and in salt solutions, the osmotic pressure of several proteins is proportional to the concentration, up to concentrations of several per cent. These solutions do not display very high viscosity. Both phenomena indicate that the interaction of the particles with each other and the energetic interaction with the solvent are much less marked than in solutions of chain polymers. This indicates that the particles are more compact and more or less spherical in shape (see Chap. H, sect. III5). Other proteins, on the other hand, show marked deviations.

Duclaux and Dobry[514] have found that the same protein may behave very differently in various solvents. Gliadin in alcohol shows only slight deviation from van't Hoff's law, while in lactic and acetic acids the deviations are very marked, as with chain molecules, that is, the osmotic pres-

[514] J. Duclaux and A. Dobry, *Compt. rend. trav. lab. Carlsberg*, **22**, 155 (1938).

sure increases with increasing concentration more rapidly than is predicted by the van't Hoff relation. Donnan effects are also manifested and have been studied in particular by Sørensen, Loeb, and Adair.[515]

(*b*) *Viscosity of Protein Solutions*

The viscosity of solutions of globular proteins is not abnormally high; some examples are given in Table XXII. Using the equation of Herzog, Kudar, and Perrin (see page 27) for elongated ellipsoids, Polson[516] and Oncley[517] have calculated the axial ratio a/b for several proteins from the experimental values of the frictional ratios, f/f_0.

TABLE XXII

Relative Viscosities of Globular Proteins

Protein	Molecular weight	Concentration, per 100 cc.	η_{rel}	Axial ratio, a/b, according to Polson
Ovalbumin	43,800	1 g.	1.03	—
Carboxyhemoglobin	63,000	1 g.	1.023	—
Serum albumin at *p*H 4	70,100	1 g.	1.05	—
5.	—	1 g.	1.03	—
6	—	1 g.	1.038	—
Myogen at *p*H 6.3	—	1 g.	1.04	—
Pseudoglobulin	—	1 g.	1.068	—
Insulin	40,900	1 cc.	—	3.17
Ovalbumin	43,800	1 cc.	1.057	3.84
Thyroglobulin	62,800	1 cc.	1.099	7.70
Carboxyhemoglobin	63,000	1 cc.	1.0535	3.77
Serum albumin	70,100	1 cc.	1.0652	4.95
Serum globulin	167,000	1 cc.	1.090	7.40
Amandin	329,000	1 cc.	1.0705	5.49
Octopus hemocyanin	2,785,000	1 cc.	1.0902	7.04
Helix hemocyanin	6,630,000	1 cc.	1.0636	4.76
at *p*H 8.6	813,000	1 cc.	—	16.6

[515] S. P. L. Sørensen, *Z. physiol. Chem.*, **106**, 1 (1917). J. Loeb, *Die Eiweisskorper*, Springer, Berlin, 1924. G. S. Adair, *Proc. Roy. Soc. London*, **A108**, 627 (1925); **109** 292 (1925); **120**, 573 (1928); **126**, 16 (1929). See also W. Pauli and E. Valkó, *Kolloidchemie der Eiweisskorper*, 2nd ed., Steinkopff, Dresden, 1933, section 12.

[516] A. Polson, *Kolloid-Z.*, **88**, 51 (1939).

[517] J. L. Oncley, G. Scatchard, and A. Brown, in J. T. Edsall, *Advances in Protein Chem.*, **3**, Table XI, 460 (1947). Compare this chapter, page 577.

The axial ratios calculated by Polson reach values up to 16. The result for ovalbumin contradicts the observation that this protein shows no flow birefringence (Boehm and Signer) and the value for insulin is incompatible with the results of x-ray analysis (Table XXI). In making these calculations Polson ignores the possibility that, in addition to the elongate ellipsoid many other shapes are possible, and that every departure from the spherical shape must result in an increase in viscosity as compared with that calculated from the Einstein equation.

Proteins with chain molecules exhibit high viscosity typical of chain polymers:

TABLE XXIII

Relative Viscosities of Preparations of Fibrillar Proteins in 1% Solution

Casein	1.33[518]
Glutene at *p*H 4.8	1.48[519]

The action of electrolytes on viscosity is extremely complex. In general, the viscosity is least at the isoelectric point and may rise considerably on either side of the isoelectric point. According to Pauli and Matula,[520] the viscosity of gluten $\eta_{rel} = 1.48$ at *p*H 4.8, increases to $\eta_{rel} = 1.6$ at *p*H 3.8. This may possibly be associated with the unrolling of crumpled chains, as Meyer and Mark[521] suggested. In this way a more or less globular molecule would be converted into a fibrillar molecule and the viscosity of the solution would increase. As the *p*H changes, the viscosity may pass through one or several maxima or minima.

The action of salts is obscure. Comparative experiments by Pauli and Falek[522] have shown that the ions can be arranged in the lyotropic series according to their effect in lowering the viscosity. It may be that, in addition to changes in the charge on the molecule, the form of the chains is also involved.

At the present time, viscosity measurements cannot be used as more than a qualitative indication of the shape of the particles—globular or fibrillar.

[518] E. Laqueur and O. Sackur, *Beitr. chem. Physiol. Path.*, **3**, 193 (1903).

[519] From W. Pauli and E. Valkó, *Kolloidchemie der Eiweisskorper*. 2nd ed., Steinkopff, Dresden, 1933.

[520] W. Pauli and J. Matula, quoted from Pauli and Valkó, *Kolloidchemie der Eiweisskörper*. 2nd ed., Steinkopff, Dresden, 1933.

[521] K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930, page 232.

[522] W. Pauli and O. Falek, *Biochem. Z.*, **47**, 270 (1912).

Nevertheless, the change of the viscosity with time can be used to follow quantitatively certain underlying processes—decomposition, aggregation to form micelles, or denaturation.

5. Association of Protein Molecules. Molecular Compounds

(*a*) *Association and Dissociation of Proteins*

As a result of his extensive investigations, Sørensen reached the conclusion that proteins are associated in solution; the associated particles he described as reversibly dissociable systems of components. Similar conceptions have been developed by Meyer and Mark, who use the terms protein micelles and mixed micelles. The associated particles are usually held together so strongly that dissociation is detectable only at very great dilution, and can be measured only by the ultracentrifuge method, which is particularly suitable for this purpose. Svedberg has shown, for example, that hemoglobin dissociates to some extent, at considerable dilution, into particles of half the ordinary molecular weight; thyroglobulin splits up into several dissociation products. By the addition of various reagents or by solution in other solvents, the dissociation of the particles can be provoked even at moderate concentrations. Hemoglobin[523] dissociates into two components of molecular weight 34,300 if urea is added to the aqueous solution, while edestin has a molecular weight of 50,000 in a solution of urea, as compared with 300,000 in aqueous solution.

Ultracentrifuge measurements have shown that a change in *p*H can produce reversible dissociation. The hemocyanin of *Helix pomatia* has a molecular weight of 6,740,000 at the isoelectric point; on changing the *p*H dissociation occurs, step by step, into one half, one eighth, and one sixteenth molecules. At *p*H 8, all three species of dissociation products are present and these reunite to form the original molecule as soon as the *p*H is brought back to the isoelectric point (see sect. V2*c*). At *p*H 6.8–9, the dissociation may be hindered by the presence of small quantities of calcium or magnesium ions.

Phycocyan consists of particles of molecular weight 273,000 at *p*H 7–8.2; at *p*H 2.5–6 the molecular weight is half this value, while from *p*H 6 to 7 both species are present.[524]

A high salt concentration may lead to dissociation or to the formation of more highly associated molecules; 4 *N* sodium chloride solution induces the

[523] N. F. Burk and D. M. Greenberg, *J. Biol. Chem.*, **87**, 197 (1930). J. Steinhardt, *Nature*, **138**, 800 (1936); *J. Biol. Chem.*, **123**, 543 (1938).

[524] I. B. Eriksson-Quensel, *Biochem. J.*, **32**, 585 (1938).

molecules of thyroglobulin (molecular weight = 640,000) to associate, forming particles the molecular weight of which is about 16,000,000.

It appears that, in the presence of serum albumin, serum globulin is partially dissociated. Such dissociations may also be brought about by protamines; clupein, for example, effects the dissociation of serum albumin. Amino acids, and substances such as guanine, can also induce dissociation. This effect is specific; an amino acid which has a marked action on one protein may be without effect on another protein.

(*b*) *Molecular Compounds*

A number of proteins have the power of combining with certain substances of low molecular weight to form well-defined, stable, molecular compounds which often crystallize well. The best known example is that of hemoglobin, which is a molecular compound of globin and hem; other examples are provided by the conjugated enzymes.

The associated molecules of protein, such as occur in native proteins, and the molecular compounds mentioned above, are stoichiometrically well defined; the complex is saturated if the components come together in certain numerical proportions.

From the low degree of dissociation of native proteins, conjugated ferments, or hemoglobin, it is justifiable to assume that the energy of association is high. In the case of the pyridine ferment we estimated the heat of association at 18,000 cal.; similar values should be involved in the association of pure proteins. Energy changes of this order can occur only if considerable numbers of groups are saturated; as an example, the heat of association of two methylene groups in water, amounting to *ca.* 700 cal., may be quoted. We must assume, therefore, that from 10 to 40 groups take part in the association. In this process, the groups concerned must be able to approach each other until the distance between them is that corresponding to minimum potential energy. This is possible only under very definite geometr. l conditions; the associating region of one molecule must fit on to that of the other like a casting into its mold. On the surface of the globin molecule, for example, there must be a region corresponding to the flat hem molecule; in this region one group projects into the iron complex, two basic groups are exactly covered by the carboxyls of hem, and other suitable groups hold the pyrrole residues in place by secondary valences. Only on the assumption of such geometrical niceties of structure is the specificity of formation of molecular compounds understandable; extreme subtlety of structure alone can lead to an understanding of the specificity of reaction between antigen or haptene and antibody.

In addition to the specific molecular compounds hitherto described, addition compounds of proteins with fats, stearins, polysaccharides, etc., are known to occur in the body fluids. Compounds of this type are known as *symplexes*. As a result of the formation of such addition compounds, the lipoids, insoluble in water in the pure condition, are kept in solution.

It may be mentioned here that α-lactoglobulin gives a crystalline addition compound with two molecules of sodium dodecyl sulfate[524a] while human and bovine albumin combine with lower and higher alcohols (Edsall[251]).

To these more or less specific addition compounds must be added a host of nonspecific precipitates—those, for example, of basic proteins with multivalent acids or acid proteins, and, on the other hand, those of acid proteins with multivalent or high polymeric bases, or with protamines and basic proteins,[525] as well as the nonspecific compounds with polysaccharides, fats, etc.

6. Denaturation

As a result of treatment which has no effect on other organic substances, most soluble proteins undergo profound changes in their properties. The most characteristic change is the reduction in solubility which often induces coagulation and the loss of specific biological activity. At the same time, the absorption spectrum and the viscosity of the solution change and the ability to crystallize is lost. This phenomenon is known as *denaturation*.

Denaturation may be induced by mechanical treatment, by heat, by organic solvents, and by electrolytes. The sensitivity of proteins toward these influences is very varied, however, and it seems possible that, in spite of superficial similarity, the various types of denaturation are due to different processes.

The main outlines of the processes involved in the formation of the insoluble silk thread from the aqueous solution of fibroin are now clear; we have seen that the phenomenon consists essentially in the removal of a state of super-saturation—a reaction which may be brought about by orienting forces in the surface, but which also takes place slowly of its own accord. The "native" dissolved protein is in this case, therefore, in a metastable state.

It is possible that a similar explanation holds for the denaturation and

[524a] T. L. McMeekin, B. D. Polis, E. S. della Monica, and J. H. Custer, *J. Am. Chem. Soc.*, **71**, 3606 (1949).

[525] See, for example, F. Haurowitz, *Kolloid-Z.*, **74**, 208 (1936).

precipitation of proteins in surface layers observed by Ramsden[526]; by shaking, or by the passage of a stream of air bubbles, many proteins, such as ovalbumin, are precipitated at the surface in the form of films, which according to Ramsden and Bull,[527] consist of denaturated protein. Nevertheless, irreversible denaturation in surface films does not always occur. Pepsin and trypsin do not lose their activity when spread in monomolecular layers on a water surface.[528]

Denaturation also occurs as a result of heating (a good example is the heat coagulation of ovalbumin) and as a result of precipitation with alcohol or pyridine (acetone does not usually denature). Urea and formamide also cause denaturation.

TABLE XXIV

Effect of Denaturation on Sulfhydryl Groups

Protein	Sulfhydryl groups, %	Denaturation	Sulfhydryl groups, %
Ovalbumin, native	0	Coagulated by heat	0.56
Globin, native	0	Denatured by acid	0.38
Lens protein, native	0	Denatured by acid	1.23

In addition to those changes mentioned initially, denatured ovalbumin shows the following changes. Free sulfhydryl groups appear, a phenomenon noticed by Hopkins[529] and carefully studied by Anson and Mirsky[530] and by Greenstein.[531] The free sulfhydryl groups are estimated by allowing an excess of cystine to react with the protein. The S—H linkages of the denatured protein react with the S—S linkages of the cystine:

$$RSH + Cy{-}S{-}S{-}Cy \longrightarrow RS{-}SCy + CySH$$

The quantity of cystine decomposed gives the number of reactive sulfhydryl groups. Table XXIV shows the increase in the percentage of sulfhydryl groups on denaturation.

The isoelectric point is also changed, as Michaelis and Davidsohn[532]

[526] W. Ramsden, *Arch. ges. Physiol. Pflugers*, **1894**, 517; *Proc. Roy. Soc. London*, **B72**, 156 (1903).

[527] H. B. Bull, *J. Biol. Chem.*, **123**, 17 (1938).

[528] E. Gorter, *Proc. Roy. Soc. London*, **A155**, 706 (1936).

[529] F. G. Hopkins, *Nature*, 126, 328, 383 (1930).

[530] M. L. Anson and A. S. Mirsky, *J. Gen. Physiol.*, **19**, 427, 439 (1936).

[531] J. P. Greenstein, *J. Biol. Chem.*, **128**, 233 (1939). See the review by M. L. Anson in *Advances in Protein Chem.*, **2**, 361 (1945).

[532] L. Michaelis and H. Davidsohn, *Biochem. Z.*, **33**, 456 (1911).

first showed for serum albumin, the isoelectric point of which is displaced from *p*H 4.88 to *p*H 5.1–5.3. Haurowitz and Waelsch[533] have observed a similar effect with globin; according to Mainzer,[534] Bence-Jones protein exhibits the same phenomenon. Aqueous ovalbumin no longer coagulates if it has been freed from lipoids by ether extraction[535]; the role of lipoids, however, is unexplained. The activity of crystallized enzymes is reduced or lost entirely when these are denatured. Globin loses the power of combining with hem, and apoferments no longer unite with their prosthetic groups.

As a result of denaturation the protein becomes more accessible to enzyme attack; thus, native albumins are not attacked by pancreatic trypsin, while in the denatured condition they are hydrolyzed. In this respect denaturation by solutions of urea is particularly effective. Denaturation by urea may be prevented by addition of a small quantity of sodium caprylate.[536]

Reversibility of denaturation has been observed in several cases. On warming in aqueous solution, pepsin and trypsin are inactivated; on cooling, the activity slowly returns (Northrop[537]). In contrast to this, β-lactoglobulin is denatured in a solution of urea in water at 0°C., whereas the denaturation is rapidly and nearly completely reversed at 37.4°.[538]

Insulin when heated in aqueous solution precipitates in a denatured fibrillar and inactive form which is incapable of crystallization. As Waugh[539] found, activity and capacity of crystallization are restored by dissolving the fibrous form in cold alkali at *p*H 12–14 and subsequently neutralizing to *p*H 5.35.

The mechanism of denaturation has been much studied but many points still remain obscure. It is certain that no profound chemical changes are involved, since these could scarcely be induced by treatment as gentle as concentration at a surface. As a result, the chemical theories of denaturation, which according to Wu[540] and Lepeschkin[541] consists in a hydrolysis,

[533] F. Haurowitz and H. Waelsch, *Z. physiol. Chem.*, **182**, 82 (1929).

[534] F. Mainzer, *Biochem. Z.*, **246**, 156, 182 (1932).

[535] W. D. Bancroft and J. E. Rutzler, *J. Phys. Chem.*, **35**, 144 (1931).

[536] R. G. Rice, G. A. Ballou, P. D. Boyer, J. M. Luck, and F. G. Lum, *J. Biol. Chem.*, **158**, 609 (1945).

[537] J. H. Northrop, *J. Gen. Physiol.*, **16**, 33, 323 (1932).

[538] C. F. Jacobsen and L. Korsgaard-Christensen, *Nature*, **161**, 30 (1948).

[539] D. E. Waugh, *J. Am. Chem. Soc.*, **70**, 1850 (1948).

[540] H. and D. Y. Wu, *Chinese J. Biochem.*, **4**, 345 (1924); *J. Biol. Chem.*, **64**, 369 (1925).

[541] W. W. Lepeschkin, *Biochem. J.*, **16**, 678 (1922).

according to Pauli [542] and Spiegel-Adolf[543] in an internal amide formation, attract less attention than a theory of a more physical character, which we shall now consider.

Several observations suggest that denaturation consists in a transformation from the native condition, in which the chains are folded to form a *globulus*, to another condition, with unfolded chains or chains folded in a different manner. The undenatured protein molecules must indeed be folded, as is clear from the dimensions of the unit cell and the form of the dissolved particles. The links of the chains must, therefore, be held together at many well-defined points by forces of attraction between definite groups. Mirsky and Pauling[544] suggest that hydrogen bonds between two hydroxyl groups are the most important type of linkage, forgetting that these are very readily ruptured by water. We suggest that forces of attraction between lipophilic groups are the most important; salt linkages may also play a part, and it is probable that linkages between the peptide groups are involved.

The theory of unfolding is supported by the higher viscosity of denatured products (Anson and Mirsky[545]; Loughlin and Lewis[546]). The denaturation of hemoglobin, for example, in a mixture of 50% water and 50% formamide, can be followed directly by the increase in viscosity, which in an 0.8% solution increases in 68 hours from $\eta_{rel} = 1.04$ to $\eta_{rel} = 1.13$ (Jeannerat[547]). On the basis of viscosity measurements Neurath[548] has calculated the form changes of protein molecules using the formula due to Perrin. Changes in the value of the ratio f/f_0 and hence in shape could be deduced from ultracentrifuge measurements (Rothen[549]). A strong argument in favor of the unfolding theory is furthermore provided by Astbury's observation[550] that many proteins yield an x-ray diagram similar to that of silk or β-keratin after denaturation.

Considerable amounts of energy are required to unfold the chains; 67,600 cal./mole have been found for trypsin in weakly acid solution and

[542] W. Pauli and R. Weiss, *Biochem. Z.*, **233**, 381 (1931).
[543] M. Spiegel-Adolf, *Naturwissenschaften*, **15**, 799 (1927).
[544] W. T. Astbury and R. Lomax, *J. Chem. Soc.*, **1935**, 846.
[545] M. L. Anson and A. E. Mirsky, *J. Gen. Physiol.*, **15**, 341 (1932).
[546] W. J. Loughlin and W. C. McGullagh Lewis, *Biochem. J.*, **26**, 476 (1932).
[547] J. Jeannerat, *unpublished work.*
[548] H. Neurath, J. P. Greenstein, F. W. Putnam, and J. O. Erickson, *Chem. Revs.*, **34**, 157 (1944).
[549] A. Rothen, *Ann. N. Y. Acad. Sci.*, **43**, 929 (1942).
[550] W. T. Astbury and R. Lomax, *J. Chem. Soc.*, **1935**, 846.

57,300 cal./mole for soybean inhibitor (Kunitz[550a]). In spite of this, both native and denatured forms are in equilibrium at room temperature because the denatured protein has a much higher entropy than the regularly folded native product.

The unfolding of a regularly folded peptide chain would explain why individual groups become detectable which were hidden before; in the compact protein molecule these groups face the interior and in this way escape detection. Since the solubility depends on the interaction with water of the groups situated at the surface, on the one hand, and with groups of other molecules, on the other, the solubility may diminish as soon as hydrophobic groups, which in the compact molecule mutually saturated each other, are set free by unfolding and may now contribute to the linkage with other molecules. It is also possible that ionizable groups come to the exterior and now form a salt linkage with groups of other molecules (instead of uniting with charged groups of opposite sign in the same molecule) and so lead to precipitation as a multivalent salt (see Haurowitz[551]).

These considerations, however, neglect the fact that, as Wu[552] has shown, the properties of a protein denatured in various ways depend on the type of denaturation employed. Thus, the solubility of alcohol-coagulated egg albumin appears to differ from that of the heat-coagulated product. Moreover, nonprotein constituents appear to be split off, if the heat coagulation of egg albumin takes place away from the isoelectric point. It seems, therefore, that chemical changes may accompany denaturation.

Summarizing, we can say that the term *denaturation* serves to describe certain changes taking place in proteins, and that these changes are in all probability to be referred to very various causes. It appears illogical to seek a generally valid explanation of denaturation as such.

[550a] M. Kunitz, *J. Gen. Physiol*, **32**, 241 (1948).

[551] F. Haurowitz, *Intern. Physiologen-Kongress Zurich*, **1938**; *Kolloid-Z.*, **71**, 198 (1935); **74**, 208 (1936). F. Haurowitz and F. Marx, *ibid.*, **77**, 65 (1936).

[552] H. Wu, *Chinese J. Physiol.*, **5**, 321 (1931). W. Y. Lee and H. Wu, *ibid.*, **6**, 307 (1932).

H. PROPERTIES OF HIGH POLYMERS IN SOLUTION*

I. THERMODYNAMICS AND STATISTICS OF BINARY SYSTEMS

1. Introduction

Certain physical properties of chemical compounds, such as density, refractive index, optical rotation, dielectric constant, etc., depend essentially upon the number and kind of atoms or groups in unit volume. In a homologous polymeric series such properties are independent of the molecular weight, provided that the number of end groups is negligibly small by comparison with the number of groups in the chain. With respect to such properties, the individual units of each chain behave like small molecules and so high polymers exhibit no peculiarities. On the other hand, one would expect that, with regard to physical properties dependent on molecular size, high polymers would appear to be abnormal by comparison with low molecular compounds;—this is actually the case, for example, if one considers partition between two phases, solubilities, osmotic pressure, mechanical properties, etc.

In order to discover the general laws governing the behavior of high polymers, a vast amount of valuable, though purely empirical, numerical data has been accumulated. They include all possible properties—solubilities, swelling power, melting point, osmotic pressure in solution, viscosity, etc.—from which attempts have been made to deduce empirical rules and to provide a theoretical basis for them.

Unfortunately, however, lasting results obtained in this direction are few, compared with the labor involved. Very often the material used has been insufficiently characterized, the experimental conditions inadequately defined, or investigations carried out over too narrow a range, so that supposed regularities are not generally true and the theoretical explanations advanced are therefore untenable. Thus it is of the greatest importance to select as a basis for investigation a method of approach dependent on fundamental principles which are known to be valid throughout the whole

* By Kurt H. Meyer and A. J. A. van der Wyk.

realm of physical chemistry. By such logical selection one can reduce the number of investigations, but they require the utmost care and a critical attitude on the part of the experimenter and, in general, a very considerable refinement of the usual methods. Sometimes it is the very small order of magnitude of the effects to be measured which necessitates this, sometimes the extreme sensitivity to change of temperature or other factors, and sometimes the difficulties of separating the effect under consideration from others which are unavoidably superimposed. "Non multa sed multum" is a principle which is here more clearly manifest than in practically any other field of physical chemistry.

There are two essentially different methods of approach which come into consideration in the selection of experiments and their theoretical evaluation: thermodynamic and statistical. The *thermodynamic* approach starts from a few fundamental principles confirmed by the whole of our factual knowledge—the three laws of thermodynamics—and from them derive further laws in a purely mathematical manner without reference to any visual conception; these laws then serve for the deduction of very far-reaching conclusions from the immediate observations. Any number of observations found to be at variance with thermodynamic deductions must be taken as evidence of experimental error. The *statistical* approach takes a definite molecular model for each special case, endeavors to pursue the separate fates of each individual particle, to comprehend its movement in a calculable way, and then to draw conclusions with regard to the directly observed macroscopic system by the method of average values or summation. This method admits of visual conception but depends for success entirely on a correct and fortunate choice of the model selected as a basis for the calculation. In this case variance between theory and experiment may be due to inadequate assumption, as well as to faulty experiments.

Since we are here in a very obscure and quite unexplored region, we shall apply thermodynamic analysis preferably on account of its certainty. Where it is possible we shall endeavor to make the thermodynamic findings conceivable by a statistical interpretation. And so before going any further, the relationship between thermodynamic and statistical quantities will be briefly set forth. Then we shall review our knowledge concerning the thermodynamic behavior of binary mixtures of small molecules, next proceed to systems containing one polymer component of medium size, and finally come to systems with high polymers. It will be seen that the first-named systems are already very complicated, and that only by proceeding step by step can one arrive at an explanation of the properties of high polymer systems.

2. Thermodynamic and Statistical Quantities

(*a*) *Fundamental Conceptions*

A system tends always toward equilibrium. This tendency is associated with a definite quantity of energy since, in order to restore the system in equilibrium to its former state, a definite minimum amount of work must be applied. This work is designated as the change, ΔF, in the free energy, F, of the system, or more briefly as the free energy of the process considered. ΔF is positive when work is applied, *i.e.*, brought into the system. For spontaneous changes of any kind ΔF therefore is always negative.

From the first two laws of thermodynamics the following relationship holds for any process:

$$\Delta F = \Delta H - T\Delta S \tag{1}$$

in which ΔH is defined as the change in the heat content of the system for the process concerned, or more briefly the heat of reaction. If the process is associated with absorption of heat, then the heat content of the sytem is increased and ΔH is positive; when heat is evolved, then ΔH s< 0. In many cases ΔF can be measured directly, *e.g.*, electrometrically, by gas pressure, osmotic or swelling pressure, elastic force, etc. Likewise, ΔH is often directly measurable, *e.g.*, calorimetrically. On the other hand, ΔS, the change in entropy of the system, can usually only be calculated as $(\Delta H - \Delta F)/T$ or as $-\partial\Delta F/\partial T$.

The three quantities F, H, and S (and therefore also the differences ΔF, ΔH, and ΔS) are proportional to the mass of the system. In physical chemistry, one is generally concerned with definite amounts of compounds or mixtures and so these quantities usually refer to one gram molecule (F, H, S); if the molecular weight is unknown then they are referred to one gram (f, h, s).

By dividing F, H, and S, by N_A, Avogadro's number, one can consider the individual molecule as unit mass, but from the thermodynamic point of view, quantities such as F/N_A and especially S/N_A have only algebraic significance.

Molecular statistics are related to thermodynamics by means of the Boltzmann equation:

$$\Delta S = \frac{R}{N_A}\,\Delta \ln W = k \ln \frac{W_2}{W_1} \tag{2}$$

where k is the Boltzmann constant R/N_A, W is the thermodynamic prob-

ability of the reaction, and W_1 and W_2 are the probabilities of the system in its initial and final state. In the usual calculation the probability is defined as the quotient of the number of cases for which a particular condition is fulfilled (*i.e.*, favorable cases) divided by the total number of possible cases. In general, therefore, the probability will be a small fraction and it can never be greater than unity. For our present purpose, however, it has been found useful to define W as the number of favorable cases only, *i.e.*, in an assembly of molecules as the number of possible arrangements or "configurations." W will usually be a very large number. The number of arrangements must be separately calculated for each model and may be compared with the experimental value of ΔS.

By the application of Boltzmann's equation (2), we can express the thermodynamic equation (1)

$$\Delta F = \Delta H - kT \ln W \tag{3}$$

Thermodynamic quantities may now be interpreted by the application of statistics in the following way: the tendency toward the establishment of the most probable state depends ultimately upon the thermal motion of the molecules, illustrated for example in the tendency to diffusion on mixing. In isothermal processes change in heat content may be accounted for by the forces exerted upon each other by the particles; if these forces come into action in an isothermal process, then heat is evolved; whereas when heat is absorbed energy is utilized in overcoming these forces and stored as potential energy. If no change of heat content occurs then these forces are not in operation; such process is termed "athermal."

If, in a given system, the potential energy of the forces operating between particles is very much greater than their kinetic energy, a condition of strong condensation and high orientation obtains, as is found for example in the case of crystals at low temperature. If, on the other hand, the kinetic energy is dominant, then a state of low density and random arrangement obtains; in the limiting case we reach that of the perfect gases in which all intermolecular forces vanish.

(*b*) *Thermodynamic and Statistical Definitions in Theory of Solutions*

Most frequently molar quantities, either integral or partial, are used. In the following ΔF is used to signify the integral free energy of mixing, *i.e.*, the change in the free energy of the system in the formation of one mole of the mixture from the pure liquid components. Similarly, ΔH and ΔS are the terms used for the corresponding integral heat of mixing (posi-

tive by heat absorption in endothermal processes) and the integral entropy of mixing, respectively.

If the solvent is conventionally designated as component one of the mixture, then $\Delta\bar{F}_1 = \partial\Delta F_1/\partial n_1$ is called the partial molar free energy of component one, *i.e.*, the change in free energy on bringing one mole of component one into an infinite quantity of solution. $\Delta\bar{H}_1$ and $\Delta\bar{S}_1$ are the corresponding partial molar heat of mixing and entropy of mixing, respectively.

If the integral quantities are referred to one gram of the mixture instead of one mole, then they are designated Δf, Δh, and Δs, and the partials $\Delta\bar{f}_1$, $\Delta\bar{h}_1$, and $\Delta\bar{s}_1$ refer to one gram of component one.

$N_1 = n_1/(n_1 + n_2)$ and $N_2 = n_2/(n_1 + n_2)$ are termed the mole fractions of one and two, respectively, n_1 and n_2 being the number of molecules one and two.

It can be shown that the following relationships hold between integral and partial quantities:

$$\Delta H = N_1\Delta\bar{H}_1 + N_2\Delta\bar{H}_2 \tag{4a}$$

$$\Delta h = w_1\Delta\bar{h}_1 + w_2\Delta\bar{h}_2 \tag{4b}$$

N_1 and N_2 = mole fractions

w_1 and w_2 = weight fractions

and by differentiation with respect to N_1 and w_1, respectively, the Gibbs-Duhem-Margules equation is obtained:

$$N_1\frac{\partial\Delta\bar{H}_1}{\partial N_1} = -N_2\frac{\partial\Delta\bar{H}_2}{\partial N_1} \tag{5a}$$

$$w_1\frac{\partial\Delta\bar{h}_1}{\partial w_1} = -w_2\frac{\partial\Delta\bar{h}_2}{\partial\Delta w_1} \tag{5b}$$

Thus, if the variation of a partial quantity of one component is known, *e.g.*, $\Delta\bar{H}_1$, as a function of the composition, then, for the second component $\partial\Delta\bar{H}_2/\partial N_2$ is given, and since the value of $\Delta\bar{H}_2$ is zero when $N_1 = 0$, then the absolute values of $\Delta\bar{H}_2$ for all remaining concentrations can be deduced. This is of particular interest for systems involving high polymers since the direct determination of the partial quantities of the high polymer component is usually impossible. Equations of the same form as (4*a*), (4*b*), (5*a*), and (5*b*) may be obtained for ΔF and ΔS or Δf and Δs.

W_1^0 is defined as the thermodynamic probability, *i.e.*, the number of arrangements for one mole of component one in the pure (unmixed) state, and similarly W_1 is the number of arrangements for one mole of the com-

ponent one in the mixture with component two. Then W_1/W_1^0 is the relative increase in probability on mixing one mole of component one with a large quantity of solution. Then from equation (2), we have:

$$\Delta\bar{S}_1 = k \ln \frac{W_1}{W_1^0}$$

$a_1 = e^{\Delta F_1/RT}$ is called the activity. It is equal to the relative vapor pressure which substance one would exert if the vapor behaved as a perfect gas. For practical purposes it can be replaced by p_1/p_1^0 (where p_1^0 is the vapor pressure of pure component one, and p_1 its vapor pressure over the mixture).

Furthermore, following Lewis, we define a perfect solution as one for which, at all temperatures and pressures, the following equation holds:

$$a_1 = N_1$$

and from this definition of an ideal solution the following relationships can be deduced:

$$\Delta\bar{F}_1 = RT \ln N_1 \tag{6a}$$

$$\Delta\bar{H}_1 = 0 \tag{6b}$$

$$\Delta\bar{S}_1 = -R \ln N_1 \tag{6c}$$

We shall designate the expression $-R \ln N_1$ as the ideal entropy of mixing $\Delta\bar{S}_{1id}$.

A mixture which satisfies condition (6*b*) will be termed an *athermal* solution, one which satisfies condition (6*c*) will be termed a *regular* solution as proposed by Hildebrand. According to this the perfect solution is a special case: it is both athermal and regular. All these are limiting cases which are hardly ever completely realized.

(*c*) *Statistical Treatment of Crude Models of Liquid Mixtures*

As a simplified model, we can consider two liquids composed of motionless, rigid, spherical particles (molecules) of equal size and equal attractive forces. Liquid one contains n_1 indistinguishable molecules occupying the n_1 different sites in the quasicrystalline lattice of one; liquid two contains n_2 molecules in n_2 sites. The condition of the unmixed state can only be fulfilled by one arrangement (configuration): all n_1 sites occupied by n_1 particles of type one, all n_2 by two. The number of arrangements, $W_{unmixed}$, therefore equals one. In the mixture, on the other hand, both

kinds of molecules may be distributed randomly in the $(n_1 + n_2)$ sites of the mixture; many configurations are thus possible, *e.g.*, the first two sites may be occupied as follows: one-one, one-two, two-one, two-two. Everything else remaining constant, these two sites alone can thus give rise to four different configurations.

The total number of possible arrangements is given by:

$$W_{mixt} = \frac{(n_1 + n_2)!}{n_1! n_2!} \tag{7a}$$

By Stirling's transformation one obtains:

$$\ln W_{mixt} = -n_1 \ln \frac{n_1}{n_1 + n_2} - n_2 \ln \frac{n_2}{n_1 + n_2} \tag{7b}$$

Thus, from (7b) and (2), one finds the integral entropy of mixing for $(n_1 + n_2)$ molecules:

$$\Delta S = k \ln \frac{W_{mixt}}{W_{pure}} = k\,(-n_1 \ln N_1 - n_2 \ln N_2)$$

and for one mole:

$$(n_1 + n_2) = N_A$$

$$\Delta S = -RN_1 \ln N_1 - RN_2 \ln N_2 \tag{7c}$$

In order to obtain the partial entropy of mixing, $\Delta \bar{S}_1$, we compare with (4a):

$$\Delta \bar{S}_1 = -R \ln N_1 = \Delta \bar{S}_{1id}$$

This crude model thus gives the *ideal entropy of mixing*. Mixtures of very similar substances such as $C_6H_6 + C_6D_6$ will give this entropy value or one very near it.

This value $\Delta \bar{S}_{1id}$ is only reached if the mixture is perfectly random, *i.e.*, if all possible configurations are equally probable. If, in contrast to this, certain configurations are excluded or very rare (very improbable) for any reasons, then the number of configurations will be reduced and $\Delta \bar{S}_1$ and W fall below the ideal value. If, on the other hand, certain configurations are especially frequent, then also a particular order is enforced on the system and has the same effect, namely, a reduction of $\Delta \bar{S}_1$ and W. In a general way, it may be said that increase of S or W is equivalent to increase in randomness (disorder); any preferred arrangement is equivalent to a diminution of S or W.

In our crude model, the molecules were supposed to be motionless. This is, of course, not true for any real liquid at ordinary temperature; the

molecules are in thermal motion. At any given moment, therefore, a given molecule may not be found at its fixed point, but slightly displaced. The probability of finding this molecule displaced will be the greater, the less firmly the molecule is bound by its neighbors. The liquid seems thus to have more *sites* than molecules; the number of sites of each molecule must therefore be multiplied by a factor taking into account the space in which the molecule can move. This space will be designated by v_1 (solvent) and v_2 (solute) and be termed the *free volume* (v_1^0 and v_2^0 in the pure state).

If it is postulated that the forces of attraction to which a molecule is submitted do not vary on mixing ($\Delta H = 0$) then v_1^0 (unmixed) equals v_1 (mixed). We have then:

$$W_{pure} = (v_1^0)^{n_1}(v_2^0)^{n_2}$$

$$W_{mixt} = \frac{(n_1 + n_2)!\, v_1^{n_1} v_2^{n_2}}{n_1! n_2!}$$

$$\frac{W_{mixt}}{W_{pure}} = \frac{(n_1 + n_2)!\, v_1^{n_1} v_2^{n_2}}{n_1! n_2! (v_1^0)^{n_1}(v_2^0)^{n_2}} = \frac{(n_1 + n_2)!}{n_1! n_2!}$$

Since in this special case $v_1 v_2$ is cancelled by $v_1^0 v_2^0$, the system gives the ideal entropy of mixing.

Real mixtures are in general not composed of spherical molecules of equal size; the entropy therefore differs from the ideal value. The difference:

$$\Delta \bar{S}_1 - \Delta \bar{S}_{1id} = \Delta \bar{S}_{1exc}$$

will be termed excess entropy. It may be positive or negative. Many causes exist for the appearance of $\Delta \bar{S}_{1exc}$. If in one or both of the two liquids a definite arrangement is favored, such as a parallel disposition of rodlike molecules, which disappears on mixing, then a positive increment to the excess entropy results. Conversely, if such an arrangement were brought about by mixing, then clearly the molecules will be less randomly distributed than in an ideal mixture and the excess entropy of mixing would be negative.

If forces of attraction are involved, the mixture will no longer be perfectly random. For, if the molecules of the solute are more firmly bound to each other than to the molecules of the solvent (endothermal mixture), then clusters of solute molecules will be more frequent than in a random arrangement; if, on the other hand, the solute has more affinity for the solvent than for molecules of the solute, then *solvates, i.e.*, groups of solute and solvent, will be found. Here also the arrangement will be less random than in an ideal mixture. Both cases involve a reduction of W and S.

In addition to this, the forces of attraction also influence the free volume. If forces of attraction are operating then the molecules will have less freedom of motion. This reduction of the free volume involves a negative contribution to the entropy. Conversely, in an endothermal mixture the necessary increase of the free volume implies a positive contribution to the entropy.

The total entropy of mixing is thus composed of a series of terms. Besides the ideal entropy of mixing which is always positive, several terms have to be taken into account. They may, independently from each other, be positive or negative, large or small. In these cases, statistical treatment, based on refined models, has not yet given satisfactory results in spite of all optimistic assertions to the contrary.

3. Methods for Measurement of Partial Thermodynamic Quantities for Binary Mixtures

(*a*) *Measurement of Free Energy of Mixing*

$\Delta\bar{F}_1$ can be measured by means of the relative vapor pressure. In the free state, the pure component one (solvent) is in equilibrium with its own vapor with pressure p_1^0 and must therefore possess the same free energy; in a mixture it is in equilibrium with its vapor which is at reduced pressure p_1.

If the vapor behaves as a perfect gas, then:

$$\Delta\bar{F}_1 = RT \ln a_1 = RT \ln \frac{p_1}{p_1^0} \tag{8}$$

The vapor pressure of a mixture can be measured in various ways. If one component is nonvolatile, then the partial pressure of the other is equal to the total vapor pressure. This can be determined using the special apparatus described by Boissonnas and van der Wyk.[1] Otherwise, the partial pressure can be calculated from the total pressure according to a procedure suggested by Boissonnas,[2] or Scatchard's distillation method can be used, in which the total vapor pressure is measured and the composition of both the liquid mixture and the distillate (for analysis of the vapor) is determined pycnometrically. Lastly, the method of isothermal distillation is available and has been applied by Scatchard more especially for aqueous mixtures where the solution is brought into equilibrium with an

[1] C. G. Boissonnas and A. J. A. van der Wyk, *Helv. Chim. Acta*, **20**, 779 (1937).

[2] C. G. Boissonnas, *Helv. Chim. Acta*, **22**, 541 (1939).

aqueous solution of an electrolyte, usually sulfuric acid, as the activity of the water in the latter can be determined electrometrically with great accuracy.

Neglecting compressibilities of the liquid phases, the osmotic pressure or the swelling pressure P is related to the free energy or activity, respectively, as follows:

$$-P\bar{V}_1 = \Delta\bar{F}_1 = RT \ln a_1 \tag{9a}$$

$$-P = \frac{RT}{\bar{V}_1} \ln a_1 \tag{9b}$$

where $\bar{V}_1$ is the partial molar volume of the solvent.

The determination of osmotic pressure has been treated in Chap. A, sect. I2*a*. An apparatus for the measurement of very high osmotic or swelling pressures has been described by Posnjak.[3]

(*b*) *Determination of Entropy and Heat of Mixing*

Values for $\Delta\bar{S}_1$ can be arrived at by determining the free energy temperature coefficient, since from the second law the following relationship holds at constant pressure:

$$\frac{\partial \Delta\bar{F}_1}{\partial T} = -\Delta\bar{S}_1 \tag{10a}$$

And, similarly, for determination of the heat of mixing, we have.

$$\frac{\partial \Delta\bar{F}_1/T}{\partial 1/T} = \Delta\bar{H}_1 \tag{10b}$$

The necessary values for the free energy temperature coefficient can be obtained by measurement of $\Delta\bar{F}_1$ at various temperatures either by relative vapor pressure or osmotic pressure methods.

Values for $\Delta\bar{H}_1$ can also be obtained either from the sum:

$$\Delta\bar{H}_1 = \Delta\bar{F}_1 + T\Delta\bar{S}_1$$

or by direct calorimetric measurement.

[3] E. Posnjak, *Kolloid-Beihefte*, **3**, 417 (1912).

II. EXPERIMENTAL RESULTS FOR SUBSTANCES OF LOW MOLECULAR WEIGHT

1. Mixtures of Substances of Equal Molecular Size

(*a*) *Athermal Mixtures*

It has been shown experimentally that athermal mixtures of substances of low molecular weight behave almost like perfect solutions, the following systems coming into this category: 1,2-dibromoethane–1,2-dibromopropane[4]; methanol–ethanol[5]; stannic chloride–carbon tetrachloride[6]; benzene–toluene[5]; ether–benzene[5]; chlorobenzene–bromobenzene[5]; carbon disulfide–carbon tetrabromide[7]; and the still more exhaustively investigated system carbon tetrachloride–cyclohexane.[8] The activities of the components, measured by relative vapor pressure, are approximately equal to their mole fractions and so in these cases the entropy of mixing is approximately equal to the ideal entropy of mixing.

On the other hand, Scatchard[9] has shown that in some cases a very small variation of H is associated with an appreciable excess entropy which may be due to the breakdown of a highly oriented arrangement present in one of the two components. An example is furnished by the system benzene–cyclohexane.

(*b*) *Endothermal and Exothermal Mixtures*

In many cases of low molecular substances when only a small change in H occurs on mixing, the entropy change differs only slightly from the ideal value; such solutions are termed *regular* according to Hildebrand.[6] The deviation in activity from the ideal state is caused here by the heat of mixing. If ΔH is negative, *i.e.*, forces of attraction become saturated on dilution, then component one is drawn into solution, so to speak, and its activity sinks below the value of its mole fraction. If ΔH is positive, then $a_1 > N_1$ and $a_2 > N_2$ and the components may be said to be "expelled" from the mixture. For a series of systems, these deviations have been ex-

[4] J. Zawidski, *Z. physik. Chem.*, **35**, 124 (1900).

[5] G. C. Schmidt, *Z. physik. Chem.*, **A121**, 246 (1926).

[6] J. H. Hildebrand, *Solubility of Non-Electrolytes*. Reinhold, New York, 1936, pages 26, 60, 65.

[7] A. E. Korvezee, *Rec. trav. chim.*, **53**, 464 (1934).

[8] G. Scatchard, S. E. Wood, and J. M. Mochel, *J. Am. Chem. Soc*,. **61**, 3206 (1931).

[9] G. Scatchard, *J. Phys. Chem.*, **43**, 119 (1939).

perimentally investigated by Schmidt[10] and found to be in the direction required by the theory.

More precise measurements show, however, that the entropy always differs from the ideal values if $\Delta H \neq 0$ so that truly regular solutions are unknown. According to Boissonnas[11] the weakly endothermal system benzene–carbon tetrachloride, which Scatchard[12] considered as regular, is not really so but possesses a small but definitely positive excess entropy. Besides that, both ΔH and ΔS are found to vary with temperature. For the strongly endothermal system benzene–carbon disulfide, the positive excess entropy is considerably higher; in terms of statistical thermodynamics this must be attributed to an increase in the free volume. For still more strongly endothermal systems a miscibility gap appears—two phases result: phase I, a dilute solution of component two in one, and phase II, a solution of one in two. Each component has the same free energy $\Delta \bar{F}_1$ in both phases; but the entropy of one is greater in II than in I, and its heat of mixing must therefore also be greater in II according to:

$$\Delta \bar{F}^{\mathrm{I}} = \Delta \bar{F}^{\mathrm{II}} \text{ and } (\Delta \bar{H}_1 - T\Delta \bar{S}_1)_{\mathrm{I}} = (\Delta \bar{H}_1 - T\Delta \bar{S}_1)_{\mathrm{II}}$$

In case of a miscibility gap one can say that the tendency for diffusion ($T\Delta S$) is not strong enough to overcome completely the forces of attraction (ΔH). Usually such gaps diminish and finally disappear with increase in temperature.

For the strongly exothermal system chloroform–acetone the entropy of mixing is found to be much less than the ideal value. The molecules of one component strongly attract the other component whereby the free volume is reduced, a sort of compound being formed in which each molecule is preferably surrounded by molecules of the other component. For very strongly exothermal systems $\Delta \bar{S}_1$ becomes negative: it may be said that a real compound appears. It is usually found that $\Delta \bar{H}_1$ has the same sign over the complete range of concentration, *i.e.*, the mixture is consistently either endothermal or exothermal. In any case, a simple relationship holds at small concentrations between $\Delta \bar{H}_1$ and the concentration w_2 of component two:

$$\Delta \bar{H}_1 = \alpha w_2^2$$

where α is a constant.

[10] G. C. Schmidt, *Z. physik. Chem.*, **A101**, 286 (1922); **121**, 221 (1926).

[11] C. G. Boissonnas and M. Cruchaud, *Helv. Chim. Acta*, **27**, 994 (1944).

[12] G. Scatchard, *Trans. Faraday Soc.*, **33**, 160 (1931). G. Scatchard, S. E. Wood, and J. M. Mochel, *J. Am. Chem. Soc.*, **62**, 713 (1940).

But systems are also known for which the sign of H is reversed by a change of concentration, *e.g.*, for chloroform-alcohol. Heat is evolved on mixing a small amount of chloroform with much alcohol, whereas heat is absorbed on addition of a little alcohol to much chloroform, the mixture being athermal at a definite concentration and temperature. This system has been analyzed thermodynamically by Scatchard and Raymond,[13] their results being shown in figure 1. It is important to notice that the remarkable properties of this system could not be deduced from a consideration of the

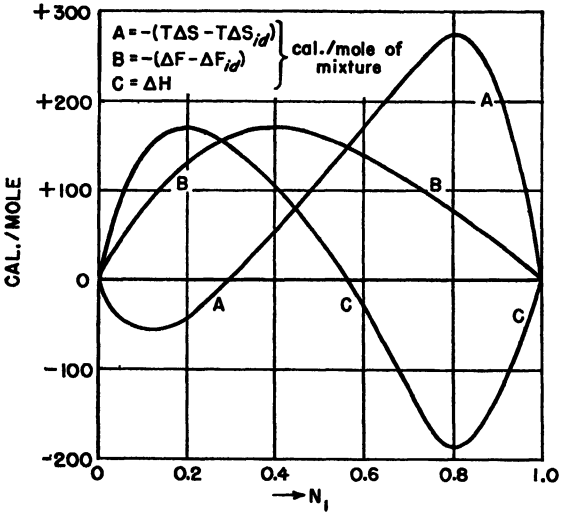


Fig. 1. Integral thermodynamic quantities for the system ethanol (component 1)–chloroform (Scatchard and Raymond[13]).

free energy curve alone, whereas the appearance of maxima and minima in both the excess entropy and heat of mixing curves is significant. The absorption of heat and the positive excess entropy on adding small quantities of alcohol to a large quantity of chloroform may be ascribed to the dissociation of double molecules of alcohol and to the disorder which results therefrom. On the other hand, chloroform can form a sort of compound with the alcohol with evolution of heat, accompanied by a negative excess entropy. This *solvate* formation is at its maximum when the chloroform–alcohol proportions are 1:4. The formation of such solvates and association complexes will be referred to in a later section.

[13] G. Scatchard and C. L. Raymond, *J. Am. Chem. Soc.*, **60**, 1278, 3099 (1938).

(c) *Dependence of ΔH on Temperature*

Owing to the lack of experimental data the heat of mixing is often considered to be relatively insensitive to temperature. This, however, is not really justified as can be seen for example in the case of water–methyl alcohol and also water–cane sugar. For the former system it is found that at higher temperatures less heat is absorbed on mixing; for the system water–cane sugar the change is in the reverse direction. In Table I are given the partial heats of dilution $\Delta\bar{H}_1$ (per mole of water) and $\Delta\bar{H}_2$ (per mole of methanol) which have been calculated by van der Wyk from the total heat of mixing, ΔH, determined experimentally by Bose.[14]

TABLE I

Heats of Dilution and Mixing

System: Water (1)–Methanol (2)

	T = 0.00°			T = 19.68°		
N_2	$\Delta\bar{H}_1$	$\Delta\bar{H}_2$	ΔH	$\Delta\bar{H}_1$	$\Delta\bar{H}_2$	ΔH
0.25	−216	−304	−237	−195	−292	−219
0.50	−316	−102	−209	−294	− 96	−195
0.75	−425	− 41	−137	−390	− 41	−128

In Table II the partial heats of dilution, $\Delta\bar{H}_1$, in calories per mole of water for the system water–cane sugar have been calculated as approximate mean values (for different concentrations and temperatures) from the data of Vallendar and Perman, Hunter and Gucker.

TABLE II

Partial Heats of Dilution

Water–Cane Sugar

m[a]	0°	12°	20°	40°	60°
1	−0.4	− 1.7	− 2.2	− 3.1	− 3.9
2	−0.9	− 6.0	− 7.0	− 8.8	−10.5
3	−4.3	−12.0	−13.5	−16	−18

[a] m = moles of cane sugar in 1000 g. of water.

For some systems the sign of the heat effect is reversed as is shown, for example, by water–propanol (Table III).

[14] E. Bose, *Z. physik. Chem.*, **58**, 585 (1907).

TABLE III

Heats of Dilution

Water–Propanol

	$T = 0°$			$T = 21.03°$		
N_2	$\Delta\bar{H}_1$	$\Delta\bar{H}_2$	ΔH	ΔH_1	ΔH_2	ΔH
0.25	−214	+200	−111	−111	+200	−47
0.50	−149	+ 85	− 32	− 68	+112	+20
0.75	− 36	+ 8	+ 2	+ 83	+ 18	+35

2. Mixtures Involving Molecules of Different Sizes

(*a*) *Athermal Mixtures*

One would not expect to find an ideal entropy of mixing for a system involving molecules of very different sizes and shapes, even if it were athermal since the necessary condition cannot be fulfilled that the molecules should be completely interchangeable. Actually, for athermal systems containing elongated molecules admixed with small molecules, the entropy of mixing is found to be greater than the ideal entropy, as illustrated by carbon tetrachloride-dioleyl thapsiate for which the experimental results of Meyer and Lühdemann are given in figure 2.[15]

(*b*) *Weakly Endothermic and Exothermic Mixtures*

The following systems have been completely analyzed thermodynamically:

Weakly endothermal systems	Weakly exothermal systems
Cyclohexane–oleyl oleate	Chloroform–oleyl oleate
n-Hexane–oleyl oleate	Chloroform–dioleyl thapsiate
Carbon tetrachloride–oleyl oleate	Propyl bromide–dibutyl valerianate
n-Hexane–dioleyl thapsiate	Propyl bromide–dibutyl sebacate
Cyclohexane–dioleyl thapsiate	Benzene–dibutyl sebacate
n-Heptane–*n*-hexadecane[15a]	

A system which may be exothermal or endothermal according to composition:

Benzene-dibutyl valerianate

In all these systems there is a positive excess entropy. As can be seen from figure 3 the course of $\Delta\bar{H}_1$ is sometimes very complex; it is therefore

[15] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **27**, 845 (1944).

[15a] J. H. van der Waals and J. J. Hermans, *Rec. trav. chim.*, **68**, 181 (1949).

not permissible to extrapolate values for $\Delta\bar{H}_1$ on the basis of measurements over a limited range of concentration. Up to the present time systems of this type with large heats of mixing have not been investigated.

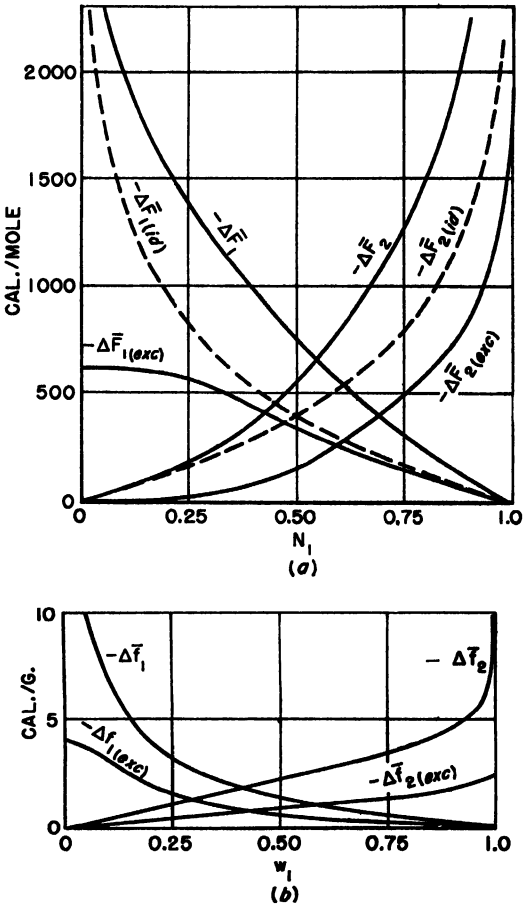


Fig. 2. Partial free energies for the system carbon tetrachloride (1) dioleyl thapsiate (2): (*a*) molar fractions; (*b*) weight fractions (Meyer and van der Wyk[15]).

(*c*) *Statistical Treatment of Nonideal Solutions and Comparison with Experiment*

For a statistical treatment of slightly endothermal and exothermal solutions, the assumption has been made that the arrangement in the mixture

is the same as in an ideal solution and therefore $\Delta\bar{S}_{1exc} = 0$ (*regular solutions*). Further, it has been assumed that the simple relationships $\Delta\bar{H}_1 = bN_2^2$ and $\bar{H}_2 = bN_1^2$ hold over the whole concentration range.[16] But since neither of these conditions is fulfilled in real solutions, the relationship derived herefrom:

$$a_1 = N_1 e^{bN_2^2/RT} \quad \text{and} \quad a_2 = N_2 e^{bN_2^2/RT}$$

$$\Delta\bar{F}_1 = RT \ln N_1 + bN_2^2$$

cannot be considered as a satisfactory statistical approach.

Fowler and Guggenheim[17] base their calculations on the model of the *strictly regular solution*. It is assumed: (*1*) that both types of molecules

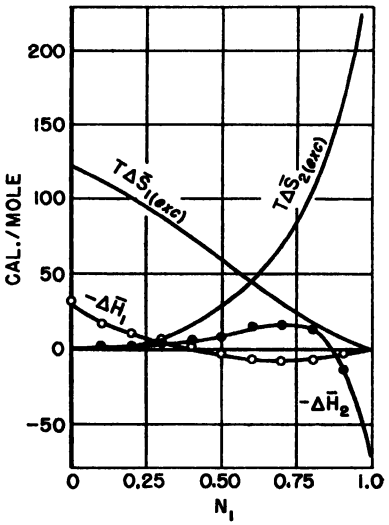


Fig. 3. System benzene (1) – butyl valerianate (2) (Meyer and van der Wyk).

pack in the same way so that they have a common value for the coordination number (number of close neighbors); (*2*) that the ratio of the diameter of the molecules is between 1 and 1.26 so that a mixture of both kinds of molecules will pack in the same way as in each of the pure liquids; (*3*) that

[16] A. W. Porter, *Trans. Faraday Soc.*, **16**, 336 (1920). J. H. Hildebrand, *J. Am. Chem. Soc.*, **51**, 69 (1929). G. Scatchard, *Chem. Revs.*, **8**, 321 (1931).

[17] R. H. Fowler and E. P. Guggenheim, *Statistical Thermodynamics*. Cambridge Univ. Press, London, 1939.

the ratio of the free volumes v_1^0/v_2^0 does not differ from unity by more than 30%; (*4*) that on mixing the molecular volumes and the free volumes remain unaltered: $v_1^0 = v_1$ and $v_2^0 = v_2$; and (*5*) that in any given configuration the potential energy of any molecule may be regarded as the sum of contributions of its closest neighbors. The authors derive from this model a very complicated relationship for the dependence of $a_1 = p_1/p_1^0$ on composition.

If we now consider these assumptions, *1*, *2*, *3*, and *5* may be realized in certain exceptional cases, but *4* is only possible for an athermal mixture ($\Delta H = 0$), for in all nonathermal mixtures hitherto investigated with sufficient precision ΔH is found to have its effect on the free volume. The calculations of Fowler and Guggenheim are therefore only true for systems which are not and cannot be realized.

Furthermore, models of athermal mixtures of molecules of different sizes have been the object of statistical treatment by these same authors and also by Hildebrand.[18] Following the former, a positive excess entropy is to be expected for mixtures of spherical molecules of very different sizes; systems which approximate their model have not yet, however, been thermodynamically investigated.

Hildebrand calculates that a mixture of rigid rodlike molecules of different lengths should exhibit no excess entropy. Here also precise measurements over the whole concentration range are lacking.

III. MIXTURES CONTAINING A HIGH POLYMER COMPONENT

1. Globular and Fibrillar High Polymers

(*a*) *Centers of Disturbance in Solution*

It is generally accepted that for "ideal dilute" solutions a number of simple relationships hold which depend on the fact that the solute particles are so far apart that there is no question of interaction between them. Theoretically, this condition is only fulfilled at zero concentration since, at a measurable concentration at a given moment, some definite fraction of the dissolved molecules will not be completely surrounded by solvent molecules but will be proximate to other solute molecules. This phenomenon is responsible for the observed deviations from the simple relationships such as that of van't Hoff, and any one point where two solute molecules are in juxtaposition may be regarded as a *center of disturbance.*

[18] J. F. Hildebrand, *J. Am. Chem. Soc.*, **59**, 794 (1937).

For any particular molal concentration it is clear that the number of centers of disturbance will increase in direct proportion to the coordination number of the molecule, *i.e.*, the number of molecules or groups which can verge upon it. For, the greater the number of adjacent groups, the greater must be the probability that among them one group of another solute molecule will be found.

Thus, the *degree of disturbance* will increase with increasing coordination number of the solute. It is quite evident therefore that the mutual disturbance for chain polymers will be much greater than for globular polymers.

As an illustration, we can consider a benzene solution of a slightly degraded rubber of molecular weight 200,000. The length of the chain of 3000 isoprene residues would approximate to 12,000 Å. If one such chain were entirely surrounded by solvent, then it would verge upon roughly 10,000 benzene molecules, its coordination number being some twenty-five times that of a spherical particle of the same volume. Owing to the internal mobility of these chain molecules, the segments of the chain will be fairly evenly distributed throughout the solution, and so isoprene residues of one chain will find themselves in contact with those of others. Calculation of the order of magnitude of this effect shows that, roughly speaking, in 1% solution 100 of the 10,000 isoprene residues in a chain would be adjacent to other isoprene residues, and in 0.1% solution there would be 10 such residues of the chain not completely surrounded by solvent. Thus, it can be seen that, even at great dilution, the chain molecule is to some extent under the influence of other polymer molecules. A similar calculation of the disturbance effect in the case of cellulose indicates that, in a 10% viscose solution of D.P. 100, 10 residues of each chain would be found in contact with other polymer residues. It is clear, therefore, that dissolved chain molecules cannot move freely and independently of each other. At any given moment there will be a number of points of contact along the length of any one chain. Because of this, it is to be expected that very considerable differences in behavior will be observed between solutions of the globular and fibrillar types.

(*b*) *Interaction with Solvent*

The interaction of each chain molecule with the solvent will amount approximately to the additive effect of the individual groups, and so it will increase with the number of groups, *i.e.*, the chain length. The molar heat of mixing may therefore reach very high values, thus giving rise to an

abnormally high free energy of mixing. This is only true if the groups are accessible to the solvent. Clearly, this is not the case for the inner groups of globular molecules, like most proteins, and so, for them, observed effects due to molecular interaction are small by comparison with those found for fibrillar molecules.

We shall deal first with the various aspects of chain polymer solutions and subsequently with those for globular polymers.

2. Free Energy of Solution and Swelling of Chain Polymers

(a) *Review of Activity Curves*

By introducing a chain polymer into a suitable solvent, the latter is slowly absorbed; swelling occurs which is limited in some cases, whereas

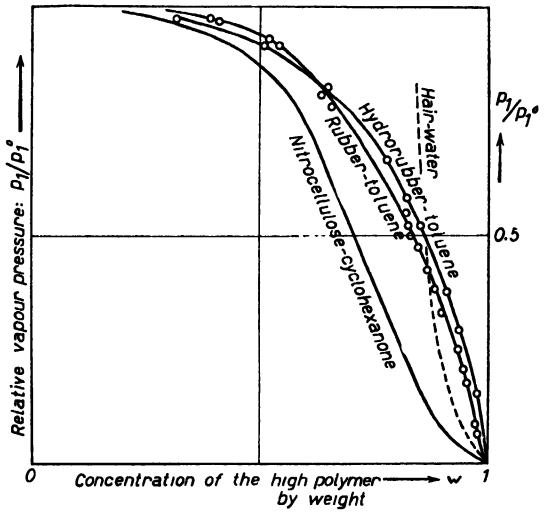


Fig. 4. Activities of the volatile components in various systems as functions of the concentration by weight.

in others it continues until complete solution results. In concentrated solution where the lowering of the vapor pressure is considerable, the activity $a_1 = p_1/p_1^0$ and, respectively, $\Delta\bar{F}_1$, can be directly estimated; for solutions of medium or low concentration the osmotic pressure is measured. If the system is not liquid but only a swollen solid, then the osmotic pressure is measured as swelling pressure since there is no difference between

these two quantities. At present, osmotic pressure data are available for many systems but vapor pressures have been measured for relatively very few; complete or nearly complete activity curves are therefore known only for a few systems. Even so the available material suffices to show how varied the shape of activity curves can be. The following groups may be distinguished (Fig. 4):

(*1*) Toluene–rubber,[19] benzene–rubber,[20] heptane–rubber,[21] and toluene-hydrogenated rubber[19] are all athermal or slightly endothermal systems and exhibit unlimited swelling. Δf_1 is therefore completely accounted for by the tendency for diffusion (see Fig. 4). By plotting activity against concentration, a curve without inflection is obtained.

(*2*) Tetrachloromethane–rubber,[22,23] chloroform–rubber,[22,23] cyclohexanone–nitrocellulose,[24] and trichloroethylene–cellulose acetate[25] are also systems of unlimited swelling but here the activity curves are S shaped (have an inflection point) and, as far as measurements are available, the systems are exothermal ($\Delta\bar{h}_1$ negative). The comparatively low activity is explained by the solvent being bound to the solute by forces of some kind.

(*3*) Acetone–rubber[21] and methanol–rubber[21] both exhibit two phases over a certain range of concentration. The a_1 curve is similar to those for case (*1*); $\Delta\bar{h}_1$ is strongly positive. The polymer takes up a limited though sometimes very considerable quantity of solvent, with absorption of heat, the solvent phase containing only small amounts of the polymer so that the distribution of the components in the two phases is very unsymmetrical.

(*4*) Water–gelatin[25,26] and water–agar[27,28] also exhibit two phases over a certain range of temperature; here too the distribution of components is unequal, the solvent taking up very small quantities of the polymer; but the activity curve is S shaped. It is found that $\Delta\bar{h}_1$ is negative (exothermal) at high concentrations as in group (*2*), but positive at low concentrations as in group (*3*), from which it can be deduced that in the swollen phase the polymer molecules are so firmly linked together that heat is absorbed by their separation (Fig. 5).

(*5*) Water–cellulose[27,28] and water–keratin[28] are systems where only limited swelling occurs, the polymer being insoluble in the pure solvent. Here the activity curves are S shaped; $\Delta\bar{h}_1$ is strongly negative in the region of high concentration. These can be considered as special cases of (*4*) where, in concentrated solutions, the solvent is bound by attractive forces but where, in the swollen polymer, the chains are so firmly linked together that they can be considered as practically inseparable.

[19] K. H. Meyer, E. Wolff, and C. G. Boissonnas, *Helv. Chim. Acta*, **23**, 430 (1940).
[20] G. Gee and L. R. G. Treloar, *Trans. Faraday Soc.*, **38**, 147 (1942).
[21] J. Ferry, G. Gee, and L. R. G. Treloar, *Trans. Faraday Soc.*, **41**, 340 (1945).
[22] J. Lens, *Rec. trav. chim.*, **51**, 971 (1932).
[23] L. Hock and H. Schmidt, *Rubber Chem. Technol.*, **7**, 462 (1934).
[24] C. G. Boissonnas and K. H. Meyer, *Helv. Chim. Acta*, **20**, 783 (1937).
[25] I. R. Katz, *Ergeb. exakt. Naturw.*, **3** and **4** (1924 and 1925).
[26] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **27**, 845 (1944).
[27] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **23**, 488 (1940).
[28] R. Fricke and J. Lüke, *Z. Elektrochem.*, **36**, 309 (1930).

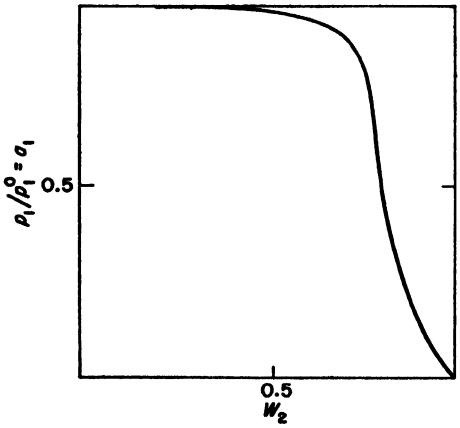


Fig. 5. Activity of water in the system water–gelatin.

(*6*) Toluene–vulcanized rubber, also a system of limited swelling, has only been partly investigated. At high concentrations the general form of the curve resembles those in group (*1*) but in the swollen system, the chains are inseparable.

The view expressed by Katz[25] and taken up later by Schulz[29] that the free energy of swelling equals the heat of swelling has to be abandoned. It has been shown untenable from the theoretical point of view and has also been disproved experimentally.

(*b*) *Osmotic Pressures of Chain Polymer Solutions*

Experiment has shown that, for systems involving chain polymers, the osmotic pressure usually differs considerably from the ideal value even at great dilution, inasmuch as it increases much more rapidly than proportionally to the concentration.

Substituting $P = -\Delta\bar{F}_1/\Delta\bar{V}_1$ and $a_1 = N_1$, the osmotic pressure of a perfect (ideal) solution can be expressed by:

$$P = -\frac{RT}{\bar{V}_1}\ln N_1 = -\frac{RT}{\bar{V}_1}\ln(1 - N_2) \qquad (11a)$$

from which we obtain by expansion into a series:

$$P = \frac{RT}{\bar{V}_1}\left(N_2 + \frac{1}{2}N_2^2 + \frac{1}{3}N_2^3 \ldots\right) = \frac{RT}{\bar{V}_1}N_2\left(1 + \frac{1}{2}N_2 + \ldots\right) \qquad (11b)$$

[29] G. V. Schulz, *Z. physik. Chem.*, **A184**, 1 (1939).

In practice, one takes care not to exceed pressures of 500 mm. which corresponds to a concentration of ~0.002 mole per liter. For a molal volume of 0.1 liter of component one $N_2/2 = 0.0001$, so that this and all subsequent terms can be neglected and we obtain the van't Hoff approximate equation:

$$P = \frac{RT}{\bar{V}_1} N_2 \rightarrow \frac{RT}{V_1^0} \frac{wM_1}{M_2} = RTc/M_2 \qquad (11c)$$

Thus for a perfect solution the osmotic pressure is proportional to the concentration c of two at great dilution; when a solution is ideally dilute and all molecules of component two are surrounded by solvent molecules, it will behave thermodynamically as an ideal solution of component two in one. In place of the osmotic pressure it is convenient to represent graphically the reduced osmotic pressure p/c or p/w (when w is the weight fraction of two) as a function of the concentration. Thus, for solutions of chain polymers which are athermal, weakly endothermal or weakly exothermal, it is found that p/w increases linearly with w. This may be expressed by the equation:

$$P = \frac{RT}{M_2} c + Ac^2 \text{ or } P = \frac{RT}{M_2} \rho_1 w + Bw^2 \qquad (12)$$

where ρ_1 is the density of the solvent and A and B are empirical constants. The molecular weight is deduced from the intercept on the ordinate, *i.e.*, the value for P/w at infinite dilution. In these cases, the P/c or P/w curves are practically parallel for solutions of polymer homologues in the same solvent, *e.g.*, nitrocelluloses of varying mean molecular weights measured in acetone; the constant B, which corresponds to the slope of the curve, is therefore independent of the molecular weight. Similar effects can be observed in the case of the chemically related substances, rubber and gutta-percha (Fig. 6). If, on the other hand, the same polymer is dissolved in various solvents, the intercept remains constant,[30] being dependent on molecular weight and T only, while the values of B vary (Fig. 7).

At high concentrations, the term $(RT/M)c$ in the van't Hoff equation practically disappears in comparison with Ac^2; the pressure becomes independent of the molecular weight as shown by Stamberger[31] in the case of rubber.

[30] A. Dobry, *Kolloid-Z.*, **81**, 190 (1937).

[31] J. Stamberger, *J. Chem. Soc.*, **1929**, 2318. *Cf.* P. J. Flory, *J. Am. Chem. Soc.*, **65**, 372 (1943).

Obviously, the abnormal values of the osmotic pressures found for chain polymers must have their counterparts in all other phenomena which depend upon the free energy. This is so for lowering of vapor pressure, depression of freezing point, and elevation of boiling point, and likewise for equilibrium in the ultracentrifuge. Here too, if one wants to deduce the molecular weight of the dissolved chain polymer, it is essential to make a number of determinations and extrapolate to zero concentration.

A glance at figures 6 and 7 shows that for the systems toluene–gutta-percha, toluene–rubber, and cyclohexane–polyisobutylene, which are all

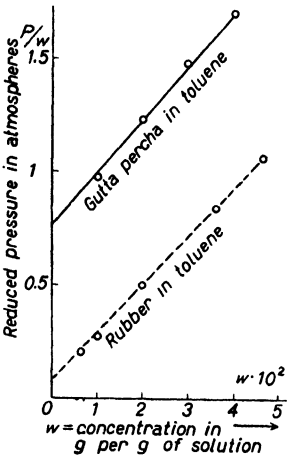


Fig. 6. Reduced osmotic pressures of rubber and gutta-percha in toluene.

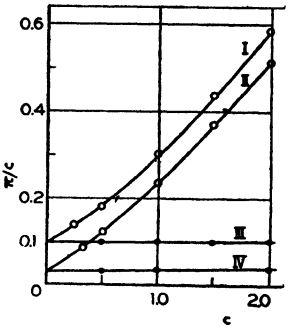


Fig. 7. Reduced osmotic pressures π/c (π in g./cm.^{2}, c in g./100 cc.) at 25°C. of two fractions of polyisobutylene, I and II in cyclohexane, III and IV in benzene, (from Flory[31])

practically athermal, the reduced osmotic pressure P/w increases very considerably with concentration; this is due to a high excess entropy. In endothermal systems, such as benzene–polyisobutylene, the effect of the excess entropy can be directly compensated by the heat of reaction, $\Delta\bar{h}_1$, so that P/w appears to be independent of concentration and the system is mistakenly considered to show a normal van't Hoff relationship. Such apparently normal behavior may also be shown by a solution to which has been added a quantity of a poor solvent, insufficient to cause precipitation, *e.g.*, alcohol to a toluene solution of rubber (Dobry).

For strongly endothermal or exothermal systems, equation (12) is no

longer valid. The P/w curve for cellulose triacetate in tetrachloroethane,[32] and likewise that of polystyrene in toluene,[33] diverges considerably from a straight line. According to Steurer[34] still greater divergence is shown in the case of ethyl cellulose solutions (approximately 2.5 ethoxyl, 0.5 free hydroxyl for each glucose residue) for which some curves have noticeable minima (see Fig. 4, page 79).

In view of the very diverse behavior observed it seems hopeless to attempt to deduce empirical equations of general validity such as Freundlich[35] has tried to do, or to apply the so-called Freundlich adsorption equation to the swelling process. Still less acceptable is the procedure adopted by Schulz in which the idea of a definite solvate envelope is used to establish formulas in order to extrapolate the reduced osmotic pressure at zero concentration.

To obtain a real insight into the complicated relationships which exist the measurement of free energy or activity is insufficient; the estimation of both its components $\Delta\bar{h}_1$ and $Td\bar{s}_1$ is necessary. In concentrated solution, this can be effected by measuring the heat of mixing (heat of swelling) or by means of the temperature coefficient of the relative vapor pressure; in dilute solution measurement of the temperature coefficient of the osmotic pressure must be relied upon as other methods are then insufficiently exact.

3. Heat and Entropy of Solution and Swelling

(*a*) *Thermodynamic Analysis by Means of Osmotic Pressure*

In equations (10*a*) and (10*b*) we can substitute $\Delta\bar{F}_1 = -P\bar{V}_1$ by $-PV_1^0$ since in very dilute solution the partial molal volume $\bar{V}_1$ is within the limits of experimental error, equal to the molecular volume of the pure solvent V_1^0 at the same temperature. As an approximation, the differential can be replaced by the difference between the values at two only slightly differing temperatures and so the following approximate equations may be obtained from (10*a*) and (10*b*), T_2 being the higher temperature:

$$\Delta\bar{S}_1 = \frac{(V_1^0P)_{T_1} - (V_1^0P)_{T_2}}{(T_1 - T_2)} \quad \text{and} \quad -\Delta\bar{H}_1 = \frac{1/T_1(V_1^0P)_{T_1} - 1/T_2(V_1^0P)_{T_2}}{1/T_1 - 1/T_2}$$

[32] O. Hagger and A. J. A. van der Wyk, *Helv. Chim. Acta*, **23**, 484 (1940).

[33] T. Alfrey, A. Bartovics, and H. Mark, *J. Am. Chem. Soc.*, **65**, 2319 (1943).

[34] E. Steurer, *Z. physik. Chem.*, **A190**, 1 (1940).

[35] H. Freundlich, Kapillarchemie. 4th ed., Vol. II, Akadem. Verlagsgesellschaft, Leipzig, 1932, p. 570.

Since relatively small differences of large numbers must be estimated, the results can only have real meaning if the greatest precision is applied in measuring both the pressure and the molecular volume.

(*b*) *Athermal System*

Within the limits of experimental error, the system toluene–gutta-percha[36] is athermal. The observed large deviation of $\Delta\bar{F}_1$ from the ideal value is therefore entirely due to a high entropy of mixing.

The system toluene–rubber[37] is very weakly endothermal and in this case data are available for the whole range of concentration: in dilute solutions, osmotic pressure measurements, in concentrated solutions, calorimetric measurements by Hock and Schmidt.[38] The great divergence between the activity and the ideal value is again to be attributed to the high entropy of mixing; the small positive term $\Delta\bar{H}_1$ only causes a small deviation in the opposite sense. Neglecting the small heat of reaction, and putting $\Delta\bar{F}_1 = -T\Delta\bar{S}_1$, we obtain for $T\Delta\bar{S}_1$ approximate values which are only slightly smaller than the exact values. The comparison with ideal values are shown in Table IV. From the values given in Table IV, it is clear that the

TABLE IV

Ideal and Actual Values of $T\Delta\bar{S}_1$

		$T\Delta\bar{S}_1$, cal./Mole	
w	N_2	Ideal	Found
0.3	0.146×10^{-3}	+0.086	>+5
0.5	0.341	0.201	> 37
0.7	0.795	0.468	>178
0.9	3.06	1.81	>637
0.95	6.43	3.79	>950

ideal entropy of mixing represents only a vanishingly small part of the observed entropy which is almost entirely made up of the excess entropy.

If mole fraction and molecular weight of rubber are calculated from the values of $\Delta\bar{S}_1$ by means of (6c), it appears that with increasing concentration of rubber its molecular weight decreases to the limiting value of 400. Thus, pure rubber behaves in this respect like a solvent of molecular weight 400, forming with toluene a perfect solution.

Using the Gibbs-Duhem equation $\Delta\bar{s}_2$ (the entropy variation for the rubber) can be calculated from the curve for $\Delta\bar{s}_1$ (entropy variation for the

[36] E. Wolff, *Helv. Chim. Acta*, **23**, 440 (1940).

[37] K. H. Meyer, E. Wolff, and C. G. Boissonnas, *Helv. Chim. Acta*, **23**, 430 (1940).

[38] L. Hock and H. Schmidt, *Kautschuk*, **10**, 33 (1934).

toluene), the values of $\Delta\bar{f}_2$ and $\Delta\bar{f}_1$ are given in figure 8. Since the very small heat absorption may be neglected, these values can also be regarded as $T\Delta s_{exc}$ for concentrations between 0.02 and 0.98. The excess entropy of mixing developed by dissolving rubber in a pure solvent amounts to 4

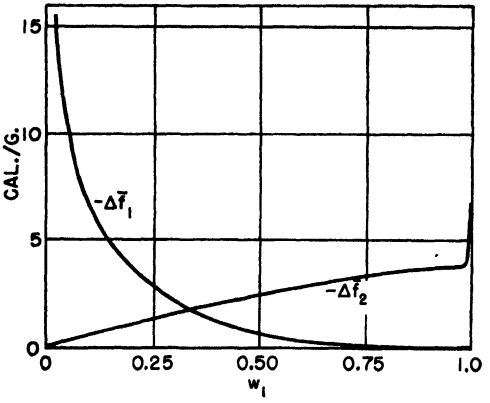


Fig. 8 System toluene (1)–rubber (2).

cal. per gram; multiplying this by the molecular weight, the value for $T(\Delta\bar{S}_2 - \Delta\bar{S}_{2id})$ is $\sim 10^6$ cal. per mole for the above-mentioned sample. According to Gee and Treloar[39] the somewhat more strongly endothermal system benzene rubber behaves very similarly.

(*c*) *Exothermal Systems*

An example of a weakly exothermal system is furnished by cyclohexanone–nitrocellulose[24] where $\Delta\bar{H}_1$ and $T\Delta\bar{S}_1$ are effective on the activity and the osmotic pressure in the same sense. The entropy curve resembles those for the systems previously considered, there being a considerable excess entropy of mixing in dilute solution. At higher concentrations the entropy curve cannot be determined with sufficient accuracy, but from the vapor pressures at two different temperatures one can deduce that $T\Delta\bar{S}_1$ is not very different from $-\Delta\bar{F}_1$. Probably, however, the entropy of mixing is negative at high concentrations since compound formation occurs as indicated by x-ray evidence.

The systems chloroform–rubber and carbon tetrachloride–rubber are

[39] G. Gee and L. R. G. Treloar, *Trans. Faraday Soc.*, **38**, 147 (1942).

similar, activity measurements having been carried out by Lens[40] and the small heats of mixing determined by Hock.[41]

The system tetrachloroethane–cellulose acetate[42] which is strongly exothermal, presents quite a different picture (Fig. 9). Here the entropy of mixing is less than the ideal value in dilute solution and becomes negative at concentrations greater than 2%. The high free energy and the abnormally high osmotic pressure are here furnished by the heat of mixing; the effect of the entropy of mixing on the free energy is in the reverse direction.

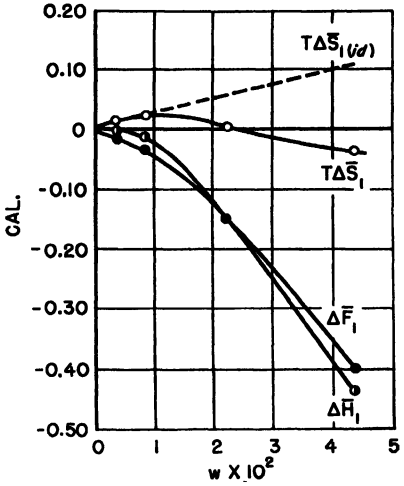


Fig. 9. System tetrachloroethane–cellulose triacetate.

This negative entropy means that the negative entropy term corresponding to the reduction in the free volume is greater than the positive term $\Delta\bar{S}_{1id}$. The solvent has less freedom in the mixture than in the pure state, and therefore is bound. Solvation is not limited to an envelope one molecule thick, for even in a mixture containing 97.5% solvent all of the latter is bound, which amounts to 70 molecules of tetrachloroethane linked to each glucose residue.

(*d*) *Endothermal Systems*

For endothermal systems such as benzene–polyisobutylene, the excess entropy effect can be exactly compensated by the heat of reaction, Δh, so

[40] J. Lens, *Rec. trav. chim.*, **51**, 971 (1931).
[41] L. Hock and H. Schmidt, *Rubber Chem. Technol.*, **7**, 462 (1934).
[42] O. Hagger and A. J. A. van der Wyk, *Helv. Chim. Acta*, **23**, 484 (1940).

that P/w appears to be independent of concentration and can be mistakenly taken as normal van't Hoff behavior:

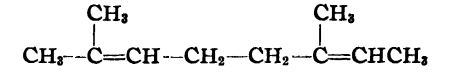
$$P = RT\left(\underbrace{\frac{\rho_1 w}{M_2}}_{\Delta\bar{S}_{1id}} + \underbrace{Bw^2}_{\Delta\bar{S}_{1exc}}\right) - \underbrace{aw^2}_{\Delta\bar{H}_1}$$

where:

$$RTBw^2 \cong aw^2$$

Osmotic pressure measurements have been carried out by Steurer[43] on the endothermal system benzene–ethyl cellulose; the P/w curve has a minimum at $w_2 = 0.001$. Unfortunately, no exact thermodynamic analysis is available, but it is to be expected that both $\Delta\bar{H}_1$ and $\Delta\bar{S}_1$ will show complicated variation with concentration.

According to Ferry, Gee, and Treloar[44] the systems acetone–rubber and methanol–rubber must be strongly endothermal since dihydromyrcene:



which can be regarded as a very low homologue of rubber gives endothermal mixtures with acetone or methanol. Methanol–rubber has been analyzed thermodynamically by these workers; only 2% methanol is taken up by the rubber with a considerable absorption of heat and a great increase in entropy. As we shall see later, the solubility is limited when $\Delta\bar{H}_2$ exceeds a certain limit.

(e) *Systems for which Sign of* $\Delta\bar{H}_1$ *Varies with Concentration*

The system water–gelatin is found to be endothermal at low concentrations but exothermal at high concentrations. Figure 10[45] gives a survey of the thermodynamic behavior. In concentrated solution $\Delta\bar{s}_1$ is negative which means that gelatin forms a hydrate, but on the other hand, in dilute solution, gelatin is associated. From the $\Delta\bar{h}_2$ curve, which shows a minimum, it can be seen that more heat is liberated on bringing gelatin into a 25% gelatin solution than by bringing it into pure water. This is due to the fact that, in the former case, the gelatin becomes hydrated without any breakdown of the micelles, so the heat of hydration has its full value. On the other hand, in pure water, dissociation of the hydrated micelles occurs,

[43] E. Steurer, *Z. physik. Chem.*, **A190**, 1 (1941). See Figure 4, page 19.

[44] J. Ferry, G. Gee, and L. R. G. Treloar, *Trans. Faraday Soc.*, **41**, 340 (1945).

[45] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **27**, 845 (1944).

involving absorption of heat which detracts from the heat of hydration. In this way solutions of gelatin afford a good example of solvate formation of associates, a phenomenon previously encountered in the system chloroform–alcohol.

Over a definite range of concentration and temperature gelatin takes up only a limited quantity of water, the excess water being saturated with gelatin; on addition of a large quantity of water all the gelatin dissolves, so

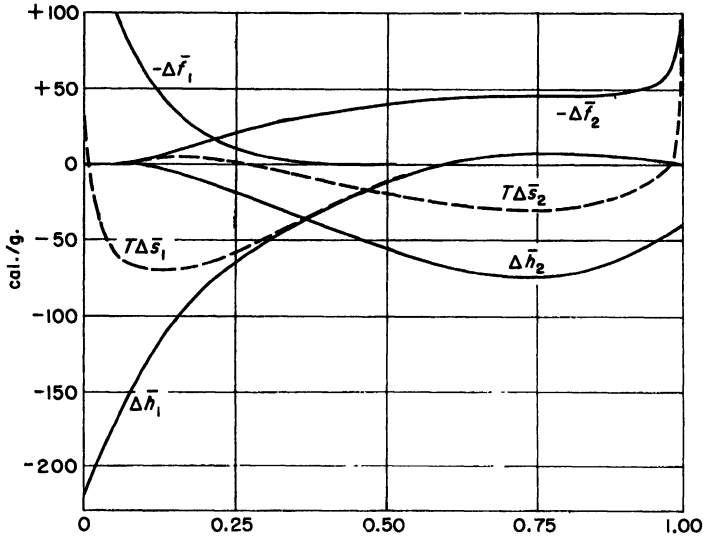


Fig. 10. Partial thermodynamic quantities; system water–gelatin. Abscissa, w_1 weight fractions of water.[45]

that, strictly speaking, gelatin cannot be put into the category of substances of limited swelling.

The system dioxane–cellulose acetate[46] exhibits a similar though less pronounced behavior. At low concentrations ($w_2 \rightarrow 0$) $\Delta\bar{h}_1$ is positive; becomes zero at $w_2 \cong 0.005$, and is negative at higher concentrations. Association and solvation must therefore be superposed.

(*f*) *Systems with Limited Swelling*[47]

A solution of benzene in vulcanized rubber is weakly endothermal, and so the swelling is only due to the tendency for diffusion. On the other hand,

[46] F. Kunze, *Z. physik. Chem.*, **A188**, 90 (1941).

[47] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **23**, 494 (1940).

from experimental evidence quoted in the literature, it can be deduced that agar, casein, hair and wool keratin, and cellulose bind water with considerable evolution of heat and decrease in entropy[47] (Table V).

TABLE V

Solvent H_2O Cal per mole of H_2O:	w = 0.943 (H_2O = 5.66%)			w = 0.892 (H_2O = 10.8%)		
	$\Delta\bar{H}_1$	$\Delta\bar{F}_1$	$T\Delta\bar{S}_1$	$\Delta\bar{H}_1$	$\Delta\bar{F}$	$T\Delta_1\bar{S}_1$
Agar-agar at 5°	−3200	−1850	−1350	−1850	−1000	− 850
Casein at 5°	−2050	− 900	−1150	−1000	− 350	− 650
Hair keratin at 5°	−2250	−1200	−1050	−1450	− 500	− 950
Wool keratin at 20°	−2800	−1100	−1400	−1650	− 450	−1200
Cellulose (cotton) at 50°	−1000	− 230	− 770	− 200	− 30	− 175

(g) *Systems for Which Sign of $\Delta\bar{h}_1$ Varies with Temperature*

For benzyl alcohol–methyl amylose the partial heat of dilution definitely varies with temperature and can indeed undergo a reversal in sign[48] (Table VI). This phenomenon is even more striking in the system water–methyl

TABLE VI

System Benzyl Alcohol–Methyl Amylose
(Mol. wt. 70,000; concentration 0.991%)

t	20°	30°	40°
ΔH_1	−16	− 7.5	+ 2 millical./mole

amylose where the polymer precipitates at higher temperatures, indicating that the heat of dilution (absorption of heat) is so great that the tendency for diffusion is no longer sufficient to obtain a solution.

From all these examples, it can be seen that the thermodynamic properties of binary systems containing a polymeric component are extraordinarily diverse and complicated, a fact which is not always borne in mind. The thermodynamic analysis of ternary systems is well-nigh impossible.

4. Phenomena of Swelling

(a) *Swelling Power*

The swelling power, *i.e.*, the quantity of solvent absorbed when a gel of limited swelling capacity is in equilibrium with pure solvent, has repeatedly

[48] A. J. A. van der Wyk, *unpublished data.*

been used for the characterization of swelling phenomena. Among others, measurements of this quantity have been made on gelatin by Hofmeister[49] and Ostwald[50] and on rubber by Flusin[51] and Le Blanc[52]; the general conclusion can be drawn that it is very dependent on the solvent. Such investigations are often of practical value, *e.g.*, to establish the stability of rubber goods toward petroleum, etc.

Theoretically, this phenomenon can be explained as follows. When the polymer consists of a network of primary valence linkages, such as in vulcanized rubber or collagen fibers, liquid is imbibed until the swelling pressure is equal to the contractile forces brought into play. For rubber chains extended by reason of the swelling process, this tendency for contraction—which is directly comparable with that of stretched rubber—increases with the deformation so that a large increase in volume indicates a high swelling pressure. It is known that in benzene and in toluene the swelling pressure is due solely to the tendency to diffusion. If, therefore, the swelling is greater in a given solvent than in toluene, then the pressure is intensified by forces of attraction (heat evolution); this is the case for rubber in chloroform, whereas in ether the swelling is less as heat is absorbed.

The behavior of gelatin can be explained similarly. It is a network of crystalline fringed micelles. When swelling occurs in a solution of an electrolyte, $\Delta\bar{\Pi}_1$ may be increased by the chemical interaction of the electrolyte with the hydrophilic groups of the gelatin; in addition to the diffusion tendency of the chains in a swollen phase, there is also the diffusion tendency of the gegenions (Donnan effect) so that the phenomenon is very involved.

There is yet another peculiar feature of this system. At low temperatures, gelatin undergoes limited swelling in water, while the water phase becomes saturated. If the whole system is brought into solution by warming and the solution is cooled, then the whole mass solidifies as a uniform jelly. In effect, we can obtain two different final states at will, either a swollen slab of gelatin surrounded by a saturated aqueous solution or a homogeneous jelly. As far as their energy contents are concerned the difference between them is as great as that between a heap of minute inorganic crystals and one large crystal.

[49] F. Hofmeister, *Arch. exptl. Path. Pharmakol.*, **28**, 210 (1890).

[50] W. Ostwald, *Arch. ges. Physiol. Pflügers*, **108**, 563 (1908); **111**, 581 (1909).

[51] M. G. Flusin, *Ann. chim. et phys.*, **8**, 480 (1908).

[52] M. Le Blanc and M. Kröger, *Kolloid-Z.*, **33**, 168 (1923). *Cf.* J. R. Scott, *Trans. Inst. Rubber Ind.*, **5**, 95 (1929).

(*b*) *Kinetics of Swelling*

It has been seen that pure rubber in the presence of toluene behaves like a hydrocarbon of molecular weight 400. This is revealed not only in the activity of toluene in rubber, but also in the velocity of diffusion at the rubber–toluene interface. It is observed that the surface of rubber advances into the toluene with a high velocity of diffusion which is characteristic of small molecules. As the dilution of the rubber continues, the diffusion coefficient falls and reaches a constant value depending on the real molecular weight only at very great dilutions.

The steep fall in concentration at the rubber–toluene boundary, so often observed in diffusion experiments, is due to the relationship between the coefficient of diffusion and the concentration. Macroscopically, the diffusion of rubber and toluene or similar systems of soluble high polymers and solvents appears as swelling; fluid penetrates from the surface into the high polymeric, coherent phase, so that the surface advances uniformly into the solvent without macromolecules passing into the liquid at first. The phenomenon can be explained crudely by the fact that the chains form a felt.

While it is true that mixing will eventually be complete at equilibrium in dilute solutions, owing to the low diffusion velocity of the large molecules, this state will only be reached after a very long period. In preparing homogeneous solutions of high polymers, it is thus always necessary to stir.

(*c*) *Swelling of Crystalline Bodies*

At this point, it is convenient to consider briefly the swelling of crystalline bodies. It is possible to determine from x-ray analysis whether liquid penetrates only into the spaces between the crystallites—intermicellar swelling—or into the crystallites themselves—intramicellar swelling (see section on the swelling of cellulose, Chap. E, sect. II).

Oriented preparations always exhibit anisotropy of swelling: one-dimensional intramicellar swelling occurs in graphitic acid and in montmorillonite; this swelling may also be termed laminar swelling, since the solvent separates the laminae of the primary valence nets. Predominantly one-dimensional swelling is encountered in viscose films, for example. Since in these the micelles lie for the most part in the plane of the film, and water is deposited between the micelles, the film increases mainly in thickness. Two-dimensional swelling is found in fibrous structures.

If the swelling agent penetrates the crystallites, it may lead to the formation of a compound insoluble in the swelling agent (as in the swelling

of cellulose in alkali), or the swelling agent may dissolve the chains. In the latter case, the lattice is first enlarged, and the swelling agent is adsorbed, usually until a stoichiometrically defined quantity has entered. The lattice of the addition compound then breaks up and the substance passes into solution. This phenomenon has been observed in the solution of nitrocellulose in ketones. The vapor pressure isotherms show no sharp inflection, which might suggest the formation of a stoichiometrically defined compound.

5. Statistical Approach of Abnormal Entropy of Mixing

(*a*) *Qualitative Considerations*[53]

It has been seen that the abnormal osmotic behavior of chain polymers can be attributed either to marked forces of attraction, *e.g.*, tetrachloroethane–cellulose acetate, or to a high entropy of mixing, *e.g.*, carbon tetrachloride–dioleyl thapsiate and toluene–rubber, or to a combination of both, *e.g.*, cyclohexanone–nitrocellulose. An explanation of the abnormally high energy interchange has been given in sect. III 1*b*. In attempting to explain statistically the high entropy of mixing, we shall limit ourselves to athermal systems and so cut out the effect on entropy caused by variation in heat content.

As has been pointed out previously, the thermodynamic probability of an athermal mixture of small molecules can be deduced on the assumption that both kinds of molecules can occupy all sites of the lattice equally. The ideal entropy of mixing can then be calculated by use of the Boltzmann equation (7*a–c*). Similarly, a high polymer system such as rubber in toluene can be envisaged as a sort of lattice occupied partly by toluene molecules (white spheres) and partly by segments of flexible rubber molecules (black spheres). Let us now compare the number of possible configurations of white spheres (toluene molecules), if they can change places with black spheres united by links to form chains (rubber molecules), with the number prevailing in a system containing the same number of unlinked black spheres (representing small molecules).

A glance at figure 11 shows that the number of configurations is the same in both cases when the concentration of white is low. In the special case of figure 11*a* and *b*, the two toluene molecules may occupy any two places. It is also clear that the length of the chains (that is, the true molecular weight of the high polymer) is without influence. The change in entropy

[53] K. H. Meyer, *Z. physik. Chem.*, **B44**, 383 (1939).

for white (toluene) when the two toluene molecules are transferred from the pure toluene phase to the chain system is the same as it would be on mixing with an equal number of free chain links. When the concentration of white (toluene) is higher, the picture changes. Figure 12 shows that the number of possible arrangements of white (toluene) in the system white spheres (toluene)–black chains (rubber) is much smaller than in the system

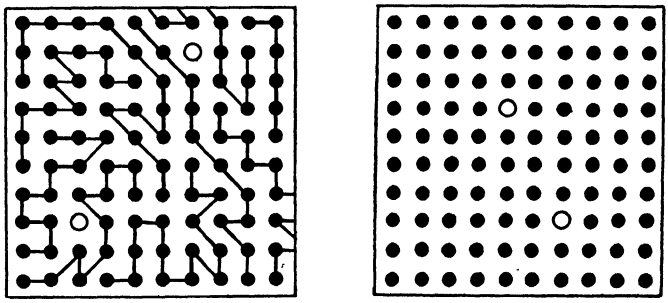


Fig. 11. Possible arrangements of white spheres in: (*a*) a system of chains of black spheres, and (*b*) in a system of free black spheres. At high concentrations of black the numbers of possible arrangements for white are equal.

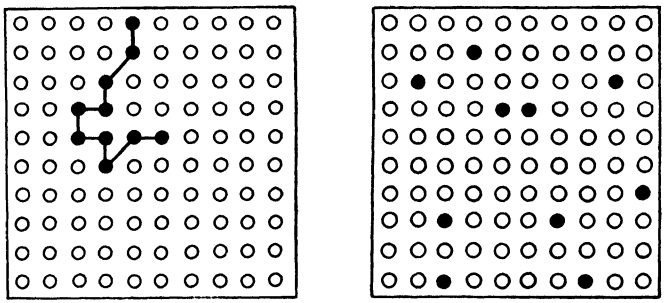


Fig. 12. At low concentrations of black the possible arrangements for white are less numerous in (*a*) than in (*b*).

white spheres (toluene)–black spheres (hydrocarbon of low molecular weight). The possible configurations are limited in the system white spheres–black chains by the condition that the links of the chains must remain joined together. In this way all configurations are excluded in which this condition is not satisfied (arrangement such as that in Fig. 12*b*, for example).

If now these considerations are applied to the systems under discussion, it is to be noted first of all that a flexibility such as was assumed in the model is not encountered in chains of methylene or isoprene groups. The position of an isoprene group in a rubber chain is largely determined by the neighboring links—within the chain a short-range order prevails. The farther a link is removed from any particular link, however, the less is its position influenced by the latter, until finally the two links behave independently of each other. One cannot, therefore, assume that methylene groups or similar small chain fragments are able to exchange places with molecules of a substance of low molecular weight, but must expect the chain fragments capable of this exchange to be larger. As is shown by experiment, in very high concentrations of rubber, toluene has the same activity as in a solvent of molecular weight 400, forming an ideal solution with toluene. Since all other influences on entropy have been excluded, it is possible to ascribe the high entropy of mixing of toluene, ΔS_{1exc} to the fact that fragments of rubber chains of molecular weight 400, corresponding to six isoprene units, are able to change places freely, like free molecules, with toluene molecules. This is in good agreement with the qualitative considerations discussed above. These chain fragments have been variously designated as "kinetically effective units" by Meyer and Lühdemann, as "submolecules" by Huggins, and as "segments" by Powell. In Table VII the molecular weights of these units (the *apparent molecular weight*) are listed for different compounds.

TABLE VII

Real Molecular Weights, M, and Molecular Weights of Segments, M^*[51]

Compound	M	Solvent	M^*
Butyl valerianate.....	158	Benzene.....	128
		Propyl bromide.....	150
Dibutyl sebacate.....	314	Benzene.....	105
		Propyl bromide.....	222
Dioleyl thapsiate.....	787	Carbon tetrachloride.....	271
Rubber (degraded).....	250,000	Toluene.....	400
		Benzene.....	160

These considerations can be completed by considering $\Delta\bar{S}_2$, for, as already shown, the entropy change of one mole of rubber on addition to a large quantity of dilute solution, is much greater than the ideal entropy. This can be attributed to the fact that in the pure compound, not only is the center of gravity of a particular chain molecule determined by its neigh-

[51] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **27**, 845 (1944).

bors, but also its shape, which to a certain extent must conform to those of its neighbors. In the dilute solution, however, a chain molecule can assume all possible shapes quite independently of the shapes of other chain molecules. The molecule has therefore more freedom (more possible configurations) than it had before mixing; this involves a positive term to $\Delta\bar{S}_2$.

(*b*) *Quantitative Approaches*

On the basis of these qualitative explanations proposed in 1939,[53] many authors have attempted to develop quantitative relationships. Flory[55] and Huggins[56] have calculated the thermodynamic probability in the following way. The first segment of the first chain can obviously occupy any site, for the next segment only the adjacent sites are available, *i.e.*, actually z positions if the coordination number is z. For the next segment, there will be $(z - 1)$ sites available. A similar procedure can be applied to the segments of other chains always remembering the number of sites already occupied by the chain first considered. For an athermal mixture of n solvent molecules with N chain molecules each containing x segments, the entropy is found according to Flory:

$$\Delta\bar{S}_1 = -R\left[\ln v_1 + \left(1 - \frac{1}{x}\right)v_2\right] \text{ and } \Delta\bar{S}_2 = -R\,[\ln v_2 - (x - 1)\,v_1]$$

where v_1 and v_2 are the volume fractions of the components. As Flory himself points out, the agreement between calculated and observed values is not very good, the divergences being possibly due to oversimplification or to the unjustified neglect of later terms in the mathematical series.

If Flory's equation is used to calculate x, the number of segments in each chain molecule of dioleyl thapsiate dissolved in carbon tetrachloride, the following values for x are obtained from the experimental values of $\Delta\bar{S}_1$:

$v_1 = 1 - v_2$	$\Delta\bar{S}_1$	x
0.015	6.1	−9.8
0.2	1.31	−5.7
0.6	0.216	0
0.9	0.035	+8.8

[55] P. J. Flory, *J. Chem. Phys.*, **9**, 660 (1941); **10**, 51 (1942); **12**, 425 (1944).

[56] M. L. Huggins, *J. Chem. Phys.*, **9**, 440 (1941); *J. Phys. Chem.*, **46**, 1 (1942); *Ind. Eng. Chem.*, **35**, 216 (1943); *Ann. N. Y. Acad. Sci.*, **43**, 1 (1942); **44**, 431 (1943); *J. Am. Chem. Soc.*, **64**, 1712 (1942).

This shows that Flory's equation is far from being satisfactory. For non-athermal solutions, Flory adds to his expression a further term in which he assumes a simple relationship between ΔH and the composition; he further neglects its effect on the free volume. Both assumptions are certainly inadmissible.

Miller[57] postulates the following:

$$\Delta\bar{S}_1 = -R\left[\ln(1-v_2) - \frac{z}{2}\ln\left\{1 - \frac{2v_2}{z}\left(1-\frac{1}{x}\right)\right\}\right]$$

with z = coordination number, and v_1, v_2, and x as above.

Other quantitative relationships have been proposed by Powell, Clark, and Eyring,[58] Münster,[59] Guggenheim,[60] Orr,[61] Gee,[62] Alfrey and Doty,[63] and Zimm.[64]

By comparison with the amazing output of theoretical work which has developed from the original investigations at Geneva, progress in the experimental field has fallen lamentably behind. Apart from work on various rubber systems, carried out in the laboratories of the Rubber Producers Association, no new work can be cited and one is continually obliged to resort to old results. It is to be hoped that other systems will be completely investigated thermodynamically; *e.g.*, carbon tetrachloride–octadecane and diisobutylene (or cyclohexane)–polyisobutylene, for both of which fairly simple behavior would be anticipated, and also the more complicated system water–methyl cellulose. In our opinion new impetus should be directed to experimental investigation rather than calculations based on oversimplified models.

6. Mixtures Containing a Globular Polymer Component

Solutions of those proteins whose molecules (according to the results of x-ray analysis) are compactly built, exhibit but slight deviations from

[57] A. R. Miller, *Proc. Cambridge Phil. Soc.*, **39**, 54, 131 (1943).

[58] R. E. Powell, C. R. Clark, and H. Eyring, *J. Chem. Phys.*, **9**, 268 (1941).

[59] A. Münster, *Kolloid-Z.*, **105**, 1 (1943).

[60] E. A. Guggenheim, *Proc. Roy. Soc. London*, **A183**, 203, 213 (1944); *Trans. Faraday Soc.*, **41**, 107 (1944).

[61] W. J. C. Orr, *Trans. Faraday Soc.*, **40**, 320 (1944).

[62] G. Gee, *Rubber Chem. Technol.*, **17**, 672 (1944).

[63] T. Alfrey and P. Doty, *J. Chem. Phys.*, **13**, 77 (1945).

[64] B. H. Zimm, *J. Chem. Phys.*, **14**, 164 (1946).

van't Hoff's rule even when the molecular weight is very high. Figure 13 shows the P/w curve of hemoglobin[65] in water and, for comparison, that of a nitrocellulose of approximately the same molecular weight in cyclohexanone. Small deviations, similar to those of hemoglobin, are shown by hemocyanins. The difference in behavior, as compared with the chain polymers, leads one to suppose that the anomalous entropy is much less con-

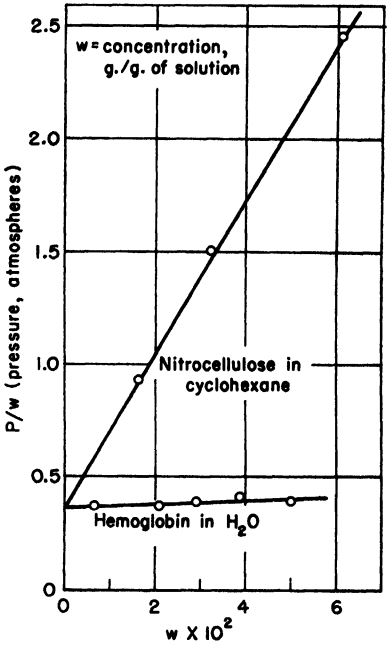


Fig. 13. Reduced osmotic pressure of nitrocellulose and hemoglobin as a function of the concentration.

spicuous here than with chain polymers; with regard to the heat of solution, we have seen above that only the groups at the surface interact with the solvent, so that summation of the energy increments due to the links of the chain, encountered in chain polymers, does not enter into consideration here.

[65] From measurements by G. S. Adair, quoted by Pauli-Valkó, *Kolloidchemie der Eiweisskorper*. 2nd ed., Steinkopff, Leipzig, 1933, page 183.

IV. STATE OF HIGH POLYMERS IN SOLUTION

1. Solvates and Associates in Solution of Compounds of Low Molecular Weight

(*a*) *Consequences of the Third Law*

Equilibrium in solution is determined by the two terms ΔH and $T\Delta S$. Now the third law requires that the entropy shall disappear if temperature approaches absolute zero. In the neighborhood of absolute zero, the attractive forces practically control the equilibrium: where $\Delta H > 0$ the two components separate completely; where $\Delta H < 0$ a compound results. At ordinary temperatures $\Delta H > 0$ will give rise to a state between complete disorder and complete separation; for $\Delta H < 0$, a state between disorder and a regular compound. Solutions can thus be classified as follows:

Athermal Solution.—If the heat of mixing is zero over the whole range of concentration then the statistically most probable distribution will be established, *i.e.*, completely random. That is to say, there will be neither associates of solute nor solvent molecules and equally there will be no groups formed by solvent and solute molecules (solvates).

Exothermal Solutions.—If heat is evolved on mixing then there is no longer a completely random distribution but a definite order is brought about in which solvent molecules are adjacent to those of the solute. The heat liberated can be called *the heat of solvation* and the dissolved molecules are said to be *solvated.*

Endothermal Solution.—If heat is taken up then we are dealing with *associates, i.e.*, arrangements involving groups of similar molecules; by comparison with the completely random distribution a certain degree of separation must have occurred. The heat evolved if a molecule two, originally surrounded by solvent molecules, becomes linked to a second molecule two or a pre-existing group of two, is called *the heat of association.*

Now the process of mixing a substance two with a large quantity of one can be analyzed as follows. (*1*) The creation of a temporary *cavity* in the one phase (consisting wholly or largely of one); forces of attraction must be overcome and thus a definite amount of energy, λ_1, a kind of heat of vaporization, must be brought into the system. (*2*) A molecule of two must be removed from the pure phase two which requires that its heat of vaporization, λ_2, shall also be brought into the system. (*3*) The introduction of the vaporized molecule into the cavity which entails the liberation of a kind of heat of condensation, λ_3.

Heat evolution on solution means that $\lambda_3 > (\lambda_1 + \lambda_2)$, *i.e.*, the attractive

force between the unlike molecules is greater than that between like molecules; solvation therefore occurs. When heat is absorbed the position is reversed and associates are formed. Here $(\lambda_1 + \lambda_2) > \lambda_3$ where either λ_1 or λ_2 may be the predominating factor.

A relationship between the heat and degree of association is provided by statistical considerations. For a mixture of equally sized and similarly shaped molecules, the probability that a particular molecule adjacent to a particular molecule two is itself a molecule two is equal to the molar fraction N_2; where the number of neighboring molecules is n, the probability that one neighbor of a particular molecule two is itself two, becomes nN_2. If heat is evolved on mixing, this product must be multiplied by a factor of about $e^{\lambda/RT}$.

(*b*) *Polydisperse Association*

If, by the addition of a third molecule to a diassociate the same amount of energy is liberated as in the formation of the diassociate itself, and if this is true for the addition of subsequent molecules, then 3,4, and higher associates will be formed without any particular stoichiometric relationship being favored, *i.e.*, with respect to the size of the associates, the solution will be polydisperse. According to Meyer and van der Wyk[66] such polydispersity is to be expected in systems where the quantity λ_1 (due to the attractive forces of the solvent) outweighs λ_2 (the heat of evaporation of the solute). The association of the solute is then largely brought about by the fact that the solvent squeezes together the dissolved molecules. In such cases it can be assumed the same amount of free energy is gained each time a molecule two becomes attached to a pre-existing group of two. Then, by the law of mass action, the association constant K given by:

$$K = \frac{[\text{Associates of } \nu \text{ molecules}]}{[\text{Associates of } (\nu - 1) \text{ molecules}][\text{free molecules}]}$$

is independent of the degree of association.

On this basis, the distribution of the sizes of the associates may be computed from:

$$m_\nu = \nu\, n_1^\nu\, K^{\nu-1} \qquad \text{and} \qquad n_1 = \frac{1}{K}\,\frac{2KS + 1 - \sqrt{4KS + 1}}{2KS} \tag{13}$$

[66] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **20**, 1321 (1937).

where m_ν is the weight concentration of micelles formed from ν individual molecules, n_1 the molar concentration of free molecules, and S is the total molar concentration with respect to the monomer, *i. e.*, $S = w/\rho M_2$ where ρ = density of the solution; w and M_2 = the weight concentration and molecular weight of the dissolved molecules. Figure 14 shows the expected distribution for different values of K at a constant total concentration of $S = 0.5$ mole per liter.

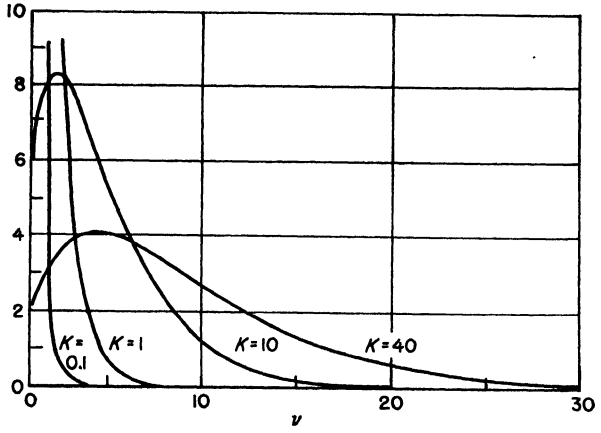


Fig. 14 Size distribution for various values of K at total concentration of $S = 0.5$ mole/liter. Ordinate, $m\nu \times 10^{-2}$.

Such equilibria are found for dilute aqueous solutions of soap, paraffinsulfonic acids, and the like.

(*c*) *Associates of Definite Composition*

In contrast to the polydisperse association, it can also happen that, when a definite number of molecules have formed an associate, a kind of saturation sets in, for example in the case of the double molecules of carboxylic acids or alcohols. An apolar solvent like cyclohexane dissolves in an excess of alcohol with only a very small heat effect since it is interposed between the apolar groups. On the other hand, dissolving alcohol in a large quantity of hexane involves dissociation of the alcohol diassociates and heat is taken up to the extent of 5800 cal. per mole of ethanol in cyclohexane.[67,68] At low alcohol content, the concentration of alcohol associates will be about $e^{5800/RT}$, *i.e.*, 20,000 times as great as in a random mixture.

[67] G. von Elbe, *J. Chem. Phys.*, **2**, 73 (1934).

[68] K. L. Wolf, H. Pahlke, and K. Wehage, *Z. physik. Chem.*, **B28**, 1 (1935).

Polar molecules associate in polar and apolar solvents in different ways. The medium and higher fatty acids form di-, tri-, and higher associates in aqueous solution, whereas in an apolar medium, association is practically complete at the bimolecular stage. On the other hand, complete dissociation may occur in solvents containing both polar and apolar groups.

(*d*) *Solvation of Associates*

Associates which are at the same time solvated are found in systems which exhibit a change of sign for ΔH on varying the concentration, one such case, chloroform–alcohol, having been cited in sect. II1*b*. When mixing chloroform with much alcohol, heat is given off, indicating the formation of solvates between the alcohol bimolecules and the chloroform, while heat is absorbed on mixing it with a little alcohol, thus indicating dissociation of the alcohol molecules.

Since the tendency for diffusion increases with temperature, the formation of both associates and solvates at higher temperature will be diminished in a general way. At the same time, a variation in ΔH may occur, involving a change in heat of solvation or association. It has been found for propanol–water and water–cane sugar in sect. II1*c*. In the former system solvates disappear at higher temperatures and are replaced by associates; in the latter system solvate formation is favored at higher temperatures.

2. Solvates and Associates of Globular Polymers (Proteins)

As yet, the only polymers known to have a compact nonfibrillar molecular structure are proteins. Evidence has been afforded by their behavior in the ultracentrifuge and by x-ray analysis of crystalline proteins. The basic structure is that of a peptide chain with side groups. Formamide dissolves in water with absorption of heat and accordingly must be associated; it is probable therefore that peptide groups also have a marked tendency to associate. But there another effect comes into play, for many side groups have a strongly hydrophilic character; it may be assumed that aqueous protein solutions will contain micelles which are solvated. This view is supported by the observation that the micelles (associates) are split up by addition of urea or formamide; the hydration of proteins has been repeatedly confirmed.

From the very high cohesion of protein associates it follows that considerable heat is liberated in their formation. To prevent a dissociation of more than 2% a protein of molecular weight 100,000 must possess a heat of

association of about 20,000 cal. Actually, the hemocyanins exhibit still higher heats of association.

3. Nature of Solutions of Fibrillar Polymers

(*a*) *Chain Polymer Solutions as Swollen Solids*

The deviations from ideal behavior discussed in sect. III 1*a* show that even very dilute chain polymer solutions cannot be looked upon as ideal in the sense of the solute molecules being completely surrounded by solvent.

The mechanical properties of the solutions also manifest the interaction of chain molecules at many points along their length. This was established as early as 1932 by Eisenschitz and Rabinowitsch[69] in the case of dilute solution of cellulose acetate. They calculated the modulus of shear from the variation of viscosity with shearing stress and obtained values of 10^3 dynes per sq. cm. for 0.5% solutions of cellulose acetate; the value was found to increase with concentration, reaching 5×10^4 dynes per sq. cm. for a 12% solution. These investigators expressed the view that: "With regard to their elasticity cellite solutions down to 1% concentration are better described as swollen solids than as dilute solutions." Erbring[70] has demonstrated the rubberlike elasticity of more concentrated solutions (solutions which can be spun).

The felting of fibrillar molecules has also been shown by the results of diffusion experiments. For instance, Krüger and Grunsky[71] have reported diffusion measurements on cellulose nitrate in dilute solution. They found that the marked local decrease in concentration in the region of the original interface, solvent solution, is not appreciably modified with progressive diffusion. The 1% acetone solution behaves like a sponge soaked to capacity, so to speak. In contrast, Herzog[72] pointed out, as early as 1907, that various proteins did not show these anomalies; the proteins concerned were precisely those ovalbumins which are now known to have a compact "globular" structure.

Still more concrete evidence of mechanical contact between fibrillar molecules in very dilute solution has been provided by investigations on sedimentation in the ultracentrifuge. Except when extremely dilute polystyrene mixtures undergo sedimentation, the boundary line is sharp because the longer chains carry down the smaller chains. The separation of

[69] R. Eisenschitz and B. Rabinowitsch, *Ber.*, **64**, 2522 (1932).

[70] H. Erbring, *Kolloid-Z.*, **98**, 164 (1942).

[71] D. Krüger and H. Grunsky, *Z. physik. Chem.*, **A150**, 115 (1930).

[72] R. O. Herzog and H. Kasarnowski, *Z. Elektrochem.*, **13**, 533 (1907).

the higher from the medium polymer chains only sets in at concentrations below 0.02%, giving rise to a diffuse boundary. For natural cellulose too there is no fractionation even at a concentration as low as 0.05%. The same phenomenon is also shown by the results of estimations of molecule size distribution. On the same polystyrene sample, measurements have been taken in small but varying concentration by Signer and Gross,[73] also by Lansing.[74] The molecular weight distribution curve for the lower concentration, 0.89 g. per liter shows a maximum for molecules of the 30,000 mol. wt. order (by sedimentation equilibrium). This contrasts with the curve obtained for a concentration of 4.79 g. per liter, which has its maximum at 80,000 with an average mol. wt. 90,000 (by sedimentation velocity). Obviously, even at such dilutions, the fibrillar molecules are not freely mobile and so, much too high figures for the molecular weight are obtained. We may therefore say that for concentrations at which sufficiently exact measurements are possible, chain polymer solutions must be regarded as completely soaked felts of flexible rods or threads. In no way can they be considered to be solutions of molecularly dispersed solute.[75] Expressions such as "solutions where the colloid particles are macromolecules" are incorrect since neither free colloid particles, nor free macromolecules are present, the molecular felt or framework persisting even at great dilution.

Investigations of dilute solutions of long chain polymers by means of light scattering (see Chap. A, sect. I2*f*), viscosity (see Chap. A, sect. I4*b* and Chap. J, sect. V), and flow birefringence (Chap. A, sect. I4*c* and Chap. J, sect. VII5) have shown that molecules of cellulose esters in good solvents are relatively stiff and extended up to molecular weights of 20,000 to 50,000; if the M.W. is higher, progressive curling is observed.[76] Hydrocarbons appear to be less stiff.

(*b*) *Formation of Chain Polymer Solvates and Associates*

In the previous discussion, no account has been taken of thermal effects. For some high polymer systems the heat factor is small and can be neglected, but this would obviously lead to false conclusions in cases where the

[73] R. Signer and H. Gross, *Helv. Chim. Acta*, **17**, 726 (1934).

[74] Cited by T. Svedberg, *The Ultracentrifuge*. Oxford Univ. Press, London 1940.

[75] K. H. Meyer. *Hochpolymere Verbindungen*. Akadem. Verlagsgesellschaft, Leipzig, 1940, page 531.

[76] R. M. Badger and H. Blaker, *J. Phys. & Colloid Chem.*, **53**, 1056 (1949).

heat effects are considerable. The system tetrachlorocthane–cellulose triacetate is strongly exothermal, the solvent being completely bound in a kind of solvate even at a concentration as low as 2.5%. The whole system behaves like a swollen solid but the reason is different from apparently similar cases where ΔH is small. Other examples of solvate formation are provided by nitrocellulose in acetone, or cyclohexanone.

Strongly endothermal effect on dilution is characteristic of solutions containing associates, as illustrated by water–gelatin, dioxane–polyvinyl chloride[77] and benzene–ethyl cellulose.[78] In the latter there is evidence for association even in 1% solution. In these associates, only a small number of groups of each chain are in contact with other chains.

Fibrillar proteins can undergo both solvation and association as shown by gelatin in water. According to Signer and Mosimann[79] there is evidence for the existence of associates in this system; but at the same time, the evolution of heat which accompanies the moistening of dry gelatin indicates that the gelatin binds water, *i.e.*, gelatin micelles become solvated. Solutions of fibrillar myosin and likewise fibrinogen also contain solvated micelles.

(*c*) *Hysteresis Phenomena and Formation of Supersaturated Unstable Solutions*

For high polymer solutions equilibrium is often reached very slowly. This is so for methyl cellulose–water[80] where the extremely slow establishment of equilibrium is manifest in a remarkable hysteresis phenomenon. The following effects were observed in a 1.56% solution. On warming turbidity develops; at 46° the viscosity increases, over several hours' duration after an initial fall; at 50° gelatinization begins, but on cooling the gel does not liquefy until the temperature has fallen to 36°. It is difficult to explain the initial temporary fall in viscosity at 46° which, in the opinion of Heymann, should be ascribed to reduction of particle size or diminution in hydration, the latter being the more favored view. But certainly the packing of chain molecules to give larger bundles could be accompanied by a decrease in viscosity; further aggregation with the formation of a coherent structure would result in increase of viscosity. It is interesting to observe the effect of ultrasonics on such a *structure viscous* solution prepared by warming at 46° and cooling. The considerably enhanced vis-

[77] P. Doty, H. Wagner, and S. Singer, *J. Phys. & Colloid Chem.*, **51**, 32 (1947).
[78] E. Steurer, *Z. physik. Chem.*, **A190**, 1 (1941).
[79] R. Signer and H. Mosimann, *Helv. Chim. Acta*, **24**, 1058 (1941).
[80] E. Heymann, *Trans. Faraday Soc.*, **31**, 846 (1935).

cosity is seen to sink below the original and equilibrium value. The equilibrium is very slowly restored. It would appear that the coherent micellar structure is largely disintegrated by ultrasonics so that only micellar bundles remain, which dissociate very slowly, undergoing complete hydration, and so finally reach the equilibrium state.

Hysteresis is also observed when cellulose acetate is dissolved in a mixed solvent.[81] Dissolution in a pure solvent such as chloroform with subsequent addition of the nonsolvent benzene gives rise to a solution of lower viscosity than by dissolving directly in the previously prepared solvent mixture. In the latter case dispersion of aggregates proceeds very slowly and perhaps the associates present in the mixture are only slowly developed when nonsolvent is added to a molecularly dispersed solution in chloroform.

A solution of amylose initially quite clear is found to develop a cloudiness on long standing and finally the whole of the polysaccharide precipitates. Even before there is any visible effect, changes have occurred since the clear solution becomes more and more resistant to amylase. Undoubtedly this *aging* consists of an aggregation process by which micelles develop in the originally molecularly disperse solution. As the process continues, ultramicrons appear and finally the solution becomes turbid.

Some years ago, Lieser[82] succeeded in preparing supersaturated solutions of cellulose. Such supersaturated solutions seem to occur frequently in living organisms; inulin is present in that form in cell sap, similarly fibroin in the viscous fluid of the silk worm spinning gland.

A review of the several discussions above shows how very diverse and numerous are the phenomena encountered. Clearly, no simple generalized treatment is possible for high polymer solutions, especially chain polymers. In their consideration it is essential that due regard be paid to the multiplicity of the factors involved.

V. EQUILIBRIA IN SOLUTION

1. Chain Length and Partition between Two Phases

A substance distributed between phases I and II is in partition equilibrium when, for each component, the relation

$$\bar{F}_2^{\mathrm{I}} = \bar{F}_2^{\mathrm{II}} \tag{14a}$$

holds, and hence:

[81] I. Sakurada, *Kolloid-Z.*, **61**, 50 (1932). H. Erbring, *ibid.*, **108**, 16 (1944).
[82] T. Lieser, *Kolloid-Z.*, **98**, 142 (1942).

$$\bar{H}_2^{\mathrm{I}} - TS_2^{\mathrm{I}} = \bar{H}_2^{\mathrm{II}} - TS_2^{\mathrm{II}} \tag{14b}$$

or omitting index 2:

$$\Delta\bar{H} = T\Delta\bar{S} \tag{14c}$$

$-\Delta\bar{H}$ is the heat evolved when one mole of the substance is transferred reversibly from phase I to phase II, the masses of which are supposed to be so large that no change in their composition will result from this transference.

If we are concerned with the partition of a substance between two solvents and if association or solvation does not occur to any considerable extent, the entropy of solution is known from experience to approach the ideal entropy of mixing; the entropy of transference therefore becomes:

$$\Delta\bar{S} = R \ln \frac{N^{\mathrm{I}}}{N^{\mathrm{II}}} \tag{15a}$$

where N^{I} and N^{II} are the mole fractions of the substance in phases I and II. Then:

$$\Delta\bar{H} = RT \ln \frac{N^{\mathrm{I}}}{N^{\mathrm{II}}} \tag{15b}$$

Let us now consider a series of homologous chain polymers. Each link of the chain is supposed to interact with the solvent, so that if one link is transferred from phase I to phase II, an amount of heat q is involved and, if a substance consisting of z links is transferred, the heat involved will be zq. We have the relation:

$$zq = RT \ln \frac{N^{\mathrm{I}}}{N^{\mathrm{II}}} \tag{16}$$

and since we postulated dilute solutions:

$$zq \approx RT \ln K \frac{w^{\mathrm{I}}}{w^{\mathrm{II}}} \tag{17}$$

where w^{I} and w^{II} are the equilibrium concentrations by weight and K is the ratio of the molecular weights of the two solvents, $M_1^{\mathrm{I}}/M_1^{\mathrm{II}}$.

The ratio of the concentrations at equilibrium $w^{\mathrm{I}}/w^{\mathrm{II}}$ is Nernst's partition coefficient, τ. Accordingly:

$$\tau = \frac{1}{K} e^{zq/RT} \tag{18}$$

The partition coefficient therefore increases by the factor $e^{-q/RT}$ when a polymer of $(z + 1)$ links is substituted for one of z links, since:

$$\frac{\tau_z}{\tau_{(z+1)}} = e^{-q/RT}$$

In a similar manner, it can be shown that the equilibrium constant for distribution between solvent and gas phase changes by a given factor if a link is added to the chain. Table VIII gives several examples illustrating the validity of this rule.

TABLE VIII[83]

Partition Coefficients

Homologous alcohols			Homologous fatty acids		
Alcohol	Cottonseed oil water	Factor	Acid	Benzene water	Factor
CH_3OH.....	0.00966		CH_3COOH....	0.055	
		3 66			5.16
C_2H_5OH.....	0 0357		C_2H_5COOH....	0.28	
		4.42			3.85
C_3H_7OH.....	0.156		C_3H_7COOH....	1.08	
		3 77			3.47
Iso-C_4H_9OH....	0.588		C_4H_9COOH....	3.73	
		3.62			4 32
Iso-$C_5H_{11}OH$...	2.13		$C_5H_{11}COOH$...	16.1	

Since in general higher chain polymers dissolve with considerable positive excess entropy, $\Delta\bar{S}_{2exc}$, it is not permissible to replace the transfer entropy by the ideal value. For higher polymers this entropy can be represented by zs, where z is the number of chain residues and s the excess entropy of transference per residue. The increment s is necessarily very small since it is the difference between two excess entropies.

Equations (15), (17), and (18) then become:

$$\Delta\bar{S} = \Delta\bar{S}_{id} + \Delta\bar{S}_{exc} = R \ln \frac{N^{\mathrm{I}}}{N^{\mathrm{II}}} + \Delta\bar{S}_{exc} \qquad (15c)$$

$$\Delta\bar{H} - T\Delta\bar{S}_{exc} = RT \ln \frac{N^{\mathrm{I}}}{N^{\mathrm{II}}} \qquad (15d)$$

[83] Data from A. Frumkin, *Z. physik. Chem.*, **116**, 501 (1925), and B. B. Worth and E. E. Reid, *J. Am. Chem. Soc.*, **38**, 2316 (1916).

$$z(q - Ts) \approx RT \ln K \frac{w_1}{w_2} \tag{17a}$$

$$\tau = \frac{1}{K} e^{z(q - Ts)/RT} \tag{18a}$$

Thus, passing from a degree of polymerization z to the next higher $(z + 1)$ the partition coefficient is multiplied by the factor $e^{(q - Ts)/RT}$. On the whole, therefore, the higher polymers fall into line with those of lower molecular weight except that the heat of transference, q, per residue must be replaced by the lower value $(q - Ts)$.

2. Chain Length and Solubility

(a) *Solubility of Amorphous Polymers*

For the solubility of a crystalline or amorphous polymer (subscript 2) in a solvent (subscript 1) we have the equation:

$$\Delta\bar{H}_2 = \underset{\text{dissolved}}{\bar{H}_2} - \underset{\text{undissolved}}{H_2^0} = \underset{\text{dissolved}}{T\bar{S}_2} - \underset{\text{undissolved}}{TS_2^0} = T\Delta\bar{S}_2 \tag{19}$$

Let us consider first the case of a substance of low molecular weight giving approximately a regular solution. Then the following holds (approximately):

$$\Delta\bar{H}_2 = -RT \ln N_2^s \tag{20}$$

where N_2^s is the mole fraction of 2 in saturated solution.

If all the links come into contact with the solvent, the heat of solution of a chain of z links is equal to the sum of the heats of solution q, of each individual link and therefore:

$$\Delta H_2 = zq = -RT \ln N_2^s \tag{21a}$$

If q is negative, the compound is miscible with the solvent since by definition $N_2 < 1$. Equilibrium between a molten phase and a solution, however concentrated, is impossible in this case; the former must disappear. When, on the other hand, the heat of solution is positive, the value of N_2^s rapidly diminishes as z increases. An example is provided by the solubility of homologous normal paraffins in water; each new methylene group diminishes the solubility by two thirds (Table IX).

As mentioned above, when $\Delta\bar{H}_2$ is positive a considerable positive excess entropy appears on dissolving high polymers and so the equations above,

TABLE IX

Solubility in Water of Homologous Normal Paraffins (Moles/Liter)[84]

Paraffin		Factor
C_5H_{12}	0 005	3.1
C_6H_{14}	0.0016	3.1
C_7H_{16}	0.00052	4.0
C_8H_{18}	0 00013	

as they have been used by Brønsted[85] and Schulz,[86] cannot be applied without modification. As in section 1, Eq. 15*c*, the positive entropy term must be included, so that the equation becomes:

$$N_2^s = e^{-z(q-Ts)/RT} = e^{-zq/RT}\, e^{zs/R} \qquad (21b)$$

Since small positive values for q can be compensated by Ts, even polymers of very high degree of polymerization may dissolve with heat absorption as shown by the systems benzene–rubber and water–gelatin. On the other hand, where $q > Ts$, the higher polymer homologues are practically insoluble whereas their lower homologues may be soluble.

The solubility, if small, may be expressed as weight fraction by putting:

$$w_2^s = N_2^s \frac{M_1}{M_2} = N_2^s \frac{M_1}{zm_2}$$

where m_2 is the M.W. of the repeating links. Then, from Eq. 21*b*:

$$w_2^s = \frac{m_2}{M_1}\, ze^{-z(q\,-\,Ts)/RT}$$

It is clear that not only does w_2^s rapidly (though more slowly than N_2^s) diminish as the chain length increases, but also that its variation soon becomes very small, because:

$$\frac{dw_2^s}{dz} = \frac{w_2^s}{z}\left(1 - z\,\frac{q - Ts}{RT}\right)$$

which tends rapidly to a value of $-w_2^s(q - Ts)/RT$ for high values of z. Therefore a fractionation of very long chain material is not possible by this method. Powers[87] demonstrated experimentally for polystyrene solutions that this is indeed the case.

[84] H. Fühner, *Ber.*, **57**, 510 (1924).
[85] J. N. Brønsted, *Compt. rend. trav. lab. Carlsberg, Sørensen-Festband*, **22**, 99 (1938).
[86] G. V. Schulz, *Z. physik. Chem.*, **A179**, 321 (1937).
[87] P. Powers, *Ind. Eng. Chem.*, **41**, 2213 (1949).

The effect of temperature is approximately given by the following relationship:

$$\frac{1}{N_2^s}\frac{\partial N_2^s}{\partial T} = \frac{zq}{RT^2} \tag{22}$$

which indicates that the temperature solubility coefficient increases with increase in chain length. If z is very large a temperature can be found below which the polymer is only slightly soluble and above which it is freely miscible. Below this temperature two phases are present. As a result of equation (22) the solution becomes rapidly poorer in solute as the temperature falls. On the other hand, the gel phase contains a considerable quantity of solvent and remains swollen. Here, as a first approximation, we have:

$$\frac{1}{N_1^s}\frac{\partial N_1^s}{\partial T} = \frac{\Delta \bar{H}_1^s}{RT^2}$$

Since $\Delta \bar{H}_1^s$ is very small by comparison with zq the temperature coefficient in this case, *i.e.*, for the solubility of the solvent in the gel, is very small. In consequence the composition of the swollen phase will change only very slowly on cooling.

(*b*) *Fractional Precipitation*

If a precipitant is added to a solution of a high polymer, similar phenomena can be observed. When a certain concentration is reached, the homogeneous liquid separates into a mobile, liquid phase and a gel. If a mixture of chains differing in length is present, it is possible—by cautiously adding the precipitant—to produce a kind of fractionation, in such a way that the most highly polymerized fraction is precipitated first as a much swollen gel, while the shorter chains remain in solution and are induced to flocculate only by the addition of more precipitant.

This process is readily understandable in a qualitative way. The addition of precipitant may be regarded as a gradual change in the heat of solution, q, or, better, of the expression $(q - Ts)$. The high polymer distributes itself between two phases, one richer in solvent (gel I) and one poorer in solvent but richer in precipitant (phase II). The heat of transfer:

$$\bar{H}_2^{\mathrm{I}} - \bar{H}_2^{\mathrm{II}} = zq$$

and the excess entropy of transfer may be introduced as in sect. V 1. It follows from equation (18) differentiating with respect to q:

$$\frac{1}{\tau}\frac{\partial \tau}{\partial q} = \frac{z}{RT} \tag{23}$$

The relative change of the partition coefficient is thus greater for the more highly polymerized fraction than for the less polymerized fraction. In the addition of a small quantity of precipitant, therefore, the longest chains become concentrated in phase I, while the molecules of a lower degree of polymerization exhibit no marked tendency to separate out. By separating the gel from the solution and repeating the operation, it is possible to achieve a considerable fractionation (Rocha,[88] Mardles,[89] Herz,[90] and Daneš.[91]

(c) *Influence of Electrolytes on Solubility*

The considerable influence of changes in *p*H or addition of salts on the solubility of high polymers may be explained in the following way. If a molecule contains a considerable number of groups reacting with dissolved substances with evolution of heat, the heats of reaction of all groups are added together, so that the total change in the heat of solution and hence the influence on the solubility may be considerable. Thus, the ions H^+, Li^+, Na^+, K^+, Ca^{++}, and the like, may react with the groups $-NH_2$, $-NH\cdot CO-$, $-OH$, etc., with evolution of heat. The hydroxyl ions react with carboxyl and give COO^- and water.

TABLE X

Increment, cal./mole	Factor
− 500	2
− 1,400	10
− 2,800	120
−12,000	5×10^8

It is possible to estimate by what factor the solubility of a relatively insoluble substance increases for a negative increment of the heat of solution ΔH_2 (Table X). The heat of reaction of the proton transfer:

$$-COOH + OH^- \longrightarrow -COO^- + H_2O$$

[88] H. J. Rocha, *Kolloidchem. Beihefte*, **30**, 230 (1930).
[89] E. W. J. Mardles, *Kolloid-Z.*, **49**, 4 (1929).
[90] W. Herz, *Cellulosechemie*, **15**, 95 (1934).
[91] V. Z. Daneš, *Kolloid-Z.*, **68**, 110 (1934)

amounts to ~12,000 cal. A relatively very insoluble compound containing carboxyl groups will therefore be 500 million times more soluble in a medium containing excess hydroxyl ions than in water.

(*d*) *Solubility of Crystalline High Polymers*

For a crystalline substance, the heat of solution is the sum of the heat of fusion, ΔH_f, and the heat of solution of the melted substance, ΔH_l. From equation 20 we then have:

$$\Delta H_f + \Delta H_l = -RT \ln N_2^s \tag{24}$$

It is a well known fact that, for two apolar liquids, the heat of mixing is usually very small, at any rate in comparison with the heats of fusion. It follows that the numerical value for $\Delta \bar{H}_2^s$ is almost entirely determined by the heat of fusion, since the heat of dilution can be neglected as a first approximation. For such simple cases the solubility can therefore be expressed as a function of the heat of fusion:

$$-\Delta H_2^f \approx RT \ln N_2^s$$

This has been verified experimentally in the case of naphthalene–benzene and many other systems.

We may consider, for example, the solution of a crystalline higher paraffin in a lower liquid paraffin. It is known by experiment that the heat of dilution is very small, a fact which is readily understood since a particular molecule of the solute paraffin will be completely surrounded by methylene groups whether it be in the pure state or in solution. If the solvent, *e.g.*, C_6H_{14}, is mixed with the fused substance to be dissolved, *e.g.*, $C_{20}H_{42}$, there is practically no thermal effect, so that in this case the full value of ΔH_2 can be reckoned as the heat of fusion. Now King and Garner[92] have established experimentally that for normal paraffins the heat of fusion increases in proportion to the number of methylene groups, z, the increment being approximately 610 cal. per methylene group.

We can therefore write:

$$\Delta H_f = zq \tag{25}$$

It further has been found that the melting point of normal paraffins, T_f, increases with chain length according to the empirical relationship:[93]

[92] A. M. King and W. E. Garner, *J. Chem. Soc.*, **1936**, 1372.

[93] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **20**, 1321 (1937).

$$\frac{1}{T_f} = a + \frac{b}{z}$$

where $a = 2.395 \times 10^{-3}$ and $b = 17.1 \times 10^{-3}$. If equation (21a) is now applied to the solution process, then if T' is not greatly different from T'':

$$-\ln N_2'/N_2'' = \frac{zq}{R}\left(\frac{1}{T'} - \frac{1}{T''}\right)$$

Taking the temperature as T'' and the melting point T_f as the higher temperature T', we obtain:

$$\ln N_2^s = \frac{zq}{R}\left(\frac{1}{T_f} - \frac{1}{T}\right) \tag{26}$$

and by substitution of $(a + b/z)$ for $1/T_f$:

$$\ln N_2^s = \frac{zq}{R}\left(a + \frac{b}{z} - \frac{1}{T}\right)$$

and taking the antilogarithm:

$$N_2^s = Ae^{-Bz}$$

with $A = e^{bq/R}$ and $B = -\frac{q}{R}\left(a - \frac{1}{T}\right)$.

Table XI gives the observed and calculated solubility values for two higher hydrocarbons in decalin (mol. wt. 138) solution: w signifies the

TABLE XI

Solubility of Hydrocarbons in Decalin

t, °C.	N_2^1 (calc.)	w (calc.), %	w (found), %
	$C_{34}H_{70}$		
25	8.49×10^{-3}	2.88	—
35.5	2.79×10^{-2}	8.8	7.14
40	4.56×10^{-2}	14.15	14.9
46	8.69×10^{-2}	24.7	26
	$C_{60}H_{122}$		
25	4.05×10^{-6}	0.0025	<0.01
40	7.66×10^{-5}	0.05	0.04
64.5	1.03×10^{-4}	3.3	4.09
68.5	1.26×10^{-3}	7.3	8.19
75	2.84×10^{-2}	15.2	23.4

saturation concentration in weight per cent. The agreement is quite satisfactory on the whole although by comparison with the lower homologue the values for $C_{60}H_{122}$ are not very good at higher concentrations. This is to be expected in view of the excess entropy, for the above considerations would only hold for concentrated solution if the entropy approaches the ideal value. In the above equations q must be replaced by $(q - Ts)$ for high polymers. But, since this expression is always positive and since in general the heat of fusion is much greater than the excess entropy commonly found for chain polymers, N_2^s becomes very small for the higher members unless it happens that the heat of fusion is compensated by heat being evolved in the course of solution. Crystalline high polymers will therefore be insoluble in all solvents except those which have some special affinity for particular groups in the solute. For example, at temperatures well below its melting point a higher polymeric crystalline paraffin must be practically insoluble in all solvents since no interaction between methylene groups and a solvent, whatever its nature, can give off any considerable amount of heat.

Of some importance for the solubility of high polymers is the size of the crystallites. A quantitative relationship between size of crystallite, surface tension of solid–liquid interface, and several other factors was developed in a general form by Gibbs and has been applied to the problem of solubility by Ostwald and Freundlich.[94] For spherical particles we have the relation:

$$\frac{RT}{M} \ln \frac{a_2}{a_1} = 2 \frac{\sigma}{\rho} \left(\frac{1}{r_2} - \frac{1}{r_1} \right) \tag{27}$$

where M is the molecular weight, a_2/a_1 is the ratio of the activities, and hence the solubility quotient of particles of with radii r_2 and r_1; ρ is the density and σ is the surface energy. For large crystals $1/r_1$ is negligible in comparison with $1/r_2$. If L_1 is the solubility of the larger crystallites, we obtain the equation:

$$\frac{RT}{M} \ln \frac{L_2}{L_1} = \frac{2\sigma}{\rho r_2}$$

L_2/L_1, the solubility compared with the normal solubility, therefore increases exponentially with $1/r_2$. Moreover, the larger the expression $2M\sigma/RT\rho$ becomes, the more sensitive is the solubility to changes in r. For sub-

[94] See H. Freundlich, *Kapillarchemie*. Vol. I, 4th ed., Akadem. Verlagsgesellschaft, Leipzig, 1930, page 218.

stances of very high molecular weight, therefore, the difference in solubility between large and small particles is particularly striking. A difference in solubility between small, submicroscopic crystallites and larger ones of 1000 times is not impossible for substances of high molecular weight (see also the observations on amylose, Chapter F, section VII2*e*).

(*e*) *Solid Phase Rule*

A preparation which is a mixture of high polymers exhibits properties which are often regarded as peculiar to all high polymers and have led to the formulation of the solid phase rule.[95] This peculiarity lies in the fact that the solubility appears to increase with the amount of solid, undissolved phase present. Such behavior is explained by the fact that the fractions of lower molecular weight are more readily soluble than the higher fractions, and that mixtures of crystallites of different sizes, differing in solubility, may be present. The solution may therefore be saturated with respect to molecules of high molecular weight or to larger crystals, but still unsaturated with respect to molecules of lower molecular weight or to small crystallites. If, therefore, more of the mixture is added, substance passes into solution. Moreover, the solubility of higher fractions may be considerably increased by the presence of lower fractions. An example is given by Waentig and Peschek.[96] The solubility of palmitic acid in carbon tetrachloride at 0°C. is 0.5%, while in a solution of 1% lauric acid in the same solvent, palmitic acid is twice as soluble.

In the case of truly homogeneous, well-crystallized high polymers, such as pure proteins, solubility is independent of the quantity of solid phase present.

Many commercial preparations of high polymers consist of mixtures of substances differing in solubility and are therefore partially soluble in certain solvents. In contrast to normal usage, the term solubility is frequently used to describe the proportion of soluble substance present and is expressed as a percentage of the material. Thus, "10% alcohol-soluble nitrocellulose" describes a sample containing 10% of alcohol-soluble constituents.

3. Influence of Individual Groups on Solubility

Roughly speaking, the various groups may be divided into hydrophilic groups (which are always polar) and hydrophobic groups; the hydrophilic

[95] W. Ostwald, *Kolloid-Z.*, **41**, 163 (1927); **43**, 249 (1927). A. von Buzágh, *ibid.*, **41**, 169 (1927); **43**, 215, 220 (1927).

[96] P. Waentig and G. Peschek, *Z. physik. Chem.*, **93**, 529 (1919).

groups are lipophobic, that is, are insoluble in apolar, "lipoid" solvents, while the hydrophobic groups are lipophilic. The ratio of hydrophilic to hydrophobic groups in a molecule determines its solubility (and the swelling power of the substance) in water, on the one hand, and in organic solvents on the other, since the heat of solution will be derived, approximately additively, from the increments due to the individual groups. (Chelation or steric factors may be of importance in hindering the reaction of certain groups with the solvent.)

It is therefore understandable that polymeric hydrocarbons dissolve in hydrocarbons; high polymeric olefins dissolve particularly readily, with evolution of heat, in halogenated hydrocarbons such as chloroform, which are also miscible with olefins of low molecular weight. Substances with many hydrophilic groups, such as polyacrylic acid, proteins, and polyvinyl alcohol, are soluble in water, formamide, and similar solvents; on the other hand, substances which, like the esters of cellulose, contain hydrophobic residues along with hydrophilic groups, dissolve in solvents or mixtures also containing both hydrophilic and lipophilic groups. These general points have been emphasized by Whitby,[97] Lee,[98] and Sheppard and Sweet,[99] and a large body of experimental evidence has been collected. On the other hand, the search for a relationship between solvent powers and dipole moment of the solvent appears to be hopeless, since both substances with apolar groups and those with polar groups may, in spite of profoundly different solvent powers, possess the same dipole moment. Dioxane, in which the dipole moments of the two polar ether groups exactly compensate each other, offers an example of a solvent markedly hydrophilic in character but with zero dipole moment.

4. Gelation[100]

Solutions of many polymers and of many substances of low molecular weight (such as soaps) set, under certain conditions, to form translucent gels. In spite of a high liquid content, these have considerable mechanical rigidity; on the other hand, the mobility of the ions dissolved in the gel is inappreciably affected, as is evident from conductivity measurements. This leads one to conclude that such gels consist of a wide-meshed, solid structure, in the meshes of which ions and fluid particles are free to move.

[97] G. S. Whitby, *Colloid Symposium Monograph*, **4**, 203 (1926).

[98] W. B. Lee, *J. Soc. Chem. Ind. Japan*, **49**, 226T (1931).

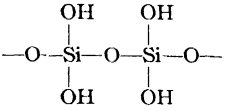
[99] S. E. Sheppard and S. S. Sweet, *J. Phys. Chem.*, **36**, 819 (1932).

[100] Review: E. Heymann, *The Sol-Gel Transformation*. Act. Scientif. 396, Hermann, Paris, 1936.

In such structures or three-dimensional meshworks the rods or threads are interlocked at particular points which can be called points of adhesion. A framework consisting of submicroscopic, mechanically interlocked crystalline needles will be much more brittle than a meshwork of flexible, threadlike molecules, where the links are formed by crystallites or chemical bonds; the latter type of framework can exhibit very high reversible deformation and behave like rubberlike bodies. If the points of adhesion can be severed, then the gel may be liquefied and is said to be *reversible.*

(*a*) *Irreversible Gelation*

Typical irreversible gelation is exhibited by silicic acid. When liberated from solutions of alkali silicates, the acid polymerizes—as is shown by the increase in viscosity of the solution. This polymerization, accompanied by the elimination of water, leads from free, orthosilicic acid, $Si(OH)_4$, to shorter and longer chain molecules of polysilicic acids:



and finally to a three-dimensional network of silicic acid.

A further example is the gel formation brought about in dilute rubber solution by sulfur chloride. Here the interlocking is brought about by the formation of sulfur cross linkages between the rubber chains. The high elasticity of these rubber gels results, as in the case of unswollen rubber, from the flexibility of the rubber chains.

Irreversible gelation is due, in both these cases, to an irreversible chemical reaction. Irreversible gelation of supersaturated solutions of biological origin will be discussed in connection with reversible processes.

(*b*) *Reversible Gelation*

The formation of the framework of the reversible gels, *e.g.*, soaps, starch, gelatin, pectin, and agar, is due to a kind of crystallization. The specific heat increases considerably in the temperature range at which gel formation or liquefaction occurs. In the course of gelatinization, therefore, heat is given off. Moreover, in many gels ultramicroscopic crystallites have been detected by x-ray interferences and numerous observations have shown that gelatinization is preceded by the formation of ultramicrons. The beginning of gelatinization, therefore, is equivalent to crystallization *i.e.*, it sets in when the saturation concentration is exceeded. The *temperature of*

gelatinization must therefore be regarded as that at which the solution is saturated.

From the relationship between the temperature of gelatinization and the concentration, the heat of gelatinization of gelatin has been found to be 21,000 cal. per mole.[101] This heat is the sum of the molecular heat of fusion of crystalline gelatin and the heat of solution of liquid gelatin. Calorimetric determination yielded a value of 12.5 cal. per gram from which a "molecular weight" of about 1700 is deduced. This figure corresponds to about 15 amino acid residues, whereas the osmotic determination indicates several hundred such residues per molecule. The "molecules" taking part in the crystallization can therefore only be segments of the chains; other segments remain amorphous or more correctly dissolved, *i.e.*, completely surrounded by solvent. Gerngross, Herrmann, and Abitz,[102] who first put forward this conception of a gelatin gel, speak of "fringed micelles." A single chain molecule runs successively through several crystallized regions, which represent, so to speak, the knots of the meshwork. Owing to the great length of the molecules, it is sufficient if only a relatively small part "crystallizes," *i.e.*, enters into more or less regular packing. This kind of crystallization is but one example of the quite general phenomenon that segments of chain molecules can behave thermodynamically as independent entities.

Transparent gels are obtained particularly easily from polymers with chain molecules of irregular constitution, *e.g.*, gelatin, pectins, amylopectin, agar, and fibrin, where a certain number of the chain segments do not fit into the lattice. Substances with chain molecules of strictly periodic constitution form gels from concentrated solution, but from dilute solution microcrystalline precipitates are deposited. In the more concentrated solutions the chain molecules are in contact at many points, so that segments of one chain can crystallize together with those of another, thus forming a gel; in dilute solution each chain behaves like a single molecule. Gels formed from chain polymers with regularly built molecules are usually opaque, an indication that the crystallites are comparatively large.

In a gel of a given concentration the thicker the bundles of chain molecules which constitute its framework the larger are the meshes and therefore its porosity. A qualitative indication of the porosity is given by the rate of flow of solvent under pressure through a cylinder of the gel fixed in a tube.[103]

[101] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **20**, 1331 (1937).

[102] O. Gerngross, K. Herrmann, and W. Abitz, *Z. physik. Chem.*, **B10**, 871 (1930); *Biochem. Z.*, **228**, 409 (1930).

[103] R. Signer, *Bull. soc. chim. France* (5), **16**, 69 (1949).

The coarseness of fibrin fibers in clots depends on the *p*H of the solution, on its ionic strength, and on temperature, as has been demonstrated by measuring the turbidity.[104]

Under the influence of certain reagents solvent is squeezed out from many gels, a phenomenon known as *syneresis*; frequently, this process is simply the consequence of progressive crystallization, *i.e.*, the formation of larger crystallites at the expense of the molecular network of the gel. In other cases, it is only the result of lack of miscibility of the two components analogous to what happens in a miscibility gap.

Crystallization is not always reversible. Such is the case for gelating viscose solutions; another example may be found in the contents of the spinning gland of the silk worm which gelates spontaneously in dilute aqueous solution. The clotting of blood also belongs to this class, the fibrin micelles forming the framework of the gel.

[104] J. D. Ferry and R. Morrison, *J. Am. Chem. Soc.*, **69**, 388 (1947).

I. ELASTICITY, VISCOSITY, AND PLASTICITY OF HIGH POLYMERS*

When a solid is subjected to a deforming stress it may either undergo elastic deformation, *i.e.*, the body resumes its original shape as soon as the stress is released, or—in the other limiting case—it may behave like a viscous liquid undergoing plastic "flow" with the development of heat of friction. Actually, the two phenomena are superimposed, for any plastic deformation, such as a flow process in a liquid, is accompanied by some reversible (elastic) change in shape; conversely, for changes of shape loosely termed elastic, heat of friction is evolved. In treating these phenomena, we shall, as we did in the preceding chapter, first establish the thermodynamic basis and then try to develop from the objectively exact thermodynamic results, a picture of the phenomena on a molecular scale.

I. ELASTICITY OF HIGH POLYMERS

1. Thermodynamics[1]

(*a*) *Introduction*

We shall be concerned with processes taking place so slowly that the heat of friction can be neglected entirely and there is a perfect heat exchange be-

* By Kurt H. Meyer, C. Weissenberg, and A. J. A. van der Wyk.

[1] Experimental researches on thermodynamics of elastomers: J. Joule, *Phil. Trans.*, **149**, 107 (1859); H. Bouasse, *Ann. faculté sci. Toulouse*, **6**, 177 (1904); K. H. Meyer, G. v. Susich, and E. Valkó, *Kolloid-Z.*, **59**, 208 (1932); W. B. Wiegand and I. W. Snyder, *Trans. Rubber Inst.*, **10**, 234 (1934); K. H. Meyer and C. Ferri, *Helv. Chim. Acta*, **18**, 570 (1935); V. Hauk and W. Neumann, *Sitzber. Akad. Wiss. Wien, Math. naturw. Klasse* (IIb), **1938**, 147; R. L. Anthony, R. J. Caston, and E. Guth, *J. Phys. Chem.*, **46**, 826 (1942); L. A. Wood and E. L. Roth, *J. Applied Phys.*, **15**, 781 (1944); K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **29**, 1842 (1946); A. R. Mayer and C. G. Boissonnas, *ibid.*, **31**, 1514 (1948) (rubber); F. L. Roth and L. A. Wood, *J. Applied Phys.*, **18**, 749 (1947) (butadiene-styrene copolymer); K. H. Meyer, W. Lotmar, and G. W. Pankow, *Helv. Chim. Acta*, **19**, 930 (1936) (polyphosphonitrilic chloride); K. H. Meyer and C. Ferri, *Arch. ges. Physiol. Pflügers*, **238**, 78 (1936) (tendon and elastic ligament); K. H.

(*footnote continued on next page*)

tween the sample and a constant-temperature bath by which the sample is surrounded. Under these conditions, elastic processes can be considered as being reversible and isothermal; for such processes:

$$\Delta E = \Delta A + T\Delta S$$

where ΔE is the total energy change, which equals the sum of the mechanical work, ΔA, and the heat exchange or entropy term, $T\Delta S$.

An elastic body which has been strained may in recoiling produce a certain amount of mechanical, elastic, work, ΔA_{el}; the sample is said to have acquired the free energy, ΔA_{el}. If its volume V changes at the same time, it also does a certain amount of work against the external pressure P, usually the atmospheric pressure. Thus:

$$\Delta E = \Delta A_{el} + P\Delta V + T\Delta S$$

It is legitimate to neglect the work against the atmospheric pressure which is very small compared with the work done upon the elastic body. We may then write, approximately:

$$\Delta A_{el} = \Delta E - T\Delta S \tag{1}$$

In other words, the free energy which gives rise to the elastic force consists of two parts, an energy and an entropy term; both the magnitude and the sign of these two must be established for every particular case.

As a rule, both terms do appear, having either the same or opposite signs. We may, however, according to (1), distinguish two ideal cases, which are of importance because they may be found experimentally. The first case occurs when ΔS disappears. Then the changes of elastic free energy on deformation or recoil equal the changes of the total energy content; in other words, the total work done on the sample is stored as potential energy. In this case no heat exchange between the sample and the bath takes place during deformation. In the second case, where ΔE disappears, the increase of the free energy acquired by the sample during deformation equals the increase of the product of temperature and entropy of the sample. Conversely, when the sample recoils, the decrease of the free

Meyer and W. Lotmar, *Helv. Chim. Acta*, **19**, 78 (1936) (cellulose and cellulose ester); K. H. Meyer and L. E. R. Picken, *Proc. Roy. Soc. London*, **B124**, 29 (1937) (muscle); L. Misch and L. E. R. Picken, *Z. physik. Chem.*, **B36**, 378 (1937) (polyvinyl acetate); L. E. R. Picken, *J. chim. phys.*, **34**, 764 (1937) (elastoidin); E. Wöhlisch, *Kolloid-Z.*, **96**, 261 (1941) (muscle); H. J. Woods, *Nature*, **157**, 229 (1946); H. B. Bull, *J. Am. Chem. Soc.*, **67**, 533 (1945); K. H. Meyer and C. Haselbach, *Nature*, **164**, 33 (1949) (keratin).

energy equals the increase of the entropy product. Here the totality of the energy expended on deformation is given off by the sample as heat, and taken up again from the bath on recoil. But this is by no means a limiting case. The amounts of heat exchanged may be considerably larger than the free energy changes, *i.e.*, ΔA_{el} and ΔE may have opposite signs. Instances of such behavior have been observed and will be discussed at a later stage.

(*b*) *Thermodynamic Analysis*

The object of thermodynamic analysis is the quantitative determination of the two terms ΔE and $T\Delta S$. In most experiments direct calorimetric measurement of the amount of heat evolved during deformation and recoil cannot be used, partly because the thermal effects are much too small. A better method can be found if we consider, not processes at constant temperature, but those at constant deformation. By deformation, Δl, we will understand the change in length of the specimen if it is submitted to extension or unilateral compression, and for shearing, the distance of translation; for torsion Δl is the angle of rotation of the mobile part of the apparatus which transmits the force or torque. If K signifies the acting force or torque we have per definition:

$$\frac{\partial \Delta A_{el}}{\partial \Delta l} = K \tag{2}$$

ΔA_{el} is to be taken positive when deformation is imposed on the specimen and negative in recoil.

Let us suppose that an elastic object, *e.g.*, a rubber strip or a steel spring, has been fixed in a dynamometer in such a way that the deformation can be kept constant while the temperature can be varied. In general, the force will not be independent of the temperature, and we can measure its variation at constant length of the sample. According to equation (2) this variation of K may be written as:

$$\left(\frac{\partial K}{\partial T}\right)_{\Delta l} = \frac{\partial^2 A_{el}}{\partial \Delta l \partial T} \tag{3}$$

The right-hand side can be interpreted as the derivative with respect to Δl of the temperature coefficient of the free energy, that is:

$$\frac{\partial}{\partial \Delta l}\left(\frac{\partial \Delta A_{el}}{\partial T}\right)_{\Delta l}$$

According to a general thermodynamic relationship, the temperature coefficient of the free energy equals the negative value of the entropy and thus:

$$\frac{\partial}{\partial \Delta l}\left(\frac{\partial \Delta A_{el}}{\partial T}\right)_{\Delta l} = \frac{\partial}{\partial \Delta l}(-\Delta S) = -\left(\frac{\partial \Delta S}{\partial \Delta l}\right)_T$$

and by comparison with equation (3) we find that:

$$\left(\frac{\partial K}{\partial T}\right)_{\Delta l} = -\left(\frac{\partial \Delta S}{\partial \Delta l}\right)_T \tag{4}$$

If we now differentiate equation (1) with respect to Δl:

$$\left(\frac{\partial \Delta A_{el}}{\partial \Delta l}\right)_T = \left(\frac{\partial \Delta E}{\Delta \partial l}\right)_T - T\left(\frac{\partial \Delta S}{\partial \Delta l}\right)_T$$

and introduce equations (2) and (4), then:

$$K = \left(\frac{\partial \Delta E}{\partial \Delta l}\right)_T + T\left(\frac{\partial K}{\partial T}\right)_{\Delta l} \tag{5}$$

Since K and T may be directly measured and $\partial K/\partial T$ represents the slope of a K, T curve, we can now calculate, by equations (4) and (5), the two derivatives of ΔE and ΔS. It is at once apparent from equation (4) that the slope at any point of a K, T curve is equivalent to the direction of change of ΔS on recoil. The tangent to the K, T curve at this point inter-

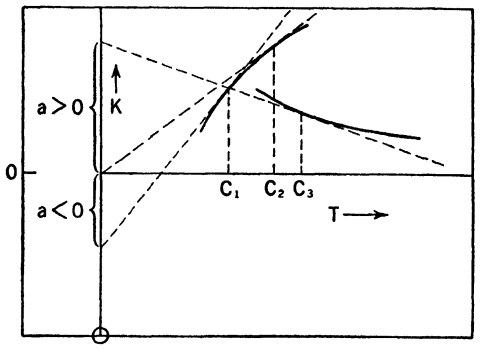


Fig. 1. Graphic evaluation of (5).

cepts on the K axis a length which is equivalent to the change of ΔE on deformation (see intercept a in Fig. 1). The two ideal cases discussed above now correspond to the following types of K, T curves.

If, on deformation, the energy is not affected ($\partial \Delta E/\partial \Delta l = 0$) the tangent of the K,T curve will pass through the origin.

If the entropy remains unchanged ($\partial \Delta S/\partial \Delta l = 0$), the K, T curve is horizontal and the tangent intercepts a positive length of the K axis, *i.e.*, the internal energy increases on deformation. Clearly, a negative intercept can only occur if the slope of the curve is positive, *i.e.*, if K increases with T. The energy, therefore, can only decrease on deformation if the entropy decreases simultaneously and then only if the decrease of the product $T\Delta S$ is larger than that of ΔE (Fig. 1). This means that under such circumstances the amount of heat evolved is larger than the work done on the sample, a possibility which we had already foreseen in sect. II*a*. The excess of heat must be considered as heat of crystallization. As we shall see, the crystallization due to deformation can be demonstrated by x-rays.

It has been shown experimentally that K, T curves often are straight lines over a wide range of temperature. They may then be represented by:

$$K = a + bT$$

where the constant a is independent of the temperature.

By comparison with equations (4) and (5) it is seen that a represents $(\partial \Delta E/\partial \Delta l)_T$. Thus, over the straight portion of a K, T curve, this quantity must be independent of the temperature, or in analytical notation:

$$\frac{\partial}{\partial T}\left(\frac{\partial \Delta E}{\partial \Delta l}\right) = 0$$

and therefore:

$$\frac{\partial^2 \Delta E}{\partial T \partial l} = \frac{\partial}{\partial l}\left(\frac{\partial \Delta E}{\partial T}\right)_{\Delta l} = 0$$

The term in brackets is the specific heat at constant deformation. We find, therefore that if K is a linear function of T the specific heat of the sample must be independent of the deformation.[1a] The following observation is in accordance with the preceding: in regions where crystallization occurs on stretching, the K, T curve is never found to be linear.

(*c*) *Selection of Deformation*

Although the work produced by the change of volume may be neglected in the preceding discussion, it is not justifiable to neglect the variations of

[1a] W. W. Vogt, in C. C. Davis and J. T. Blake, *The Chemistry and Technology of Rubber*, Reinhold, New York, 1937. C. G. Boissonnnas, *Ind. Eng. Chem.*, **31**, 761 (1939).

the volume when we want to interpret the entropy in terms of molecular statistics by means of the Boltzmann equation:

$$S = k \ln W$$

The thermodynamic probability W depends on the number of possible arrangements of the atoms or molecules and on the number of possible movements of these which are accommodated by the so-called *free volume*. A variation of S may therefore be due to changes in free volume or in molecular arrangements, or to both. Now, a very small change in the volume of a liquid is known to produce a very marked variation of the entropy. As in this case a molecular rearrangement is not involved, the cause, in terms of molecular statistics, must be sought in a variation of the free volume only.

The variation of the entropy with the volume by isotropic compression may be readily obtained by the relation:

$$-\left(\frac{\partial S}{\partial V}\right)_T = \left(\frac{\partial V}{\partial T}\right)_P \Big/ \left(\frac{\partial V}{\partial P}\right)_T \tag{6}$$

from the coefficients of expansion and compressibility. For the usual organic liquids as well as for rubberlike substances it is of the order of 10^7 ergs per degree and per cubic centimeter. At 300°K. this corresponds to a variation of the entropy term of 3.10^9 ergs per cc., *i.e.*, 3000 times the value of the work done by the atmospheric pressure which amounts to about 10^6 ergs per cc. Thus, even if the change in volume during deformation is so small that the work resulting from it is entirely negligible, this does not mean that its influence on the entropy product can also be neglected.

It appears, therefore, that deformations should be selected which are not accompanied by any change in volume. This condition does not hold for the usually studied extension or unilateral compression, even for exceedingly small deformations. If P_l is the unilateral pressure, it may be readily seen that at vanishing deformation:

$$\left(\frac{\partial V}{\partial P_l}\right)_T \longrightarrow \frac{1}{3}\left(\frac{\partial V}{\partial P}\right)_T$$

where P is isotropic pressure. This variation of the volume does not therefore vanish even at the smallest extension. The situation is quite different if shear, torsion, or flexion is applied, by which one part of the specimen is slightly compressed, the other part, however, slightly extended. The volume of the sample thus remains practically constant. It may then be assumed that under such conditions the results will be simpler and easier to interpret.

2. Experimental Results

(a) *Shear and Torsion*

If a coiled spring, made of steel wire, is extended, the deformation of the wire itself is a torsion. Experiments have shown that the restoring force of a steel wire spiral kept at constant elongation is practically independent of temperature. Referring to equation (4) this means that the entropy does

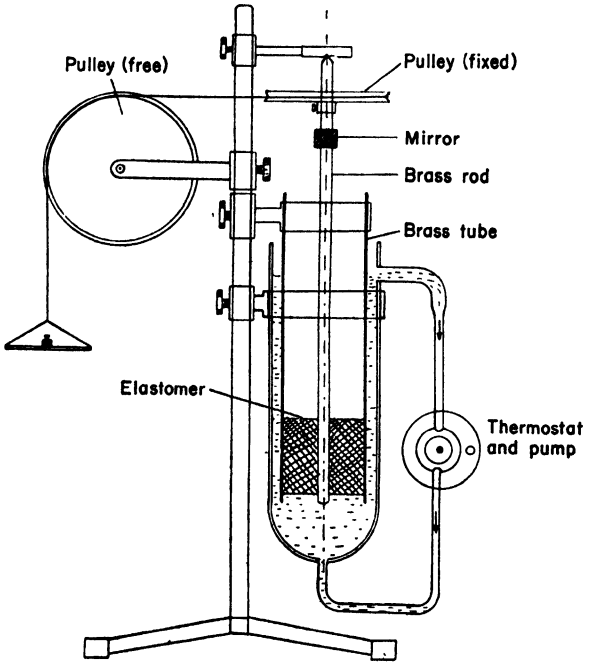


Fig. 2. Apparatus for investigation of thermoelasticity on shear (Meyer and van der Wyk[2]).

not vary under this deformation. The restoring force, therefore, must be ascribed to interatomic forces of repulsion and attraction, no atomic rearrangement being involved. The first of the ideal cases is thus realized. On the other hand, the second case has been found with vulcanized soft rubber under shear. Shear may be applied by the use of an apparatus described by Meyer and van der Wyk[2] (Fig. 2).

The sample is given the form of a hollow cylinder with thick walls. The outer curved surface adheres to the inside of a brass tube, the inner surface to that of a cylindrical rod.

[2] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **29**, 1842 (1946).

On this rod acts a torque applied by means of a weight which is fixed by a wire to a pulley on the rod. The torque is given by the product of the weight and the diameter of the pulley. The angle of rotation of the rod is measured by a mirror and scale in a small telescope. This kind of deformation could be called rotating shear; as in torsion, the deformation is given by an angle and the quantity K has the dimensions of a moment of torque.

Experiments in which the deformations were not large showed that the term in E of equation (5) disappears. The elastic force therefore is due exclusively to entropy effects. As the free volume is not appreciably affected, the variation of the entropy by this deformation must result from a molecular rearrangement. In other words, the atomic or molecular arrangement must become less random by the deformation and more random by recoil, no forces of attraction or repulsion being involved.

The increased regularity after deformation can be explained, after the theory given by Meyer, Susich, and Valkó,[1] by the stretching and more parallel orientation of the long, flexible chain molecules. The restoring force then results because the thermal movements of the segments tend to restore the original random arrangement.

While in the spring, as in all typical solids, elastic restoring forces are a result of interatomic attraction or repulsion, the elastic force in rubberlike substances (as in gases) is finally due to thermal movements. We shall call this behavior ideally rubber-elastic; according to Copeland and Mooney,[3] rubber, polychloroprene, butyl rubber, and Thiokol exhibit ideally rubber-elastic behavior on torsion and so does hair keratin swollen in aqueous thioglycolic acid (Meyer and Haselbach[1]).

(*b*) *Unilateral Compression and Extension*

If a sample of vulcanized soft rubber deformed by a unilateral compression is heated at constant length, the restoring force increases. Now, it may be shown that equation (4) remains exact, even if the volume of the sample varies by deformation. Thus, by equation (4):

$$\left(\frac{\partial K}{\partial T}\right)_{\Delta l} = -\left(\frac{\partial \Delta S}{\partial \Delta l}\right)_{T}$$

an increase of the force with increasing temperature means a decrease of the entropy with increasing deformation.

It is logical to attribute to Δl a positive sign on elongation and a negative one on unilateral compression; the stress must then have a positive sign on elongation and a negative one on compression. As the signs of both K and Δl are reversed equation (4) may be applied in its actual form.

[3] L. E. Copeland and M. Mooney, *J. Applied Phys.*, **19**, 450 (1948).

This decrease of entropy is due to a decrease of the volume, *i.e.*, of the freedom of molecular movement, and also to deformation of the chain molecules, though this latter effect should be quite small at small deformations. By unilateral compression both entropy and internal energy decrease, and, therefore, the amount of heat given off must be greater than the applied energy. Adiabatic compression would lead to an increase of the temperature of the sample; the same thing happens in a liquid on isotropic compression.

The most widely and extensively studied deformation is extension; many different kinds of rubberlike substances have been investigated by this method. Below the softening point, *i.e.*, if the substances remain hard and brittle, their behavior is not essentially different from that of

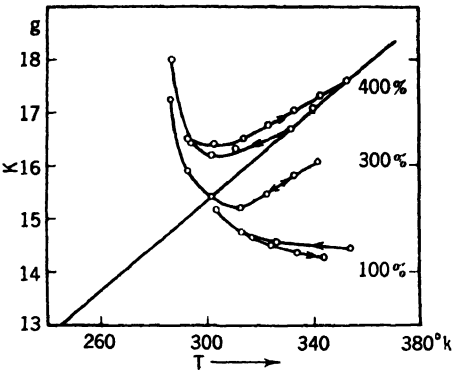


Fig. 3. Force–temperature curves for polyvinyl acetate at different elongations (Misch and Picken).

ordinary solids. The extensibility remains small and the entropy increases on extension as a result of the increase of the "free" volume. The typical rubberlike properties only appear above the softening point (Fig. 3).

Experiments with vulcanized soft rubber in Polanyi's dynamometer[4] showed that for extensions below 10% the elastic restoring force decreases if the temperature is raised: here $\partial K/\partial T$ is negative. According to equation (4) this means that the entropy increases on extension.

What is the cause of the entropy increase? The stretching of the chain molecules by the extension must provoke a decrease but this is more than compensated by the entropy increase due to volume increase. A volume increase has been observed, although at slightly higher extensions. Accord-

[4] M. Polanyi, *Z. tech. Physik*, 6, 124 (1925).

ing to Gee,[5] it amounts to only 0.03% at an extension of 80%, but it is quite large enough to account for the over-all increase of the entropy.

In the range of small extensions, the entropy thus increases on extension and decreases on recoil; therefore (compare Fig. 1, curve C_3) the internal energy must vary in the same direction. Consequently, the elastic restoring force is due to the variations of the internal energy. Up to 10% extension, the slopes of the K, T curves become less and less negative; at 10% the curve becomes horizontal. At higher extensions, the slopes have gradually increasing positive values. According to equation (4) this means that the entropy now decreases on extension and increases on recoil. Exactly as in the case of shear, these variations of the entropy can be explained ac-

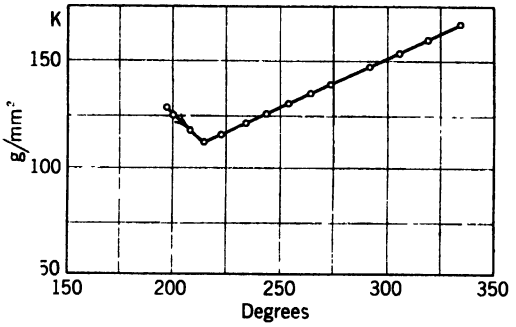


Fig. 4. Force–temperature curve of moderately vulcanized rubber (8% S) at 350% elongation. Temperature on the absolute scale (Meyer and Ferri[1]).

cording to the theory mentioned above as a consequence of the straightening, uncoiling, and orientation of the chain molecules when the sample is extended.

These phenomena are by no means a feature of rubber only. Thus, figure 3 shows the K, T curves of polyvinyl acetate which also illustrate what has been said above.

If rubber vulcanized with 8% sulfur is stretched 200 to 400%, the restoring force is found to be very nearly proportional to the absolute temperature, as long as the latter is above the softening point (see Fig. 4). The internal energy therefore remains constant on deformation and the variations of the free energy are equal to the variations of the product $T\Delta S$

[5] G. Gee, *Trans. Faraday Soc.*, **42**, 585 (1946).

as in the ideal gas. This shows that within the range of medium extensions the volume increase on extension and the associated entropy increase vanish. It appears that once the atoms have been displaced to a certain extent from their potential wells, the attractive forces increase so rapidly that no appreciably further displacement at higher extension can occur. If this type of rubber is subjected to still higher extensions (600–800%) the slope of the K, T curve decreases and finally becomes negative again.

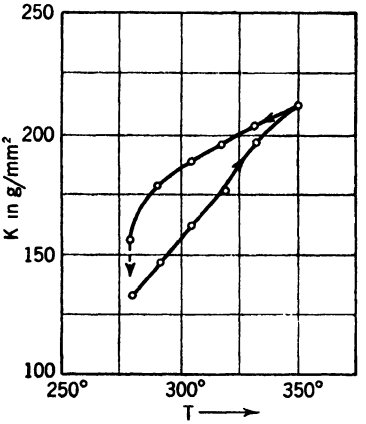


Fig. 5. Force–temperature curve of slightly vulcanized rubber showing hysteresis (Meyer and Ferri[1]).

Rubber in a completely stretched condition behaves like any ordinary solid. An extension of the already completely oriented chain molecules results in a deformation of the angles of valence, in the same way as in a cellulose fiber (see Chap. E, sect. IV2).

Besides volume increase and orientation of the chain molecules a third phenomenon appears in the case of regularly built elastomers, that is, crystallization. Usually this does not set in below an extension of 100–200%.[6] In every case for which it can be detected with x-rays, the K, T curves show an evolution of latent heat on extension and absorption on recoil. At the same time characteristic effects of delay in crystallization are observed. If slightly vulcanized rubber is stretched and then warmed, the crystalline regions melt. That is why the slope of the lower curve in figure 5 appears very steep. If this rubber is now cooled, it behaves first of

[6] Compare A. J. Wildschut, *J. Applied Phys.*, **17**, 51 (1946). L. A. Wood and N. Bekkedahl, *J. Research, Natl. Bur. Standards*, **36**, 489 (1946). J. M Goppel, *Applied Sci. Research*, **A1**, 3 (1947).

all as an amorphous (rubberlike) body and the tangent to the first portion of the upper curve passes through the origin. Gradually, however, crystallization sets in which can be recognized by the appearance and increase in intensity of the spots on an x-ray diagram, as well as by the reduction of the restoring force with time if the temperature is kept constant.

3. Moduli of Elasticity

(a) *Static Modulus*

Rubberlike substances are appreciably deformed by forces much smaller than those required to perform analogous deformations in ordinary solids.

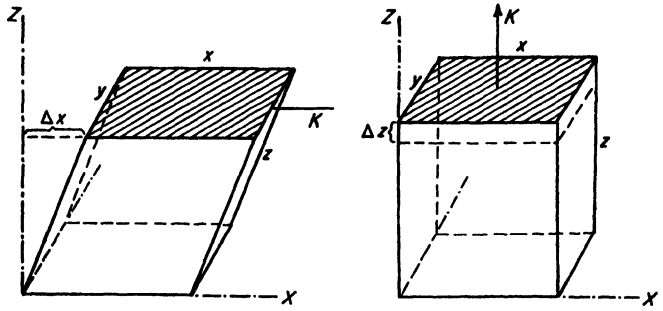


Fig. 6. Shear and extension; the force K is supposed to act uniformly over the surface xy.

A practical measure of the stiffness is the *modulus of elasticity, i.e.,* the quotient of the force acting per unit surface or *tension* and the resulting displacement per unit length or *deformation*. The dimensions of a modulus of elasticity ϵ are:

$$\epsilon = ml^{-1}t^{-2}$$

It is usually expressed in dynes per sq. cm. or equivalent units. Its value depends not only on the material but also on the kind of deformation to which it is subjected.

For shear in the direction of the x axis, the shearing tension P (Fig. 6) is given by:

$$P = K/xy$$

and the deformation S:

$$S = \Delta x/z$$

For an extension in the direction of the z axis, we have again:

$$P = K/xy$$

but now the deformation is given by:

$$S = \Delta z/z$$

The modulus of shear therefore is:

$$\epsilon_s = \frac{P}{S} = \frac{K}{\Delta x}\,\frac{z}{xy}$$

and the modulus of stretch (extension):

$$\epsilon = \frac{P}{S} = \frac{K}{\Delta z}\,\frac{z}{xy}$$

Thus, according to the kind of deformation, the shear or torsion modulus is to be distinguished from the stretch or Young's modulus; for amorphous isotropic substances at vanishing deformations, it can be shown, that:

$$\text{Shearing modulus} = \text{torsion modulus} = \frac{1}{2(1+\mu)} \times \text{extension modulus}$$

where μ stands for Poisson's number, *i.e.*, the ratio of contraction in a direction perpendicular to the deformation, to the increase in length at the same extension. If on extension the volume of the specimen is not affected, a condition which is nearly satisfied with rubberlike substances, then: $\mu = 0.5$ and therefore $\epsilon_s = \epsilon/3$.

At small deformations, ϵ amounts to 10^7 dynes per sq. cm. for rubberlike substances (compared with 2.10^{12}, which is the value for steel). Especially for rubberlike substances, the value of ϵ does not only depend on the material and the kind of deformation but also, and quite strongly, on the magnitude of the deformation to which it is subjected. Curve *I* in Figure 7 shows a force–extension curve[7] obtained by very slow deformation of a rubber vulcanized with 7% sulfur. Curve *II* refers to the same experiment but the force has been replaced by the tension, obtained by division of the actual force and the actual section, instead of the initial section of the sample. It shows that Hooke's law in the form:

$$P = \epsilon \frac{\Delta l}{l_0}$$

[7] Obtained by C. Ferri.

is approximately verified up to about 150% extension, whereas the classical form of this law:

$$K = \epsilon \frac{\Delta l}{l_0}$$

does not apply. For this reason it seems preferable to use as a more general definition of ϵ the differential quotient:

$$\epsilon = l_0 \frac{dP}{dl}$$

rather than the more frequently used quotient $l_0(dK/dl)$ which represents the slope of stress–strain curves, obtained with the usual testing apparatus.

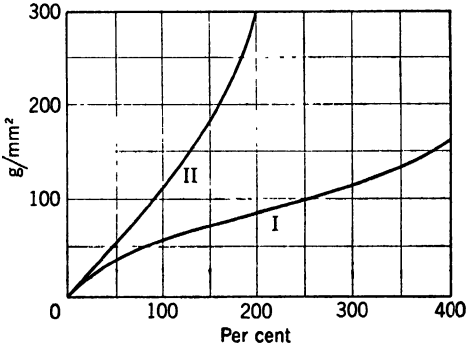


Fig. 7. Stress–strain curves of moderately vulcanized rubber. In I the force is referred to the initial cross-sectional area; in II the force is referred to the cross-sectional area at each point.

Curve *II* shows that ϵ increases first slowly, and then more and more rapidly with increasing deformation. It attains values 500 times as great as the initial modulus. The most rapid increase takes place shortly before the breaking point is attained, *i.e.*, at an extension of about 700% for this kind of rubber. Other elastomers show analogous behavior. The strong increase of ϵ corresponds with the fact that stretched rubber is a material which, because it is strongly oriented, is very different from isotropic, unstretched rubber.

It is quite remarkable that the moduli of stretch for small extensions are of the same order of magnitude for all kinds of elastomers.[7a] See Table I.

[7a] K. H. Meyer and A. J. A. van der Wyk, *J. Polymer. Sci.*, 1, 49 (1946).

Flory[8] made extensive investigations on the influence of the molecular weight (as determined osmotically before vulcanization), M, and the degree of cross linking of so-called butyl rubber (a copolymer from isobutene

TABLE I

Mean Elastic Modulus of Extension at 10–50% Extension

Elastomer	Modulus, dynes/cm.^{2}, $\times 10^{-7}$
Film of Hevea latex (mol. wt. = 3–5 $\times$ 10^6)	1.2[a]
Slightly vulcanized rubber (mol. wt. = ∞), 2% S	1.2[b]
Somewhat more highly vulcanized rubber (mol. wt. = ∞), 7% S	1.1[b,c]
Slightly degraded rubber (Feuille anglaise) (mol. wt. = 1.3 $\times$ 10^6)	0.7[d]
Buna 85 (butadiene-sodium polymer) vulcanized, 7% S	1.2[d]
Perbunan (butadiene-acrylonitrile copolymer) vulcanized	2.4[d]
Neoprene, vulcanized, 9% MgO	2.4[e]
Polyisobutylene, nonvulcanized	0.8[d]
Polyphosphonitrilic chloride	2.0[f]
Elastic sulfur	0.6[d,g]
GR-S (butadiene-styrene copolymer) vulcanized, 2% S	1.2[h]
Feather keratin (dissolved and spun)	2.7[i]
Fibrils of muscle (70% water) calculated for the dry protein	0.8[j]
Elastic fiber from ligament of ox	1.4[d]

[a] W. C. van Geel and J. G. Eymers, *Z. physik. Chem.*, **B3**, 240 (1929). L. S. Ornstein, J. G. Eymers, and G. Wouda, *Verhandel. Koninkl. Nederland. Akad. Wetenschap.*, **1932**, 1235.

[b] K. H. Meyer and C. Ferri, *Helv. Chim. Acta*, **18**, 570 (1935).

[c] R. Weise, *Kautschuk*, **8**, 106 (1932).

[d] K. H. Meyer, *unpublished work*.

[e] W. J. S. Naunton, *Synthetic Rubber*. Methuen, London, 1937, page 81 (from curves).

[f] K. H. Meyer, W. Lotmar, and G. W. Pankow, *Helv. Chim. Acta*, **19**, 930 (1936).

[g] J. D. Strong, *J. Phys. Chem.*, **32**, 1225 (1928).

[h] F. L. Roth and L. A. Wood, *J. Applied Phys.*, **15**, 749 (1944).

[i] H. F. Lundgren, A. M. Stein, V. M. Koorn, and R. A. O'Connell, *J. Phys. & Colloid Chem.*, **52**, 180 (1948).

[j] F. J. M. Sichel, *J. Cellular Comp. Physiol.*, **5**, 2 (1934).

and a small proportion of isoprene). He first discusses the influence of M^*, *i.e.*, the molecular weight of the part of the chain between two successive cross linkings, which may be calculated from the degree of cross linking. His experiments show that the Young's modulus does not vary more than 14% if M^* increases from 20,000 to 35,000; Flory's curves,

[8] P. J. Flory, *Ind. Eng. Chem.*, **38**, 417 (1946).

however, admit the conclusion that the differences in the moduli at very small deformation are even less.

He further discusses the influence of the "free ends" of the cross-linked chain molecules. These ends are able to slip back to the original disoriented condition shortly after deformation and therefore do not contribute to the restoring force exerted after equilibrium has been established. The diagram in figure 8 illustrates this conception. If in two different samples the degree of cross linking (number of cross linkings per gram of material) is the same, the concentration of free ends will be smaller if the

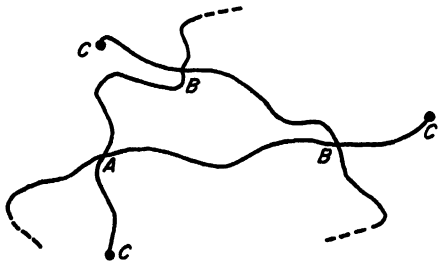


Fig. 8. Schematic representation of part of the network (after Flory); AB is the portion of the chain between cross linkings; the molecular weight is M; BC is the "free" end of the chain.

molecular weight of the material before vulcanization is higher. Flory deduces from statistical considerations, for a network as represented in the diagram:

$$K = RT\frac{\rho}{M^*}\left(1 - \frac{2M^*}{M}\right)\left(\frac{l}{l_0} - \frac{l_0^2}{l^2}\right) = RT\rho\left(\frac{1}{M^*} - \frac{2}{M}\right)\left(\frac{l}{l_0} - \frac{l_0^2}{l^2}\right)$$

where ρ is the density, l_0 the length of the sample before stretching, and l the length after stretching. In this equation the factor $\left(1 - \frac{2M^*}{M}\right)$ should account for the influence of the free ends; it should only be valid if $M \gg M^*$.

Although the experimental results are not in very good agreement with the calculations, the explanation of the variation of restoring force with molecular weight M as a result of the presence of free ends should hold qualitatively. Thus, the low modulus of slightly degraded rubber results from the fact that parts of the chains (free ends as well as broken chain

molecules) can easily slip back to the unoriented condition and thus the restoring force is not produced by the totality of all segments.

Admixtures often have a pronounced influence on the modulus and its variation with the degree of extension. Swelling agents lower, and strong vulcanization—and also addition of carbon black- increase the modulus especially at higher elongation; at the same time, the extension at the breaking point is diminished. The stiffening resulting from increased vulcanization, *i.e.*, from increase of the number of cross links is readily understood, but the causes of the stiffening obtained with carbon black have not yet been completely elucidated. The influence of aging on the modulus may vary. The moduli of copolymers of butadiene and styrene or acrylonitrile, or also of neoprene increase, while those of vulcanized butyl rubber decrease; natural rubber at first softens and afterward hardens. These changes can be explained[9] by simultaneous chain scission and cross-linking reactions, both induced by free oxygen. Depending on which of the two is faster, various rubbers soften or harden as a result. The influence of aging also depends markedly on the temperature as the velocity of the two types of reaction do not have the same temperature coefficients.

(*b*) *Dynamic Elasticity Modulus*

If one determines the elastic modulus for rapid deformations it will be found to be larger than the statically determined quantity. This "dynamic" modulus is best determined by means of an apparatus in which a vibrating plate is attached to the rubber. The modulus may be determined from the frequency, amplitude, and applied force. For a soft rubber specimen Kosten[10] found ϵ (dynamic) $= 2.7 \times 10^7$ dynes per sq. cm. at 4–20 cycles per second, while the same specimen gave $\epsilon = 1.2 \times 10^7$ dynes per sq. cm. by the static method. Thus, the tension measured after about 0.1 second diminishes rapidly to one half of its former value. We must assume that the deforming force is transmitted to a particular segment, not only through isolated points, *i.e.*, the cross links, but also, as in a liquid, through all its neighbors. By this means the chain molecules are at first constrained into a slightly folded configuration from which kinks are later smoothed out by sliding, giving rise to an increase of entropy and decrease of tension (Fig. 9).

During the sliding of the chain molecules some part of the elastic force

[9] R. D. Andrews and A. V. Tobolsky, *J. Applied Phys.*, **17**, 352 (1946).

[10] C. W. Kosten, *in Proceedings of the Rubber Technology Conference, 1938, London*, Heffer, Cambridge, 1938, p. 987. See also special issue of *J. Applied Phys.*, June, 1949.

produced during the initial rapid deformation persists while the remainder disappears. This process is called relaxation and goes faster the lower the internal friction (viscosity). With increasing temperature the latter generally decreases so that equilibrium is reached more rapidly and the dynamic modulus, and also its difference from the static modulus, decreases. Moreover, the velocity with which the final force appears varies greatly for different substances; for plastic sulfur it is strikingly small. Similar behavior is shown by polystyrene[11] and other elastomers at suitable temperatures.

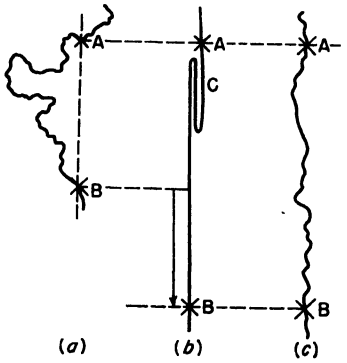


Fig. 9. Chain in unstretched condition (*a*); chain after rapid stretching (*b*); same chain partially relaxed by slipping of the loop at C (*c*).

The connection between elasticity, relaxation, and viscosity will be treated in more detail in the following sections.

(*c*) *Quantitative Relations*

Several authors have attempted to apply statistical mechanics to the problem of the deformation of an ideal rubberlike substance. In most of these studies an assumption is made which in our opinion does not correspond to the facts. It is assumed that the deforming force only acts at certain quite definite points in the chain molecule. In uncross-linked chain polymers it is supposed to act only on the ends of the molecules, in cross-linked molecules, only at the bridging links themselves which unite two chains. In some papers certain *points of entanglement* are assumed to be bridging points; in other cases a special network is postulated. Other

[11] F. H. Müller, *Kolloid-Z.*, **95**, 138 (1941).

analyses start from the assumption that the molecules are spherical in the resting condition and become cylindrical or ellipsoidal on deformation. The entropy change is calculated from the imposed form (cylinder or ellipsoid) which is less probable than the spherical form. Furthermore, all these attempts assume, expressly or implicitly, that the chain molecules in the rubber phase behave exactly as the molecules of a very dilute gas. But this assumption is not compatible with certain facts. We know, for example, that the number of possible configurations of a rubber molecule in a dilute, athermal solution, *e.g.*, in toluene, is much greater than in undissolved, amorphous rubber.[12] It may certainly be assumed, therefore, that the number in the gaseous state would be even greater than in a solution, so that one cannot accept the gaseous state as the basis of calculations affecting the rubberlike state.

With these two hypotheses, and using classical statistics, several authors have tried to compute the restoring force of deformed rubber. Depending also on different additional assumptions made, the expressions obtained differ rather widely. Table II contains a number of such expressions.

Furthermore, Huggins[13] obtained expressions for stress-strain curves for five different models; other relationships have been put forward by Green and Tobolsky,[14] and by Kubo.[15]

It may be seen that in all these equations, the restoring force depends much more on M or M^* than it should according to experiment (Table I). Only the equations of Wall, Flory, and Treloar allow for strong curvature of the stress–strain curves at small extensions. The general trend of this curve, however, is not generally corresponding to the factor $\left(\frac{l}{l_0} - \frac{l_0^2}{l^2}\right)$, as shown by Flory himself for butyl rubber, or for vulcanized rubber by Gee.[16] According to this author, however, it does agree with observed stress–strain curves from strongly swollen vulcanized rubber. This seems to indicate that this highly "loosened" network behaves much more like the model of linked chain molecules in the gaseous state, on which all calculations are based. In the swollen state the segments can move much more freely than in the bulk rubber and the deforming force is transmitted from chain to chain by the cross linkings exclusively.

[12] K. H. Meyer and A. J. A. van der Wyk, *Helv. Chim. Acta*, **27**, 845 (1944).
[13] M. L. Huggins, *J. Polymer Chem.*, **1**, 1 (1946).
[14] M. S. Green and A. V. Tobolsky, *J. Chem. Phys.*, **14**, 80 (1946).
[15] R. Kubo, *J. Colloid Sci.*, **2**, 527 (1947).
[16] G. Gee, *Trans. Faraday Soc.*, **42**, 585 (1946).

TABLE II. Equations for Elasticity of Elastomers Based on Models

M = molecular weight of the polymer before vulcanization, M^* that of the portion of the chain between the cross links; m that of the monomer residue of length b; l/l_0 = relative extension; ρ is the density, k Boltzmann's constant, K any constant, R the gas constant, T the absolute temperature, ϵ Young's modulus, F, force/initial section.

Author	Relationship	Remarks
Guth and Mark[a] (1934)	$F = KN \cdot kT \cdot \frac{L}{Zb^2}$	N = number of chains in the specimen; L = mean distance between ends of the chains; Z = number of these in one chain; valid for small extensions
Guth[b] (1941)	$\epsilon = \frac{3gkT}{(M^*/m)b\cos\vartheta/2}\left[1 + 2\left(\frac{l_0}{l}\right)^3\right]$	g = number of chains in a section normal to the extension; ϑ = valence angle (109°36′)
James and Guth[c] (1943)	$F = KT\left[\frac{l}{l_0} - \frac{1 + \alpha(T - T_0)}{l^2/l_0^2}\right]$	α = coefficient of thermal expansion of rubber, which is thought of as a cube of unit side at T_0 degrees; K is a characteristic constant for the network: $K = 3Nk/zb^2$, in which N is the number of chains active in the direction of extension and z the number of elements per chain
Pelzer[d] (1938)	$\epsilon = B\sqrt{2}\,\frac{kT}{bZ^{7/8}}\left(fN\frac{\rho}{m}\right)^{2/3}$	N = Avogadro's number; f = number of freely rotating bonds in monomeric residue; B = fraction of number of chains per sq. cm. acting along the direction of stretch; Z = number of residues between successive cross links
W. Kuhn[e] (1936)	$\epsilon = 7RT\frac{\rho}{M}$	Linear polymers without cross linkages
W. Kuhn[f] (1939)	$\epsilon = 7RT\frac{\rho}{M^*}$	Cross-linked linear polymers
W. Kuhn[g] (1945)	$\epsilon = \frac{24}{5}RT\frac{\rho}{M^*}$	Cross-linked linear polymers
Müller[h] (1941)	$\epsilon = \frac{3}{5C}nkT\left(\frac{m}{M^*}\right)^{1/2}$	Cross-linked chain polymers; C = numerical factor about unity; n = number of molecules/cc.
Wall,[i] and Flory and Rehner[j] (1942)	$F = \frac{RT}{M^*}\rho\left(\frac{l}{l^2} - \frac{l_0^2}{l^2}\right)$	
Treloar[k] (1942)	$F = \frac{7}{3}\frac{RT}{M^*}\rho\left(\frac{l}{l^2} - \frac{l_0^2}{l^2}\right)$	
Flory[l,m] (1946)	$F = \frac{RT}{M^*}\rho\left(1 - \frac{2M^*}{M}\right)\left(\frac{l}{l_0} - \frac{l_0^2}{l^2}\right)$	

In our opinion any attempt toward a quantitative theory of the rubberlike state should follow a fundamentally different line. Not the theory of the gaseous state, but the theory of the liquid state should serve as a starting point for advances toward the more complicated rubberlike state. Since, however, a satisfactory quantitative theory does not exist for liquids, we must, for the time being, content ourselves with qualitative considerations.

II. THEORETICAL TREATMENT OF VISCOSITY

1. Relaxation

The limiting case of purely elastic deformation is almost never realized; generally frictional heat is developed during deformation. Conversely, every plastic deformation, *i.e.*, flow process, is accompanied by elastic processes. As a result of the deformation, first a stress arises in the flowing mass in a direction opposed to that of the applied force; then, as the molecules give way, this stress diminishes, a process known as relaxation of stress. This is inhibited by the internal friction, η, and promoted by the restoring force in the liquid of which the elastic modulus ϵ is a measure. For the velocity of the relative decrease in stress, called the relaxation velocity, the following relationship holds:

$$\frac{d \ln P}{dt} = -\frac{\epsilon}{\eta}$$

It must however be borne in mind that both the viscosity and likewise the elastic modulus can vary during the relaxation, and indeed these variations may be extraordinarily complicated.

We can, however, postulate a fluid or a plastic body which has a constant elastic modulus and constant viscosity, and therefore also a constant veloc-

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[a] E. Guth and H. Mark, *Wien. Monatsh*, **65**, 93 (1934).
[b] E. Guth, *Ind. Eng. Chem.*, **32**, 625 (1940).
[c] H. M. James and E. Guth, *J. Chem. Phys.*, **11**, 455, 531 (1943).
[d] H. Pelzer, *Sitzber. Akad. Wiss Wien. Math. naturw. Klasse*, (IIb), **147**, 72 (1938).
[e] W. Kuhn, *Kolloid-Z.*, **76**, 258 (1936).
[f] W. Kuhn, *Kolloid-Z.*, **85**, 3 (1939).
[g] W. Kuhn, *Experientia*, **1**, 6 (1945).
[h] F. H. Müller, *Kolloid-Z.*, **95**, 170 (1941).
[i] J. T. Wall, *J. Chem. Phys.*, **10**, 132, 485 (1942); **11**, 67, 527 (1943).
[j] P. J Flory and J Rehner, *J. Chem. Phys.*, **11**, 512 (1943).
[k] L. R. G. Treloar, *Trans. Faraday Soc.*, **38**, 392 (1942).
[l] P. J. Flory, *Chem. Revs.*, **35**, 51 (1944).
[m] P. J. Flory, *Ind. Eng. Chem*, **38**, 417 (1946).

ity of relaxation; for such a so-called Maxwellian body, the stress P falls exponentially at constant deformation:

$$P_t = P_0 e^{-(\epsilon t/\eta)} \tag{7}$$

The time taken for the original stress P_0 to fall to the value P_0/e is called the relaxation time, τ. We have therefore

$$\tau = \frac{\eta}{\epsilon}$$

The magnitude of the time of relaxation is therefore equal to the reciprocal of that of the relaxation velocity. For most liquids, the relaxation velocities are very great, much higher than the deformation velocities attainable experimentally in viscometry. For this reason, the elasticity phenomena fall into the background, and to a considerable extent they can be left out of consideration in the discussion of flow processes. But high polymers and their solutions, with high viscosities and low elastic moduli, frequently exhibit quite small relaxation velocities so that the elastic deformation becomes noticeable and must be taken into account.

2. Laws of Friction for Liquids

For investigating the flow of liquids, conditions are chosen so that slow steady streaming, free from any turbulence, occurs. This is the case for purely laminar streaming, *e.g.*, in the flow through a capillary in which a stationary layer of the liquid adheres to the wall. Laminar streaming also occurs in a liquid confined between two parallel plates in opposite uniform sliding motion or, approximately, between two coaxial cylinders rotating with different speeds. Under the influence of the deforming force a shearing force in the opposite direction is set up in the liquid: the particles composing the liquid give way to this shear by slipping over one another. If we think of the flowing liquid as if it were composed of parallel layers each of molecular thickness, then the motion of each successive layer is in part transferred to the adjacent layer as momentum in the same direction, and in part converted to heat G. The heat effort, *i.e.*, the amount of heat evolved in unit time, will be indicated by $\dot{G}$, differential quotients with respect to time being indicated by a dot.

The internal friction of liquids, *i.e.*, the process of converting mechanical energy irreversibly into heat, is fundamentally different from the (external) friction of solids. As Coulomb discovered more than a hundred

years ago, friction of liquids is practically independent of pressure, whereas for solids it varies with pressure.

If, within the liquid, layers parallel to the plane zy are moving in the direction z, each layer moves at a definite rate with respect to the next one. The absolute velocity u of the layers therefore varies in a direction x perpendicular to zy. This variation, *i.e.*, the velocity gradient, du/dx is the strain velocity $\dot{S}$. Thus:

$$\dot{S} = \frac{du}{dx}$$

The strain velocity depends on the shearing force per unit surface perpendicular to x, *i.e.*, the shearing tension P. For pure liquids of low molecular weight, the relationship is, as Newton already had guessed, a simple proportionality:

$$P = \eta \dot{S}$$

in which η is a constant characteristic of the liquid under investigation. It is called the viscosity coefficient or more shortly the viscosity of the liquid; it usually decreases rapidly with increasing temperature. A liquid will have unit viscosity (on the c.g.s. scale) if a force of one dyne is necessary to maintain a velocity gradient of one cm. per second per cm. over a surface of one sq. cm.; or, in other words, if a shearing tension of one dyne per sq. cm. is needed to maintain a strain velocity of one cm. per second per cm. The dimensions are therefore:

$$\eta = ml^{-1}t^{-1}$$

To maintain steady laminar flow, work must be done as heat is constantly evolved. The mechanical effort, *i.e.*, the work per unit time, spent per unit volume, is given by the product $P\dot{S}$ and thus:

$$P\dot{S} = \dot{G}$$

This provides another interpretation of η: in unit volume of a liquid of unit viscosity, one erg per second is evolved as heat if the strain velocity is to be one cm. per second per cm.

If the liquid is Newtonian, *i.e.*, if it follows Newton's rule, then:

$$\dot{G} = \eta \dot{S}^2$$

Although a great number of pure liquids follow Newton's rule, Newtonian liquids must be considered as a special class. The quotient $P/\dot{S}$ is not a

constant for all values of P in many liquids, *e.g.*, molten sulfur and solutions of chain polymers. Such liquids are said to be non-Newtonian, the quotient $P/\dot{S}$ will be denoted η^*.

A true liquid is characterized by the fact that η^* is finite. It therefore flows even under infinitely decreasing stress. In non-Newtonian liquids η^* tends toward a finite value η_0; it can be proved that Newton's rule has general validity at infinitely small stress.

For many non-Newtonian liquids η^* seems to tend toward another limiting value at very high stress. This we will designate (after Philippoff[17]) by η_∞.

3. Continuum Mechanics of Plastoelastic Bodies

(*a*) *Mechanical Equation of State*

We shall now discuss how far it is possible to establish laws for plastoelastic systems from general considerations. The discussion will be based on the fundamental work of Weissenberg,[18] who deduced a mechanical equation of state for plastoelastic bodies from considerations based on the principals of thermodynamics.

Consider a deformable body which is acted upon by external work supplied from a reservoir of energy. It is assumed that the body suffers no change in volume and that its temperature is maintained by immersion in a constant-temperature bath. In order to apply the principal laws of thermodynamics we consider as an energetically closed system the deformable body, the reservoir of energy, and the bath. It should be pointed out that in the preceding chapters thermodynamics have been used for the discussion of systems in equilibrium, but which are not closed. The difference from the systems treated hereafter is significant.

In studying transformations of systems as described we must distinguish between three types of energy. One type of energy, A, is taken from the reservoir and supplied to the body in the form of external work. A second type, F, is the free energy which during the application of the external work is stored in the system in a completely reversible way as in an elastic spring, and may at any time be retransformed into external work. Finally, a third type, G, is the bound energy which appears in the body in the form of heat

[17] See also W. Philippoff, *Die Viskositat der Kolloide*, Steinkopff, Dresden, 1942. F. H. Müller, *Kolloid-Z.*, **95**, 138, 306 (1941).

[18] K. Weissenberg, *Abhandl. preuss. Akad. Wiss. Physik.-math. Klasse*, **27**, 605 (1931); *Mitt. deut. Materialprüfungsanstalt.*, **19**, 54 (1932); *Arch sci. phys. et nat.*, **17**, 44, 130 (1935).

and is dissipated in an irreversible way into the bath, and which cannot be retransformed into external work within the system considered. If A, F, and G denote the respective energies per unit volume of the body we find from the first law of thermodynamics that the sum maintains throughout time a constant value K, according to:

$$A + F + G = K \tag{8}$$

From the second law of thermodynamics we find that in the exchange of energy the bound energy (that is, the heat of dissipation) can in time only increase or remain constant, according to:

$$\frac{dG}{dt} \geq 0 \tag{9}$$

In the following calculations the kinetic energy will be neglected and it will be assumed that a sufficiently small volume of the body is considered to allow the stresses[19] P and the strains S to be homogeneously distributed, and that a similar homogeneity applies to the velocities, accelerations, etc., which will be denoted by $\dot{P}$, $\ddot{P}$, ... $\overset{\mu}{P}$ and $\dot{S}$, $\ddot{S}$, .. $\overset{\nu}{S}$, respectively. Finally, we assume that only infinitesimally small deformations will be considered. With these restrictions it is possible to write the thermodynamic laws as relations between the mechanical variables, that is, between the stresses and strains and their time derivatives, and to combine these relations into a general mechanical equation of state of the deformable body. For this purpose we differentiate equation (8) with respect to time and find for the energies per second, *i.e.*, *efforts*, the equation:

$$\frac{dA}{dt} + \frac{dF}{dt} + \frac{dG}{dt} = 0 \tag{10}$$

If the mechanical system is determinable then each of the efforts in equation (10) can be written as some time-independent function of the mechanical variables. The effort, dA/dt, of the external work will be the same function for bodies of all possible kinds, and will be given by the product of the stress and the strain velocity. The efforts, dF/dt and dG/dt, of the free and of the bound energies, by contrast, will be characteristic functions for the particular kind of body under examination, and will therefore change from one kind of body to another. Considering further the com-

[19] Because of the assumed constancy of the volume of the body, we find that the stress contains an amount of isotropic pressure which is indeterminate as for an incompressible body.

plete reversibility of the effort of the free energy and complete irreversibility of the effort of the bound energy, it can be deduced that a time-independent representation in terms of the mechanical variables can always be found for the free energy F, but not for the bound energy G. We express this in the subsequent formulas by writing the effort of the free energy in the form of a total differential dF/dt, and the effort of the bound energy in the form $\dot{G}$. Accordingly we find that for the three terms in equation (10):

$$\frac{dA}{dt} = - P\dot{S} \tag{11a}$$

$$\frac{dF}{dt} = \frac{dF(\overset{\mu}{P}\overset{\nu}{S})}{dt} \tag{11b}$$

$$\frac{dG}{dt} = \dot{G}(\overset{\mu}{P}\overset{\nu}{S}) \tag{11c}$$

In contrast to the treatment in other chapters the signs here are chosen so that the efforts given out by the body are counted positive, and those taken in negative. For a development of the functions F and $\dot{G}$ into power series one can show from considerations of symmetry that the mechanical variables enter these series only in even powers, in the simplest forms in squares, and that the series for $\dot{G}$ contains only positive signs in view of equation (9).

Combining equations (10) and (11) we arrive at a general equation of state:

$$-P\dot{S} + \frac{dF(\overset{\mu}{P}\overset{\nu}{S})}{dt} + \dot{G}(\overset{\mu}{P}\overset{\nu}{S}) = 0 \tag{12}$$

which allows us to derive from it as special cases the various general laws of the mechanics of deformable bodies, and to unite all these laws into one self-consistent scheme.

(*b*) *Three General Laws*

Let us consider now explicitly how the equation of state (12) accounts for the general *after-effects* and for the effects of *elasticity, viscosity, and relaxation*. We find the laws of general after-effects by integrating equation (12) over time and resolving for P and S, respectively. The integration over time accounts for the mechanical prehistory, the resolution for P expresses then the stress as a function of the preceding time development of

the strain, and the resolution for S expresses similarly the strain in terms of the preceding time development of the stress. The laws for the special cases of elasticity, viscosity, and relaxation may now be deduced in turn by putting, in equation (12), one of the three terms equal to zero.

1. Elasticity. Here we consider phenomena which are completely reversible and which are therefore characterized by vanishing of the effort of the bound energy. This leads to the general law of elasticity:

$$P\dot{S} = \frac{dF(\overset{\mu}{P}\overset{\nu}{S})}{dt} \qquad (\text{for } \dot{G} = 0) \tag{13}$$

which expresses the fact that the whole of the external work is stored as free energy in the system. We find from a development of the function F into a power series, and neglect of all terms except the first which as was mentioned earlier must be a square, that F may be expressed to a first approximation by $P^2/2\epsilon$ or $(\epsilon/2)S^2$. Substitution of either expression in equation (13) gives Hooke's law in the form:

$$P = \epsilon S \tag{13a}$$

where ϵ denotes the shearing modulus (the bulk modulus does not appear here as the volume was assumed to remain constant).

2. Viscosity. Here we consider phenomena which are completely irreversible and which are therefore characterized by vanishing of the effort of the free energy. This leads to the general law of viscosity:

$$P\dot{S} = \dot{G}(\overset{\mu}{P}\overset{\nu}{S}) \qquad \left(\text{for } \frac{dF}{dt} = 0\right) \tag{14}$$

which expresses the fact that the whole of the external work is dissipated as bound energy. We find from a development of the function $\dot{G}$ into a power series, and neglect of all terms except the first, which again must be a square, different laws as a first approximation according to whether we consider solids or liquids. For liquids the internal friction is to a first approximation independent of the strain, so that the first term in the development is either P^2/η or $\eta\dot{S}^2$. Substitution of either expression in equation (14) leads to Newton's law of viscosity:

$$P = \eta\dot{S} \tag{14a}$$

3. Relaxation. Here we consider the phenomena which are characterized by vanishing of the external work. These phenomena are discussed under the term of relaxation since it follows from the second law

of thermodynamics that the free energy here must decrease in time or remain constant. We deduce here the law of relaxation:

$$0 = \frac{dF(\overset{\mu}{P}\,\overset{\nu}{S})}{dt} + \dot{G}(\overset{\mu}{P}\,\overset{\nu}{S}) \qquad (\text{for } P\dot{S} = 0) \tag{15}$$

It is important to note that equation (15) embodies two fundamentally different laws in one as the vanishing of the external work may be achieved experimentally either by vanishing of the applied stress, or by vanishing of the strain velocity, that is, by keeping the strain constant; one therefore deduces from:

$$P\dot{S} = 0 \begin{cases} \text{either } P = 0 & (15a) \\ \text{or } \dot{S} = 0, \textit{ i.e.}, S = S_0 = \text{constant} & (15b) \end{cases}$$

Decomposing equation (15) accordingly we find that:

$$0 = \frac{dF(\overset{\nu}{OS})}{dt} + \dot{G}\,(\overset{\nu}{O}\,\dot{S}) \qquad (\text{for } P = 0) \tag{16a}$$

and:

$$0 = \frac{dF(\overset{\mu}{P}S_0)}{dt} + \dot{G}(PS_0) \qquad (\text{for } \dot{S} = 0, \textit{ i.e.}, S = S_0) \tag{16b}$$

The law (16a) may be resolved for S and integrated over time. It then describes the decay in time of the strain observed under condition of zero stress. The law (16b) may be resolved for P and integrated over time. It then describes the decay in time of the stress observed under condition of the strain being maintained at a constant value. One may again replace F and $\dot{G}$ by quadratic functions when a first approximation is required. A variety of special cases are thus formed, and of these we shall only discuss two. Consider first a solid body with a free energy F equal to $(\epsilon/2)S^2$ and an effort $\dot{G}$ of bound energy equal to $\eta\dot{S}^2$. We then find from equations (16a) and (16b) that:

$$S = S_0 e^{-(\epsilon/\eta)t} \qquad (\text{for } P = 0) \tag{17a}$$

$$P = \epsilon S_0 = \text{constant} \qquad (\text{for } \dot{S} = 0, \textit{ i.e.}, S = S_0) \tag{17b}$$

This means that the performance of relaxation experiments on the body leads either according to equation (17a), under a sudden release of the stress to an exponential decay of the strain with a time relaxation equal to η/ϵ, or according to equation (17b), under a constant value of strain to a

constant value of stress with the time of relaxation equal to infinity. Thus the solid body under consideration exhibits an appreciable relaxation of strain under zero stress but no appreciable relaxation of stress under a constantly maintained strain. Examples for such a body can be constructed, for instance, from elastic sponge rubber by filling its pores with a viscous liquid. Many plastoelastic solids show relaxation phenomena of a similar kind. Consider by contrast another type of body, this time a fluid, with a free energy F equal to $P^2/2\epsilon$ and an effort $\dot{G}$ of bound energy equal to P^2/η. Here we find from equations (16*a*) and (16*b*) that:

$$S = S_0 - \frac{P_0}{\epsilon} \qquad \text{(for } P = 0\text{)} \tag{18a}$$

and:

$$P = P_0 e^{-(\epsilon/\eta)t} \qquad \text{(for } \dot{S} = 0, \textit{i.e.}, S = S_0\text{)} \tag{18b}$$

This means that in relaxation experiments the body will either, according to equation (18*a*), under a sudden release of stress jump instantaneously back by an amount of strain equal to P_0/ϵ, and with a relaxation time equal to zero, or, according to equation (18*b*), under a constantly maintained strain lose its strain exponentially according to a relaxation time equal to η/ϵ. In many plastoelastic fluids, such as colloidal solutions of rubber, starch, gelatin, cellulose, etc., one finds relaxation phenomena of a similar type. It will be noted that in each of the two examples discussed above, there is for the same body a great difference in the times of relaxation according to whether the conditions are those of zero stress or those of constant strain.

The equation of state (12) establishes a relation between the laws for the special cases of elasticity, viscosity, and relaxation so that from any two of these one may deduce the third. This means that it is possible to calculate the constants characteristic of any one of the laws if the constants for the other two laws are known. Such considerations lead in the simplest case to Maxwell's relaxation theory of internal friction, where in accordance with equations (17*a*) and (18*b*) the viscosity modulus η is calculated as the product of the elasticity modulus ϵ and the time of relaxation τ:

$$\eta = \epsilon\tau \tag{19}$$

(*c*) *Theory of Similitude*

The equation of state (12) can also be used with great advantage as a basis of a theory of similitude between deformable bodies of various types.

One may, for instance, gather into one family all bodies whose equations of state can be transformed into one another by a change in the units of length, time, and mass. The members of one family are then mechanically similar in an exact sense so that the results of experiments can be predicted for all members of the family if the results for any one member are known. A family of this kind is exemplified by all fluid bodies which obey Newton's law of viscosity. Another family comprises, for instance, many of the plastoelastic fluids, usually termed anomalous, such as certain solutions of rubber, cellulose derivatives, gelatin, which obey, within a wide range of mechanical actions, similar equations of state. Many applications for the theory of similitude are already known. Reynolds, for instance, characterized, for all members of the family of Newtonian fluids, the transition from laminar to turbulent flow by one and the same dimensionless number. Later Weissenberg[20] and his collaborators Eisenschitz and Rabinowitsch[21] applied similitude considerations to the family of (anomalous) plastoelastic liquids. Recently Weissenberg[22] demonstrated experimentally a number of rheological phenomena in the flow of viscoelastic materials and illustrated the striking similitude in behavior between members of the same family and the complete disparity in behavior between the members of the family of Newtonian liquids and those of the family of viscoelastic liquids.

It is advantageous to gather the families of similar bodies into larger classes according to the manner in which the equation of state describes the free energy F and the effort $\dot{G}$ of the bound energy as functions of the mechanical variables. Weissenberg studied in particular two limiting cases. One extreme is represented by the class of "P bodies" for which F and $\dot{G}$ are functions only of the stress variable P, while the other extreme is represented by the class of "S bodies" for which F and $\dot{G}$ are functions only of the strain variables S. Bridging the two extremes one finds the general class of "PS bodies" where F and $\dot{G}$ depend on both stress and strain variables. One can show that the different classes obey different laws for the superposition of the reversible, elastic, and the irreversible plastic phenomena. The equation of state of any plastoelastic body:

$$-P\dot{S} + \frac{dF(\overset{\mu}{P}\,\overset{\nu}{S})}{dt} + \dot{G}(\overset{\mu}{P}\,\overset{\nu}{S}) = 0 \tag{20}$$

[20] R. Eisenschitz, B. Rabinowitsch, and K. Weissenberg, *Mitt. deut. Materialprüfungsanstalt.*, **9**, 91 (1929).

[21] R. Eisenschitz and B. Rabinowitsch, *Ber.*, **64**, 2522 (1931).

[22] K. Weissenberg, *Nature.* **159**, 310 (1947).

defines, namely, two ideal bodies, one ideally reversible and elastic, specified by the suffix ϵ, with the equation of state:

$$-P_\epsilon \dot{S}_\epsilon + \frac{dF(\overset{\mu}{P}_\epsilon \overset{\nu}{S}_\epsilon)}{dt} = 0 \tag{20a}$$

and one ideally irreversible and plastic specified by the suffix π say, with the equation:

$$-P_\pi \dot{S}_\pi + \dot{G}(\overset{\mu}{P}_\pi \overset{\nu}{S}_\pi) = 0 \tag{20b}$$

By combining the three equations one derives the laws of superposition and so specifies the relations between the mechanical variables of the plastoelastic body and the corresponding variables of the associated ideally elastic and ideally plastic bodies.

The following is found in particular for the class of S bodies:

When a plastoelastic S body is subjected to arbitrarily chosen strains the stress in this body is always equal to the sum of the stresses produced under identical strains in the ideally elastic and ideally plastic body, according to:

$$P = P_\epsilon + P_\pi \qquad \text{for } S = S_\epsilon = S_\pi \tag{21}$$

when $F = F(\overset{\nu}{S})$ and $\dot{G} = \dot{G}(\overset{\nu}{S})$. A typical representative body is characterized by:

$$F = \frac{\epsilon}{2} S^2 \text{ and } \dot{G} = \eta \dot{S}^2 \tag{21a}$$

The corresponding equation of state can be divided by $\dot{S}$ and then yields:

$$P = \epsilon S + \eta \dot{S} \tag{21b}$$

We deduce the associated ideally elastic body according to equation (20a) as one whose equation of state corresponds to that of a solid obeying Hooke's law of elasticity:

$$P_\epsilon = \epsilon S_\epsilon \tag{21c}$$

and we deduce similarly the associated ideally plastic body according to equation (20b) as one whose equation of state corresponds to that of a fluid obeying Newton's law of friction:

$$P_\pi = \eta \dot{S}_\pi \tag{21d}$$

One sees immediately from equations (21b, c, and d) and in accordance with equation (21) the stresses P_ϵ and P_π of the two ideal bodies add up here to

the total stress P observed on the S body when all the bodies are subjected to the same strains. Thus, the plastoelastic S body here considered behaves under any applied strains as partly elastic and partly plastic, with ϵS and $\eta \dot{S}$ giving the respective amounts of the two parts in accordance with equation (21*b*). One deduces further from equation (21*b*) that the S body approaches the behavior of an ideally elastic solid obeying Hooke's law in the limit of infinitely slow strains ($\dot{S} \to 0$), while it approaches the behavior of an ideally plastic fluid obeying Newton's law in the limiting case of infinitely quick strains ($\dot{S} \to \infty$).

By contrast the following is found for the class of P bodies:

When a plastoelastic P body is subjected to arbitrarily chosen stresses the strain velocity in this body is equal to the sum of the strain velocities produced under identical stresses in the associated ideally elastic and ideally plastic bodies, according to:

$$\dot{S} = \dot{S}_\epsilon + \dot{S}_\pi \qquad \text{for } P = P_\epsilon = P_\pi \tag{22}$$

when $F = F(\overset{\mu}{P})$ and $\dot{G} = \dot{G}(\overset{\mu}{P})$. A typical representative body is characterized by:

$$F = \frac{P^2}{2\epsilon} \text{ and } \dot{G} = \frac{P^2}{\eta} \tag{22a}$$

and termed Maxwell body. Its equation of state can be divided by P and then yields:

$$\dot{S} = \frac{\dot{P}}{\epsilon} + \frac{P}{\eta} \tag{22b}$$

We deduce the associated ideally elastic body according to equation (20*a*) as obeying the law:

$$\dot{S}_\epsilon = \frac{\dot{P}_\epsilon}{\epsilon} \tag{22c}$$

which after integration over time is identical with Hooke's law apart from a constant of integration. Similarly we deduce the associated ideally plastic body according to equation (20*b*) as obeying the law:

$$\dot{S}_\pi = \frac{P_\pi}{\eta} \tag{22d}$$

which is identical with Newton's law. One sees here that, in accordance with equation (22), the strain velocities $\dot{S}_\epsilon$ and $\dot{S}_\pi$ of the two ideal bodies

add up to the strain velocity $\dot{S}$ of the P body when all the bodies are subjected to the same stresses. Thus, the P body here considered behaves under all applied stresses as partly elastic and partly plastic with $\dot{P}/\epsilon$ and P/η giving the respective amounts of the two parts. Moreover, in contrast to the S body, it follows from equation (22*b*) that the behavior of the P body approaches that of an ideally plastic fluid obeying Newton's law, in the limit of an infinitely slow application of stress ($\dot{P} \to 0$), and approaches the behavior of an ideally elastic solid obeying Hooke's law in the limit of an infinitely quick application of stress ($\dot{P} \to \infty$).

It is possible to order the real materials of various kinds into the classes of S and P bodies with a higher or lesser degree of approximation but one obtains the best approach to reality for the general classes of the PS bodies. In these classes the superposition law varies from one body to another, and this makes it easy to understand the experimental fact that every real material follows particular laws of its own.

4. Molecular Theory of Viscosity of Pure Liquids

A continuous mechanical effort is required in order to maintain a steady flow. Thus the layer in most rapid motion continually loses kinetic energy. This means that the molecules in this layer may lose a part of their momentum parallel to the direction of flow by giving off kinetic energy in a direction perpendicular to that of flow. This loss of kinetic energy does not, however, mean an equal gain in kinetic energy for the neighboring layer, but an increase in temperature, inasmuch as mechanical energy is converted to heat.

As already emphasized, this internal friction is essentially different from external friction. On this account it seems misguided to attempt to understand frictional phenomena in liquids by the use of models based on the frictional process between solids, *e.g.*, solid spheres as idealized molecules. The comparison with the internal friction in gases is equally irrelevant. In the latter, the transfer of molecular momentum is effected by exchange of molecules or during collision of a molecule of one layer with one of a second. If these were the determining processes for liquid friction, then the latter would increase with temperature (at constant volume) as it does for gases. For the great majority of liquids, however, the internal friction decreases very considerably with increase in temperature. Therefore, it seems more reasonable to suppose that the transmission of molecular momentum in liquids (the diffusion of the energy in a direction perpendicular to the movement) is largely determined by the cohesion between the

particles, *i.e.*, the intermolecular forces. This is in agreement with the fact that liquids with high molecular cohesion have high viscosities; for glasslike oxides, for which all the atoms are linked by primary valences, the viscosity is extremely high.

It is clear that, in the course of flow, linkage exchange must occur since, during displacement by streaming, the neighbors on an adjacent layer of any particular molecule will be replaced by other neighbors. Now the interaction responsible for the linkage between two adjacent particles can be statistically described as if the particles were linked for a certain part of the time but not for the remainder; thus a periodic change in the potential energy appears characteristic for the linkage exchange.

If now a continuous stress is applied to this system, then at any moment some of the particles will be freely displaced relative to each other without change of their momenta, while the other particles, as a result of linkage to their neighbors, yield to the latter a part of their kinetic energy in the form of irregular motion, *i.e.*, heat.

Now, according to Boltzmann, the fraction $\Delta n/n$ of the linkages which will be found to be in a given energy state E is given by an expression of the form:

$$ae^{-E/kT} \tag{23}$$

Many years ago Guzmàn[23] and Drucker[24] had already found that the viscosity falls exponentially with increase in temperature, a fact which was later emphasized, especially by Andrade:[25]

$$\eta = Ae^{B/T} \tag{23a}$$

This equation gives the temperature variation of viscosity over a wide range. From this aspect also the deduction roughly follows that fluidity, the reciprocal of viscosity, which is a measure of the conversion of mechanical work into heat, depends on a definite energy state of the intermolecular linkages.

An empirical relationship can therefore be expressed in the following way:

$$1/\eta = ae^{-E/kT} \tag{23b}$$

These conceptions just given, developed by van der Wyk, differ in an essential point from the much discussed place exchange theory of Ewell and Eyring.[26] The latter consider the energy state of a single molecule

[23] J. de Guzmàn, *Anales soc. españ. fís. y quím.*, **11**, 353 (1913).
[24] C. Drucker, *Z. physik. Chem.*, **92**, 287 (1916).
[25] E. N. da C. Andrade, *Nature*, **125**, 309, 582 (1930).
[26] R. H. Ewell, *J. Applied Phys.*, **9**, 252 (1938).

which can move forward as soon as it attains a certain energy level; here the flow process is correspondingly regarded as a monomolecular process as these authors especially emphasize. In our view, however, the interaction between the individual molecules must be taken into account, this being analogous to a bimolecular reaction. The difference between the two conceptions is especially evident in the treatment of mixtures (see sect. IV2); whereas for pure liquids both methods lead to the same formal expression for η (which also had been deduced previously in other ways), mixtures give quite different expressions for the variation of viscosity with concentration. These can be tested experimentally and afford evidence against the monomolecular theory.

While (for substances of low viscosity) the relationship (23*a*) is quite satisfactory, glasses and substances in a glasslike state show deviations; the slope of the curve log η against $1/T$ changes rapidly in the neighborhood of the so-called second-order transition point. Various proposals have been made to explain these deviations; we would, however, point out that the usual statistics and also equation (23) have very definite limits and are not applicable at low temperatures and if high bond forces are involved.

III. EXPERIMENTAL DETERMINATION OF VISCOSITY

1. Methods of Measurement

In the case of liquids, only deformation by shearing can easily be treated experimentally; the usual definition of η relates this kind of deformation and the corresponding shearing stress. For amorphous solids which can be regarded as very viscous liquids, the increase in length under the effect of a tension can also be measured (or, respectively, the shortening on compression).

For the case in which such a body is isotropically amorphous, it can be assigned a viscosity η_{draw}; by definition it is one poise if a force of one dyne must be exerted on the face of a one-cm. edged cube, in order to obtain a stretching velocity of one cm. per second perpendicular to that surface. The values for shearing stress and tensile stress, η and η_{draw} are not equal for one and the same substance; η_{draw} is always considerably greater.

For small deformations and low velocities of deformation, the following relationship holds:

$$\eta = \frac{1}{2}\frac{1}{1+\mu}\eta_{draw}$$

where μ is the Poisson constant. If $\mu = 0.5$, as is generally the case for plastic polymers, then:

$$\eta = {}^1/_3\, \eta_{draw}$$

(a) *Method of Couette*

The liquid under investigation is disposed as a layer between two coaxial cylinders (radii r_1 and r_2, height h) one of which is rotated with a constant angular velocity Ω, while the other is maintained at rest by means of a measured moment of torque. This moment compensates the torque τ to which the resting cylinder is subjected by the shearing of the liquid. For laminar flow of a Newtonian liquid η can be calculated from:

$$\eta = \frac{\tau}{4\pi h\Omega} \cdot \frac{r_1^2 - r_2^2}{r_1^2 r_2^2} \tag{24a}$$

(b) *Flow through a Cylindrical Tube*

If for a tube of radius r and length l for a pressure head of p, the volume flow of liquid in unit time is equal to Q, and provided that the liquid is Newtonian and the flow laminar, then:

$$\eta = \frac{\pi}{8Q} \frac{p}{l} r^4 \qquad \text{(Hagen-Poiseuille's rule)} \tag{24b}$$

(c) *Falling Sphere Method*

A sphere of radius r and density ρ_1 falls through a Newtonian liquid of density ρ_2 with a constant velocity u. The following is found to hold to a close approximation:

$$\eta = \frac{2gr^2(\rho_1 - \rho_2)}{9u} \tag{24c}$$

where g = gravity constant = 981 (Stokes' rule). This expression is only applicable for determination of absolute values of η when the velocity of fall is very low and the width of the tube is large compared with the diameter of the sphere.

(d) *Method of Couette for Highly Viscous Substances*

The falling sphere method can be applied for viscosity values up to 10^4 poises; for higher viscosities, up to 10^{10} poises, an adaptation of the Couette

method can be used.[27] The angular velocity of a steel cylinder (diameter about 1 cm.), which dips about 7 cm. into the melt, is measured under a known torque. The melt is contained in a Pyrex beaker of about 2.5 cm. inner diameter and is kept at a constant temperature. Mooney[28] has described an apparatus entirely of metal which he calls a rubber rheometer as it was constructed especially for viscosity measurement of rubbers.

(e) *Flow between Two Coaxial Cylinders*[29]

The heated plastic mass is poured into a cylindrical tube (diameter $2r_1 = 10$–12 mm. and length $l = 20$–30 mm.) around a coaxial cylindrical rod ($2r_2 = 2$–5 mm.). After cooling the tube is clamped with its axis vertical and the rod is loaded with a suspended mass m of 100 to 200 grams. The rate of fall u of the rod is measured. As an approximation for small u, measured over a small distance:

$$\eta = \frac{gm}{2\pi lu} \ln \frac{r_1}{r_2} \tag{24d}$$

The method can be used for pitch and similar masses and is very suitable for the study of viscosity at different temperatures.

(f) *Torsion of a Cylinder*

For still higher viscosities, when the material is already glasslike, Parks[27] recommends the method devised by Trouton and Andrews.[30] A cylinder of the material (2.5 cm. long, diameter 0.5–0.8 cm.) is clamped at one end and set up in a vertical position. It is then subjected to torque τ at the other end. The moment μ and the angular velocity Ω of the rotation of the upper end relative to the lower end of the cylinder are measured. η can be calculated from:

$$\eta = \frac{2\tau l}{\pi r^4 \Omega} \tag{24e}$$

where r is the radius and l the length of the cylinder.

[27] G. S. Parks, L. E. Barton, M. E. Spaght, and J. W. Richardson, *Physics*, **5**, 193 (1934).

[28] M. Mooney, *Physics*, **7**, 413 (1936).

[29] A. J. A. van der Wyk, *unpublished work*.

[30] F. T. Trouton and E. S. Andrews, *Proc. Phys. Soc. London*, **19**, 47 (1909).

(g) *Stretching of a Fiber*

Another method[31] consists in measuring the rate of stretch dl/dt of a fiber of known dimensions under a given load. The following holds approximately:

$$\eta = \frac{dt}{dl}\frac{pl}{3} \tag{24f}$$

p = applied tension. At the beginning of deformation, the rate of flow is often not constant; it decreases and only becomes constant after a certain deformation has been reached which is elastic in character. For determining the viscosity it is necessary to wait until the constant value is attained.

Industrially the so-called plastometer is much used for viscosity estimations; but it is not possible to deduce absolute values for η from such measurements.

2. Flow Curve

(a) *True Liquids*

All the quantitative relationships (24) mentioned above have been established on the hypothesis that the flow is laminar and that Newton's rule is valid, *i.e.*, that the ratio between the shearing force and the rate of deformation is given by a definite constant viscosity coefficient η characteristic of the material. For many low molecular pure liquids and solutions, this assumption has been in fact experimentally verified with good approximation. For high polymer solutions, however, considerable deviations are observed. Here, for steady laminar flow a frictional law of a more general kind is valid, in which the rate of deformation is an experimentally ascertainable function of the stress:

$$\dot{S} = f(P)$$

In such cases, the viscosity is no longer a constant for a given material but is a quantity η' dependent on the shearing force:

$$\eta' = \frac{P}{\dot{S}} = \frac{P}{f(P)}$$

and this dependence makes itself evident in all flow processes of the par ticular material.

[31] E. Jenckel, *Z. Elektrochem.*, **43**, 796 (1937).

The direct measurement of η' is only possible in viscometers in which uniform stress prevails. This condition is obtained for a liquid submitted to shear between two parallel plates and approximated by the flow in a Couette viscometer. Here by determining the torque τ as a function of angular velocity ω a *flow curve* can be plotted; its slope according to equation (24*a*) equals η' (Fig. 10a).

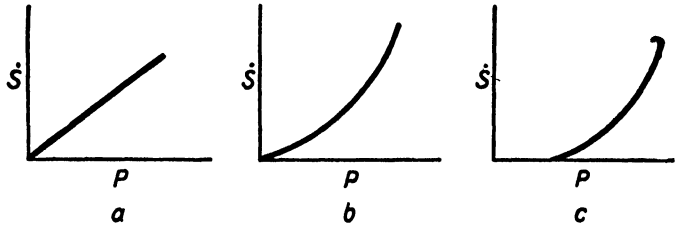


Fig. 10a. Flow curves: (*a*) Newtonian liquid; (*b*) non-Newtonian liquid; and (*c*) non-Newtonian system with yield point.

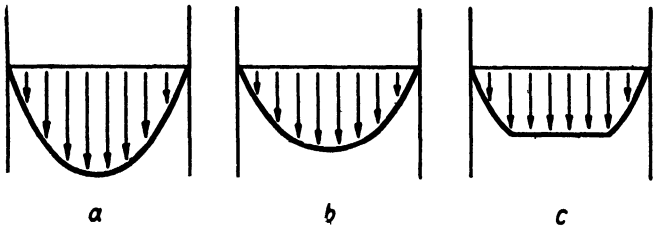


Fig. 10b. Velocity distribution in a capillary: (*a*) Newtonian liquid (parabola); (*b*) non-Newtonian liquid (any curve); and (*c*) system with yield point (discontinuity).

The conditions are more complicated for flow through a capillary inside of which the shear stress is maximum at the wall and zero in the center. Consequently η' varies along the radius (Fig. 10b). Weissenberg has given explicit formulas by which for a given liquid η' in its relation to stress can be calculated from capillary experiments.

Weissenberg and Rabinowitsch[32] define a function D:

$$\dot{S}_r = 3D + P_r \frac{dD}{dP_r}$$

where S_r is the velocity gradient (deformation velocity) at the wall and P_r the shear stress at the wall:

[32] R. Eisenschitz, B. Rabinowitsch, and K. Weissenberg, *Mitt. deut. Materialprüfungsanstalt.*, **10**, 21 (1929).

$$P_r = \frac{pr}{2l}$$

where p is the pressure head, r the radius, and l the length of the capillary. If Q is the rate of efflux through the entire capillary, then:

$$D = \frac{Q}{\pi P_r}$$

For a Newtonian liquid one obtains Poiseuille's equation. It is convenient to plot log D against log P_r; the curve thus obtained is often designated a flow curve. The dependence of S on P may be determined graphically from the flow curve, as has been shown by Eisenschitz and Rabinowitsch.[33]

It should be mentioned, however, that a certain number of plastoelastic systems cannot be treated in this way; experiments will not always yield a definite flow curve. This is the case if no real laminar flow can be realized (as in certain gels), or if slip occurs, or if the system has a definite yield value. Other complications may arise from thixotropy, in which case the constants of the material depend on time.

(*b*) *Liquids and Solids with Yield Value*

Many plastic deformable bodies and many fluids which sometimes are difficult to distinguish from true liquids show only elastic deformation when subjected to very small stresses; above a certain shearing stress, which is known as the *yield point*, they begin to flow. Hydrated cellulose fibers are examples of such substances, but all gels formed from solutions of chain polymers belong to this class, *e.g.*, pectin gels and gelatin gels. The streaming profile of such a liquid in a capillary viscometer is different from the parabolic profile of a Newtonian liquid. In very outstanding cases of such behavior, *i.e.*, when the yield point lies at a relatively high shearing stress, it is observed that, for instance, a mass flowing through a tube actually only flows at the walls of the tube, and in the middle is pushed through as an unchanged, undeformed solid body (plug flow).

In solutions of high polymers the appearance of flow above a definite shearing stress value is usually due to the fact that a framework of molecules, which is held together by forces comparable to those of a crystal lattice, *e.g.*, a network of fringe micelles, is torn apart when a certain stress has been reached and so flow sets in.

[33] R. Eisenschitz and B. Rabinowitsch, *Ber.*, **64**, 2522 (1931).

Very definite yield points are shown by so-called thixotropic substances and clay suspensions.[34]

3. Measurement of Viscosity by Means of Oscillation Damping

(*a*) *Slow Oscillations*

By means of the damping of slow torsional oscillations, it is possible to obtain the viscosity of elastic substances like rubber, at a deformation velocity of zero and deformation of zero. This value we shall indicate as η_{00}. It is a constant for the material and varies with temperature.

A horizontal beam is suspended on a cylindrical thread of radius r (r = about 0.15 cm.) and length l (about 60 cm.), and is made to oscillate in a horizontal plane. The damping of the torsional oscillations is measured. If I is the moment of inertia, Φ_1 and Φ_2 are two successive amplitudes, and τ is the period, then:[35]

$$\eta = \frac{4lI}{\pi r^2 \tau} \ln \frac{\Phi_1}{\Phi_2} \tag{24g}$$

Similar viscosity measurements have been carried out on rubber by Mooney[36]; his experimental device consisted of a kind of Couette apparatus in which rubber was sheared between the inner stationary cylinder and the outer rotatable one. The outer, heavy cylinder was rotated to a certain extent, causing the rubber to yield elastically and then let loose so that it swung a few times to reach the rest position. The viscosity can be calculated from the logarithmic decrement, the moment of inertia of the cylinder, and other constants of the apparatus.

(*b*) *Rapid Oscillations. Measurements on Elastoplastic Bodies and Liquids*

Weissenberg has developed a method for simultaneous evaluation of viscosity and elasticity in liquids in which periodic shearing stresses are measured in relation to the frequency. An apparatus for this purpose has been devised by Eisenschitz and Philippoff and is used as follows:[36a]

The substance to be examined is contained in a narrow glass tube in which a centrally suspended needle undergoes oscillations which can be controlled by an electromechanical

[34] F. Johnson Dix and G. W. Scott Blair, *J. Applied Phys.*, **11**, 574 (1940).

[35] A. J. A. van der Wyk, in *Proceedings of the Rubber Technology Conference, 1938, London.* Heffer, Cambridge, 1938, p. 985.

[36] M. Mooney, *Physics*, **7**, 413 (1936).

[36a] R. Eisenschitz and W. Philippoff, *Naturwissenschaften*, **31**, 527 (1933).

device. It can be assumed that the motion of the liquid under the effect of the oscillating needle is approximately laminar. If the frequency and amplitude are known, then the *dynamic* viscosity can be calculated from the power consumption, *i e.*, the damping.

An apparatus for the investigation of highly viscous liquids and elastomers by means of high-frequency oscillations has been described by Mason.[37] These dynamic methods are particularly interesting because one can obtain very high deformation velocities, *e.g.*, by increasing the frequency at constant amplitude. If the rate of deformation becomes greater than the relaxation velocity then elastic properties predominate.

IV. EXPERIMENTAL RESULTS IN HOMOGENEOUS SYSTEMS

1. Behavior of Pure Liquids

With very few exceptions pure liquids are Newtonian, *i.e.*, the viscosity is constant up to very high stress values. This holds equally well for liquids of low or high viscosity; even for molten supercooled glucose, no variation of η with shearing force could be detected. Table III gives the viscosities of certain widely differing substances.

TABLE III

Viscosities of Various Substances

Substance	Temp., ° C.	Viscosity, poises
Hydrogen	−252	0.00011
Benzene	20	0.0064
Water	20	0.0101
Tetra-*n*-butylmethane		0.0126[a]
n-Heptadecane, $C_{17}H_{36}$		0.0322[a]
Glycerol	20	15.0
Glucose (supercooled)	40	28×10^{9}[b]
" "	22	9×10^{13}[b]

[a] P. Karrer and C. Ferri, *Helv. Chim. Acta*, **17**, 358 (1934).

[b] G. Parks, L. E. Barton, M. E. Sparks, and J. W. Richardson, *Physics*, **5**, 193 (1934).

The case of water illustrates how the elastic modulus and the viscosity are interrelated in liquids for which the elastic deformation is negligible

[37] W. P. Mason and H. J. McSkimin, *communication* from the Bell Telephone Laboratories, Inc., 1948. W. P. Mason, W. O. Baker, H. J. McSkimin, and J. H. Heiss, *Phys. Rev.*, **73**, 1074 (1948).

compared with the plastic effect; at 20° the value for η is 0.01; the elastic modulus calculated from the velocity of sound amounts to $\epsilon = 2.10^{10}$ dynes per sq. cm. Therefore the time of relaxation $\tau \cong 5.10^{-13}$ second. Thus the rate of relaxation is so high that the elastic properties disappear in comparison with the plastic properties.

High polymers with three-dimensional network structures, *e.g.*, glass-forming oxides such as boron trioxide,[38] show Newtonian behavior in the molten state.

Nothing is actually known with regard to the rheological behavior of a substance consisting of genuinely equal chain molecules, *i.e.*, chemically pure in its strict sense. Even the behavior of highly viscous sulfur or of molten selenium which have been investigated cannot serve for this purpose as they contain chains of varying lengths. Since all other high polymers consist in fact of polymeric homologous mixtures, it is preferable to consider first the behavior of simple mixtures before discussing the results obtained with polymers.

2. Viscosity of Mixtures of Low Molecular Compounds

Experiment shows that the viscosity of a mixture may lie between the viscosities of its components, sometimes it is lower and sometimes higher; figure 11 illustrates this. Although it is not possible strictly to speak of a general rule, yet from the available data it may be said that higher viscosity is usually associated with exothermic mixtures, lower viscosity with endothermic[39]; in other words, higher intermolecular forces appear to increase the viscosity with respect to the viscosities of the components, and conversely. In order to understand the viscosity of mixtures, therefore, it is necessary to take into account not only the forces operating in the pure liquids but also those which may be exerted between different molecules. With this in mind van der Wyk[40] has considered the problem as follows. If it is first limited to the case of two ideally fluid liquids with molecules of equal size, then the steady flow of such a medium can be regarded as exactly comparable with that of a pure liquid. We will now fix our attention on a particular molecule of component one in the layer A at the moment when it is about to undergo a transfer of its momentum. The partner, in the adjacent layer B, to which energy will be transferred, is either a molecule of the same sort one or a molecule of component two. If the molar

[38] G. S. Parks and M. E. Spaght, *Physics*, **6**, 69 (1935).

[39] See R. H. Ewell, *J. Chem. Phys.*, **5**, 967 (1937).

[40] A. J. A. van der Wyk, *Nature*, **138**, 845 (1936).

fractions of the two components are N_1 and N_2 then the probability for the first case is proportional to N_1, that for the second to N_2.

The conditions for transfer are not characterized by the same energy E in the two cases. If in the first case of transfer between equal molecules we call the energy E_1, and if in the second, where different molecules are involved, we call it E_{12}, then the exchange effect between the molecule considered in layer A and all the molecules in layer B is proportional to the expression $N_1E_1 + N_2E_{12}$.

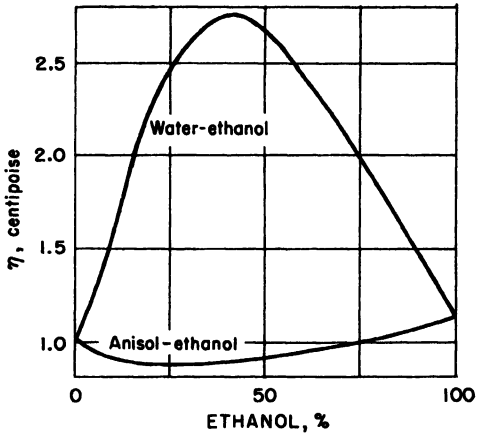


Fig. 11. Viscosity of mixtures.

The exchange effect of all the molecules in layer A with all those in layer B is then proportional to:

$$N_1(N_1E_1 + N_2E_{12})$$

Exactly the same considerations apply to molecules of component two in layer A; these will contribute:

$$N_2(N_1E_{12} + N_2E_2)$$

The whole exchange effect between the two liquid layers is thus:

$$E \cong N_1(N_1E_1 + N_2E_{12}) + N_2(N_1E_{12} + N_2E_2) = N_1^2E_1 + 2N_1N_2E_{12} + N_2^2E_2$$

Now from equation (23) $\log \eta$ is proportional to E at constant temperature. By substitution in this above expression and remembering also that $N_1 + N_2 = 1$, we arrive at:

$$\ln \eta = N_2^2 \ln \frac{\eta_1 \eta_2}{\eta_{12}^2} + 2N_2 \ln \frac{\eta_{12}}{\eta_1} + \ln \eta_1 \tag{25}$$

Application of this formula to athermal mixtures containing molecules of similar size shows good agreement with the experimental findings; some of the results are shown in Table IV.[41]

TABLE IV

Viscosity of Quasi-ideal Mixtures (in Centipoises)

Hexane(1) carbon tetrachloride(2)			Benzyl benzoate(1) -toluene(2)		
$N_1 \times 10^2$	η (found)	η (calc.)	$N_1 \times 10^2$	η (found)	η (calc)
0	0 97	0.97	0	$0\ 55_2$	0 552
9 88	$0\ 85_1$	0.852	23.7	1.18_3	1 15
20 37	0.74_2	0.752	42.6	$2\ 01_5$	2.01
43.4	$0\ 58_5$	0 582	65.0	$3\ 61_4$	3 64
69 7	$0\ 46_2$	0.456	78 9	$5\ 08_0$	5 15
84.3	$0\ 40_3$	0.405	90 0	6.66_0	6 67
100 0	$0\ 36_4$	0.36_4	100 0	$8\ 45_0$	8 45
	$\eta_{12} = 0\ 506$			$\eta_{12} = 2.75$	

From Eq. (25) it can be seen that the relationship between a simple function of the viscosity and the concentration is not linear but parabolic. In addition a new quantity η_{12} appears, called the *binary viscosity* which describes the exchange effect and so the friction between two different kinds of molecules. It is necessary to proceed from these facts to more specialized interpolation formulas if these are to have a physical meaning.

For dilute solutions, *i.e.*, when $N_2 \ll N_1$ and $N_1 \sim 1$, the expression (25) can be replaced by:

$$\ln \frac{\eta}{\eta_1} = 2\, N_2 \ln \frac{\eta_{12}}{\eta_1} \tag{25a}$$

At the same time $N_2 = w_2(M_1/M_2)$ where w_2 is the weight concentration of the (dissolved) component two, and M_1 and M_2 are the molecular weights, respectively.

On the other hand, by definition:

$$\eta/\eta_1 = 1 + \eta_{sp}$$

and from equation (25*a*) it then follows that, for dilute solutions:

[41] A. J. A. van der Wyk, *Compt. rend. soc. phys. hist. nat. Genève*, **54**, 133 (1937); *Arch. sci. phys. et nat.*, **19** (Nov.–Dec., 1937).

$$\ln (1 + \eta_{sp}) = w_2 \frac{2M_1}{M_2} \ln \frac{\eta_{12}}{\eta_1} \tag{26}$$

At sufficiently high dilution $\eta_{sp} \ll 1$ so that by development of the first member in a series, this can be further reduced to:

$$\frac{\eta_{sp}}{w_2} = \frac{2M_1}{M_2} \ln \frac{\eta_{12}}{\eta_1} = \text{constant} \tag{26a}$$

The physical meaning of the quantity η_{sp} thus becomes evident; its remarkably small dependence on temperature is readily explained by its being proportional to the log of a quotient of two viscosities.

3. Viscosity of Chain Polymers

(*a*) *Flow Viscosity*

Since it is not practicable to obtain chain polymers consisting of only one sort of molecules, investigations have only been carried out on polymeric homologous mixtures, namely, plastic sulfur, selenium, polyisobutylene, and rubber. Below a certain temperature all of these are glasslike solids; on warming they become elastic and rubberlike, eventually quite plastic. The shorter the chain length the more the plastic properties predominate.

Viscometric investigation of elastoplastic substances gives the following result. If a stress is applied, then the test sample is at first deformed at a rather high rate; on removal of the applied stress, the specimen returns to its original shape. With further deformation the rate falls—therefore the viscosity increases—and sometimes reaches a constant value; a state of steady flow sets in. Often, however, for example in unmilled rubber, with increasing deformation, the sample becomes so stiff that no further deformation is possible without breakdown of the chains so that the substance is irreversibly changed.

In figure 12 are given the observations made by Ferry and Parks[42] on a polyisobutylene of an average mol. wt. 5000, determined cryoscopically. The Couette apparatus modified for highly viscous substances as described in sect. III1*d* was used; a constant torque was applied for a period of five minutes and then removed. The angle is thus a measure of the deformation caused by the applied stress. At first there is rapid deformation, at a velocity which gradually declines (*A*) and a steady state of plastic flow sets in (*B*). On removing the applied force the system creeps back to a certain extent (*C*).

[42] J. D. Ferry and G. S. Parks, *Physics*, 6, 356 (1935).

As can be seen from the diagram, it is possible to extrapolate the linear parts of the curve, which represent steady states, to zero and so obtain segments Θ_1, Θ_2 of the ordinate which correspond to the excess deformation over the purely plastic deformation, Θ_1, while Θ_2 is the creep at the end of the experiment, resulting from the elastic properties of the material; both values increase with increasing applied stress and deformation rate; they are not identical, however, but differ more or less according to the rate of deformation, which indicates that the deformed mate ial has elastic properties different from those in the original unstretched state.

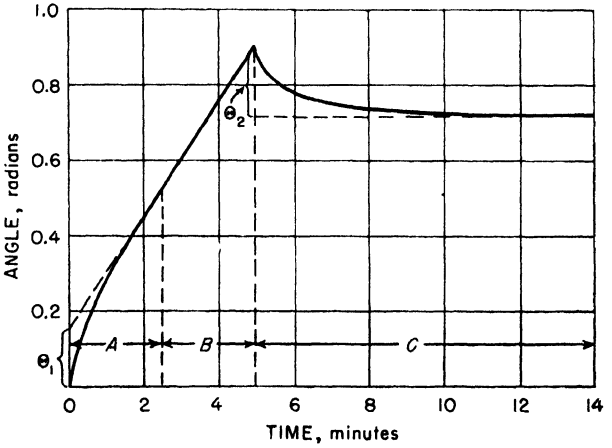


Fig. 12. Rheological behavior of a polyisobutylene (Ferry and Parks).

The restoring process, called elastic after-effect, which sets in at the end of the experiment, also proceeds at first quickly and then more slowly; if at constant deformation the fall of the stress is observed, then $d \log P/dt$, the relative rate of fall of the stress, is at first high and then becomes smaller and smaller.

The molecular interpretation of the foregoing is as follows. At first the chain segments slide freely over each other and the opposing viscous force is relatively small; the chains then become oriented and stretched until finally, at much higher shearing forces, they slide over each other, a process giving rise to a much higher viscosity, namely pure flow viscosity; the elastic tension, originating during the stretching of the chains, remains during the plastic deformation and appears as a retractive force, causing creep as soon as the shearing force disappears.

In addition to this, it is observed that all elastic melts show a marked

variation of flow viscosity with stress, *i.e.*, they are not Newtonian fluids. As an example, figure 13 shows the relationship between rate of shear and applied stress for polyisobutylene (mol. wt. 5000); it can be seen that the shearing rate increases more rapidly than the force applied; this means that the viscosity falls with increasing applied stress.

For milled rubber (mol. wt. estimated as 100,000) the effect of the applied stress on the viscosity is still much greater. Mooney,[43] using the "rhe-

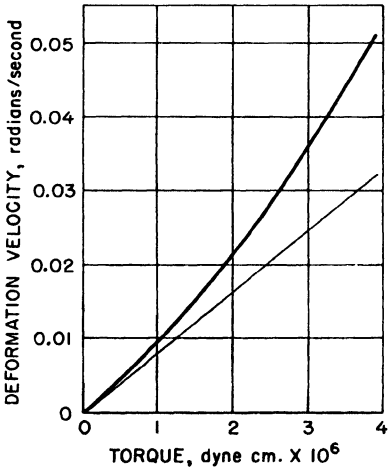


Fig. 13. Dependence of viscosity of polyisobutylene on shearing stress (Ferry and Parks).

ometer" found $\eta = 3 \times 10^7$ poises at a temperature of 97°, and, by increasing the stress eightfold, the value for η fell to 2.3×10^5 poises.

The viscosity of glassy selenium may be cited as a further example which, according to Jenckel,[44] by fivefold increase of the tension from 3.5×10^7 dynes per sq. cm. at 30°, sinks from $\eta = 11 \times 10^{12}$ to half its value. Molten sulfur above 130° also shows a fall in viscosity with increased stress, as shown by Ostwald.[45]

These phenomena may be attributed to the fact that, under high stresses and the resulting high rate of deformation, the chains are permanently stretched and therefore in a different configuration than when little strain is imposed; in the case of plastic flow through tension, they are stretched

[43] M. Mooney, *Physics*, **7**, 413 (1936).
[44] E. Jenckel, *Z. Elektrochem.*, **43**, 796 (1937).
[45] W Ostwald and H. Malss, *Kolloid-Z.*, **63**, 192, 305 (1933).

and oriented in parallel; in shearing they must behave like thread molecules in streaming solution, as will be discussed later. The substances considered here do not, therefore, obey the Newton's law of friction, nor Maxwell's law of relaxation. Fusible three-dimensional polymer networks behave in an essentially different way from chain polymers. Boron trioxide, a solid glass at room temperature, begins to soften at 260° and according to Parks and Spaght,[46] has a viscosity of 6.1×10^{11} measured in the Couette viscometer. At 360° the viscosity has fallen to 2.0×10^{7}, and by tenfold increase of the applied stress, can be shown to be independent of it; the melt is therefore a Newtonian liquid. It may be assumed that at high temperatures, the primary bonds behave like the secondary valence links in ordinary liquids so that a melt appears to consist of small individual molecules, such as are present in ordinary liquids.

(*b*) *Considerations Based on Models*

It is obvious that a polymeric homologous mixture cannot have the properties of a Maxwellian body with only one characteristic time of relaxation. Even a polymer of genuinely equal chain molecules would not be expected to show Newtonian or Maxwellian behavior since, as a result of the deformation of the molecule, the configuration of the system under stress is necessarily different from that of the unstressed system and this difference must have its effect in both flow and relaxation.

Since plastoelastic substances such as rubber or polyisobutylene do not obey Maxwell's rule, many authors[47] have endeavored to explain the observed behavior on the supposition that in such substances a number of systems, each governed by Maxwell's rule, coexist; using a model conception, they regard the body as a collection of interconnected springs with elastic moduli, $\epsilon_1, \epsilon_2, \epsilon_3 \ldots$, each of which is damped by its individual dash pot corresponding to a particular viscosity $\eta_1, \eta_2, \eta_3 \ldots$ Such a system can indeed behave like a natural polymer both in starting up and in recoil or creep, but on stationary flow the viscosity would be independent of the stress, which is never the case for plastoelastic substances.

On the basis of such or similar models attempts have been made to effect the superposition of the individual systems in a quite arbitrary way. Thus, Kuhn thinks that

[46] G. S. Parks and M. E. Spaght, *Physics*, **6**, 69 (1935).

[47] W. Kuhn, *Z. physik. Chem.*, **B42**, 1 (1939). K. Bennewitz, and H. Rötger, *Physik. Z.*, **40**, 416 (1939). W. Holzmuller and E. Jenckel, *Z. physik. Chem.*, **A186**, 359 (1940). T. Alfrey and P. M. Doty, *J. Applied Phys.*, **16**, 700 (1945). A. P. Alexandrow and Y. S. Lazurkin, *Acta Physicochim. U.R.S.S.*, **12**, 647 (1940).

the viscosity can be obtained by summation of the individual viscosities, a procedure which can have no physical meaning since, as we have seen (sect. IV2), the superposition of the viscosities characteristic of the various cohesive mechanisms must be effected in another way. Other authors, *e.g.*, Holzmüller and Jenckel[48] sum up the stresses. This leads to the above-mentioned S bodies which have properties quite different from those of elastomers, which closely resemble the P bodies.

Alexandrow and Lazurkin[49] consider the deformation at constant load as composed of two components: D_0 corresponding to the displacement of segments from their potential wells; and D_{el} the rubber-elastic component due to the orientation of the chain molecules:

$$D = D_0 + D_{el}$$

In rubber-elastic deformation it is assumed that a certain potential threshold of intermolecular forces must be exceeded. Furthermore, in contrast to Kuhn's argument, it is assumed that D_{el} tends toward a finite value, $D_{el\infty}$, so that:

$$D_{el} = D_{el\infty}(1 - e^{-t/\tau})$$

τ is a kind of relaxation time which the authors refer to as the orientation time. If the step over the potential threshold is assumed to be analogous to a unimolecular reaction we can write:

$$\tau = Ae^{q_{el}/RT}$$

where q_{el} is the heat of activation of this reaction. From previous experiments Eley[50] has calculated values between 38 and 75 kcal. per mole for this heat of activation in various polymers; these values are strikingly large; he therefore separates this heat of activation into a component related to the displacement of one segment or link from one position of equilibrium into the next one, and a component related to the rotation about carbon-carbon bonds, this rotation being necessary for the stretching of the chains.

In our opinion, these and similar attempts to superimpose arbitrarily the properties of various individual mechanisms, are superseded by the rigid treatment given by Weissenberg in 1929 (see Sect. II 3, page 744).

Kauzmann and Eyring[51] treat viscous flow as the passage of one flow unit (for simple liquids a molecule) from one potential well to the next one and calculate the activation energy of this process from the temperature coefficient of fluidity $1/\eta$. With increasing chain length this energy increases, but comes rapidly to a limiting value of about 10 kcal. per mole. From this these authors conclude that plastic flow of a chain polymer consists in movements of segments and not of entire molecules from one position of equilibrium to the next. They estimate that one segment in the rubber molecule contains 40 carbon atoms.

[48] W. Holzmüller and E. Jenckel, *Z. physik. Chem.*, **A186**, 359 (1940).
[49] A. P. Alexandrow and Y. S. Lazurkin, *Acta Physicochim. U.R.S.S.*, **12**, 647 (1940).
[50] D. D. Eley, *Trans. Faraday Soc.*, **38**, 299 (1942).
[51] W. J. Kauzmann and H. Eyring, *J. Am. Chem. Soc.*, **62**, 3113 (1940).

(c) *Viscosity of Chain Polymers at Small Deformations and Variable Rates of Deformation*

As is evident from the foregoing the viscosity of plastoelastic substances at small deformations is much smaller than the flow viscosity. The viscosity and elastic modulus at very small deformations may be determined by means of methods using oscillations. The values so obtained, which are strongly dependent upon the frequency used, are termed *dynamic viscosity* and *dynamic elastic modulus*. At extremely low frequencies one obtains the same results as by the rheometric and static measurements of viscosity and elastic modulus, respectively. As the frequency is increased, however, the dynamic viscosity decreases and the dynamic elastic modulus increases, the deformation thus approaching the purely elastic type, since the molecules have no time to generate frictional heat in moving to positions of lower potential energy. For unvulcanized and slightly vulcanized rubber, at room temperature, van der Wyk,[52] using extremely low frequencies, obtained values of 10^6 poises for the dynamic viscosity and about 10^7 dynes for the dynamic elastic modulus. At 97° Mooney[53] found a value of 10^3 poises. At 20 cycles per second and at room temperature the modulus of rubber was found to be 2.4×10^7 dynes per sq. cm. At high frequencies Mason[54] found for highly viscous liquid polyisobutylene at room temperature and 10^5 cycles per second a modulus of about 3×10^7 c.g.s., rising to 10^9 c.g.s. at 10^8 cycles per second, a value approaching that of a solid. Similar results may be expected for rubberlike polymers.

(d) *Behavior of Cross-Linked Polymers*

As an example of a slightly cross-linked polymer we may describe the behavior of vulcanized rubber. The viscosity measured at low frequencies amounts to about 10^6 poises and is little different from that of unvulcanized rubber. The same holds for the modulus.

From the value of the modulus of elasticity, $\epsilon = 10^7$ dynes per sq. cm. and of the viscosity, $\eta = 10^6$ poises, one obtains a value for the relaxation time of about 0.1 second, in agreement with the observations of Kosten. If rubber were an ideal viscous liquid, the tension produced by deformation

[52] A. J. A. van der Wyk, in *Proceedings of the Rubber Technology Conference, 1938, London.* Heffer, Cambridge, 1938, p. 985.

[53] M. Mooney, *Physics*, **7**, 413 (1936).

[54] W. P. Mason and H. J. McSkimin, *communication* from the Bell Telephone Laboratories, Inc., 1948. W. P. Mason, W. O. Baker, H. J. McSkimin, and J. H. Heiss, *Phys. Rev.*, **73**, 1074 (1948).

should vanish completely in a short but measurable space of time. The chains, however, are not capable of unlimited movement as in a liquid, and relaxation is therefore restricted, so that the tension falls to a limiting value, and not to zero as in a liquid. In properly vulcanized rubber, the network prevents further relaxation and the tension then remains constant; unvulcanized rubber and many other rubberlike polymers relax further but at a very much reduced velocity. At constant tension, the deformation therefore continues, and after removing the tension a permanent deformation persists; the specimen shows plastic flow.

The nature of the underlying molecular process is evident from the diminution in birefringence, which accompanies relaxation. This was measured by Misch and Picken[55] in the relaxation of polyvinyl acetate. The fall in birefringence indicates that chains, which were oriented in parallel, become disoriented; as a result of thermal agitation, the chains contract and slide over one another. At room temperature the relaxation time of milled rubber shortly after deformation amounted to several minutes, corresponding to a value of $\eta \sim 10^9$ when $\epsilon = 10^7$ dynes per sq. cm.; the viscosity increased constantly while relaxation continued.

The shorter the chains, the higher is the plasticity and the more do the plastic properties dominate the elastic properties, until finally the rubberlike state gradually changes to the liquid state. The relaxation of very weakly vulcanized rubber practically ceases when the tension reaches a certain level. It shows the phenomena of elastic after-effect and creep. If the tension is suddenly reduced and then observed at constant deformation, the tension increases gradually. At constant tension, the specimen contracts to a greater or less extent.

Although it is not yet possible to give a quantitative theory of these phenomena, it may be assumed that rubberlike substances showing limited relaxation consist of two interpenetrating systems[56]: a netted, nonrelaxing system, the chains of which are prevented from sliding over one another, and a system consisting of free chains which relaxes, if only slowly. On stretching such a rubberlike body, the free chain system is at first oriented along with the netted system and also contributes to the initial tension. Gradually, however, the free chains contract, sliding over their neighbors *i.e.*, relaxation takes place. If the tension is removed, the oriented portions of the vulcanized net contract and, in so doing, compress the relaxed nonvulcanized system. The tension now exerted by the whole system results from the tendency of the network to contract and the opposing tendency of

[55] L. Misch and L. E. R. Picken, *Z. physik. Chem.*, **B36**, 398 (1937).

[56] K. H. Meyer, C. Ferri, and W. Hohenemser, *Kautschuk*, **11**, 88 (1935).

the unvulcanized, compressed fraction to expand. The latter flows once more so that the resultant tension rises, or, if no external resistance is present, the rubber may slowly contract further (creep).

In the ideal case it might be expected that the relaxation of such a system would involve only a definite fraction of the tension $(P_0 - P_\infty)$ since only part of the system relaxes. If P_∞ is the permanent tension, the relation:

$$\ln \frac{P_0 - P_\infty}{P_t - P_\infty} = \frac{\epsilon}{\eta} t$$

should hold. The relaxation time of the relaxing system would then be defined as the time in which the difference $(P_t - P_\infty)$ has fallen to 0.368 $(P_0 - P_\infty)$. Such a system may be said to display limited ideal relaxation.

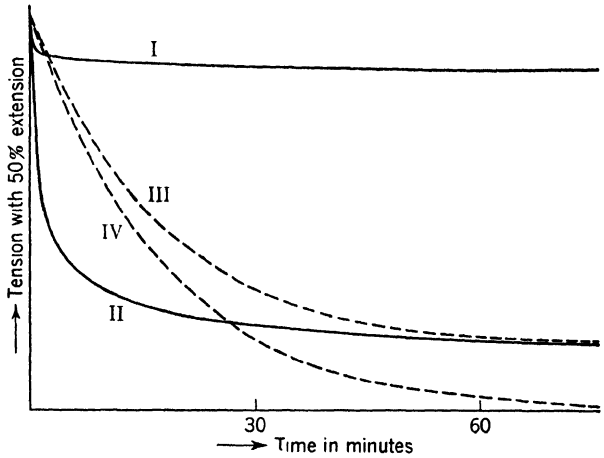


Fig. 14. Relaxation of tension at 20 °C.: (I) slightly vulcanized rubber; (II) unvulcanized rubber; (III) limited ideal relaxation; and (IV) ideal complete relaxation.

In figure 14 the fall in tension of slightly vulcanized rubber is shown (curve *I*) along with that of unvulcanized, slightly masticated rubber (curve *II*). Curve *III* shows the behavior of a body exhibiting limited ideal relaxation. Curve *IV* is the calculated curve for an ideal, completely relaxing body (that is, one with a constant velocity of relaxation). It is clear that the velocity of relaxation diminishes continually in *I* and *II* and we may suppose, therefore, that all transitional stages are present between short, swiftly relaxing chains, longer chains with a greater relaxation period, and finally a completely netted system. The mechanical properties of such a body will

then be determined by the relative proportions of the individual systems and the way in which they interpenetrate.

The dynamic viscosity of rubber is reduced by addition of suitable substances like stearic acid, but on the other hand is scarcely affected by moderate vulcanization. For objects subject to periodic stresses, *e.g.*, truck tires, the dynamic viscosity ought not to exceed a certain limit, otherwise overheating will occur. A practical measure is obtained by rebound tests, in which the kinetic energy loss of a steel ball on striking a rubber slab is determined.

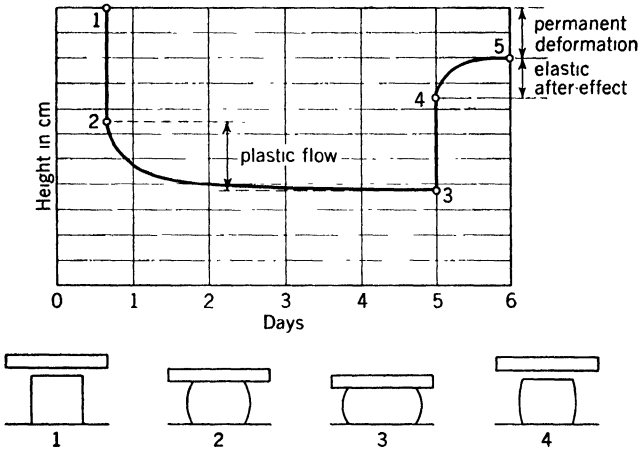


Fig. 15. Plastometer curve for Perbunan (Stöcklin).

In practice, plasticity is measured by various types of plastometers, in which the force, the time, and the deformation are recorded. A relatively simple type of measurement consists in compressing molded samples for a given period; the pressure is then removed and the recovery measured. Extremely complicated curves (Fig. 15 is an example[57]) may be obtained.

It remains to say something about the significance of crystallization in plastic and elastic processes. The crystallization which may take place when rubber is stretched, diminishes the tension; since crystallization does not take place instantaneously, the tension will gradually decrease at constant deformation. This effect may be confused with relaxation by plastic flow. The two effects can be distinguished by raising the temperature. Relaxation due to flow increases if the temperature is raised, while

[57] P. Stöcklin, *Rubber Technology Conference*, London, 1938.

reduction in tension produced by crystallization disappears on heating and the tension rises. The two effects may also be distinguished by the change in birefringence; during crystallization, the birefringence increases but, during plastic relaxation, it decreases. Figure 16 shows the behavior of slightly vulcanized rubber (crystallization[58]) and of polyvinyl acetate (plastic flow[59]).

Plasticity is diminished by crystallization, because the slipping of chains over one another is prevented in the crystalline regions, which act as points of linkage. Systems with a short relaxation period are, therefore, converted

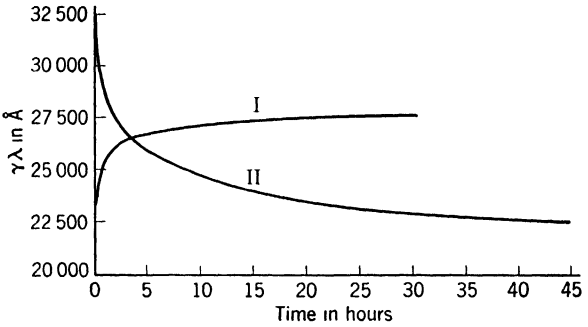


Fig. 16. Change in birefringence: (I) in stretched rubber (Thiessen); (II) in stretched polyvinyl acetate (Misch and Picken).

by crystallization into systems with a very long relaxation period. This explains the well-known fact, as was pointed out by Hinterberger and Neumann,[60] that unvulcanized rubber flows continuously under a small load, while at greater loads, flowing soon ceases. At the higher loads crystallization occurs, which prevents flow.

The molecular interpretation of these complex plastic and elastic phenomena has been considered in some detail, because it is believed that similar processes occur in living tissues and determine their mechanical behavior. The elastic and plastic phenomena in connective tissue fibers, muscles, and other contractile elements will be easier to analyze when those in material of known constitution have been clarified.

[58] P. Thiessen and W. Wittstadt, *Z. physik. Chem.*, **B41**, 33 (1938).
[59] L. Misch and L. E. R. Picken, *ibid.*, **B36**, 398 (1937).
[60] H. Hinterberger and W. Neumann, *Naturwissenschaften*, **26**, 13 (1938).

4. Solutions of Chain Polymers

(*a*) *Solutions of Nonassociated and Un-ionized Polymers*

We shall limit ourselves here to those phenomena which must be taken into account in a theoretical treatment of the subject. Apart from the extremely high viscosity values, it has been established that for chain polymers of very high molecular weight the quantity η_{sp}/c only tends to a constant value at extremely low concentrations. This implies that, even at very low concentrations, the chain molecules are not independent of each other, for, as was seen in the discussion of simple mixtures, the quantity η_{sp}/c only becomes a constant when the term containing η_2 disappears, *i.e.*, the viscosity contribution due to interaction between solute particles becomes negligible.

In the second place, for all chain polymer solutions, η' falls with increasing stress. The deviation from Newtonian behavior becomes progressively less with increasing dilution until it reaches zero for the pure solvent. In such cases, therefore, it is not possible to obtain one single viscosity figure characteristic of a given solution but only a curve corresponding to a flow curve.

In the third place, such solutions exhibit rubberlike elasticity, which may be demonstrated by appropriate methods such as the variation of the apparent viscosity with frequency. The elasticity also becomes less conspicuous with increasing dilution.

Figure 17 shows results of measurements by Philippoff[61] both for honey, which has a constant viscosity over the whole range of frequencies used, and for cellulose acetate solutions for which the "viscosity" varies very appreciably with frequency. The chief cause of these deviations is the elastic stress set up in such liquids. According to Weissenberg, and Eisenschitz and Rabinowitsch,[62] there is a close connection between this variation of η' with stress and the elastic behavior. From the flow curves of solutions of cellulose acetate of varying concentrations in acetone and in other solvents, the following empirical equation has been obtained:

$$\dot{S} = AP + BP^3$$

in which A and B are dependent on the concentration. At extremely small stress values, the second term vanishes before the first and so any solution is Newtonian with $\eta_0 = 1/A$. With increasing stress values the

[61] W. Philippoff, *Die Viskosität der Kolloide.* Steinkopff, Dresden, 1942.

[62] R. Eisenschitz and B. Rabinowitsch, *Ber.*, **64**, 2522 (1931).

deviation from Newtonian behavior increases, corresponding to P^3/B and the quantity $\sqrt{A/2B}$ can be regarded as a measure of the shear modulus.

The elastic stress set up in the liquid not only leads to marked deviations from Newton's rule but changes the whole hydrodynamic behavior. This is demonstrated most easily by experiments with liquids having a relatively large time of relaxation. Such a liquid, *e.g.*, a rubber solution contained in a cylinder rotating at high speed, is projected against the outer wall, like any ordinary liquid. If, however, a coaxial stationary rod is dipped

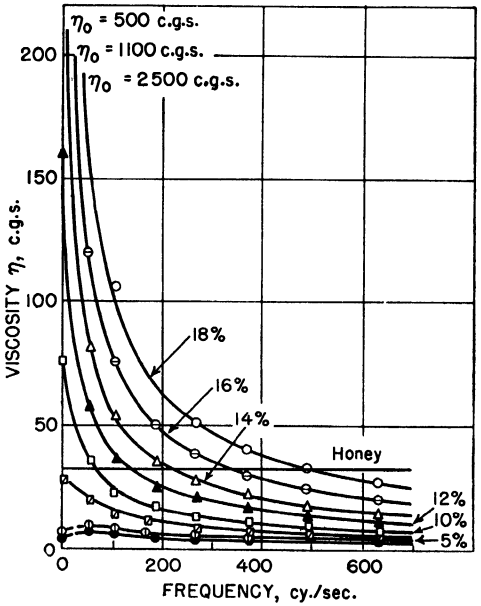


Fig. 17. Dependency of viscosity on the frequency of shearing oscillations for solutions of cellulose acetate in dioxane (Philippoff[61]).

into the liquid, the shearing stress produces a strain which causes the rubber solution to climb up on the rod, while a normal liquid does not show such a tendency.[63] Finally the dependence of $[\eta]$, the intrinsic viscosity, on solvent and on temperature deserves to be emphasized. If solutions of one and the same polymer in different solvents are compared, it is generally found that $[\eta]$ is greater, the greater the heat evolved on mixing. This confirms that $[\eta]$ is a function of the attractive forces between the solvent and solute and increases with them.

[63] K. Weissenberg, *Nature*, **159**, 310 (1947).

Examples of this are found in the values of $[\eta]$ for the athermal solutions of polyisobutylene in *n*-hexane and cyclohexane which are both greater than the value in the endothermal solution in benzene. In addition to this, the molecular weight of the solvent plays some part as shown by the values for $[\eta]$ for polyisobutylene in homologous paraffins (Table V).[64] In equation (26*a*) a relationship between molecular weight of solvent and $[\eta]$ does in fact appear.

TABLE V

Intrinsic Viscosity of Polyisobutylene Solutions

Solvent	$[\eta]$
n-Hexane	0.530
n-Heptane	0.855
n-Octane	0.923

Increase in temperature may be accompanied by rise or fall in $[\eta]$ but the variation is on the whole smaller than that in the absolute viscosity itself, which as we have seen can be readily understood (see sect. IV 2). Abnormal flow curves frequently approach Newtonian behavior if the temperature is raised.

From these results it is quite evident that intermolecular forces between solvent and solute cannot be neglected in any theoretical treatment of the viscosity of solutions.

As a rule the addition of a nonsolvent to a solution of a high polymer first depresses the viscosity, which rises again when more nonsolvent is added until finally the solute flocculates or gelates. Solutions of cellulose esters in acetone behave in this way on the addition of water; so does a solution of rubber in benzene if alcohol is added. The decrease of viscosity may be explained by the coiling of the chain molecules and the decrease of interaction with the mixed solvent; the subsequent increase results from the formation of micelles.

(*b*) *Solutions of Ionized or Associated Chain Polymers*

It is a well known fact that solutions of dipolar (Zwitterion) chain polymers such as gelatin have a pronounced minimum of viscosity at or near the isoelectric point. In 1930 Meyer and Mark[64a] pointed out that, prob-

[64] H. M. Spurlin, A. F. Martin, and H. G. Tennent, *J. Polymer Sci.*, **1**, 63 (1946).

[64a] K. H. Meyer and H. Mark, *Der Aufbau der Hochpolymeren*. Akadem. Verlagsgesellschaft, Leipzig, 1930, p. 232.

ably, here a change of shape is involved, the molecules being coiled at the isoelectric point. Kuhn, Künzle, and Katchalsky[64b] and also Hermans and Overbeek[64c] have shown that the same peculiarities can be observed in solutions of ionogen chain molecules. In a highly diluted (0.011%) aqueous solution of polymethacrylic acid (degree of polymerization Z = 650) the quotient η_{sp}/c at pH 3.5 is increased 130-fold by the addition of 0.5 cc. N NaOH per liter of solution; the amount of alkali corresponds to about $^1/_2$ neutralization.

The polyacrylic acids have been investigated by Kern.[65] The influence of concentration on viscosity is very complicated. At increasing concentration the value of η_{sp}/c at first falls steeply and then rises. The variation in viscosity with alkali concentration is also remarkable; there is a well-defined maximum at the neutral point.

Further studies on ionized polyvalent chain polymers have been carried out by Fuoss.[65a] He investigated salts of polyvinylpyridine, of copolymers from styrene and vinylpyridine, and also the quaternary salts resulting from the addition of butyl bromide to these polymers. The viscosity of a polyelectrolyte solution is much higher than that of an uncharged polymer of the same molecular weight; on the other hand, while η_{sp}/c for other polymers increases with concentration, it decreases for polyelectrolytes. According to Fuoss the following equation holds approximately:

$$\frac{\eta_{sp}}{c} = \frac{A}{1 + B(c)^{1/2}} + D$$

While the constant A is dependent upon the molecular weight, B and D are determined by the influence of the ions in solution upon the ionized groups of the polyelectrolyte. On addition of more free ions B and D diminish and finally disappear. Under these conditions the viscosity corresponds to that of an uncharged polymer at the same concentration. This example clearly shows that in the theoretical treatment of high polymer viscosity the energy interactions with the environment cannot be neglected.

Gelatin is an example of an associating polymer. The viscosity depends on the pH of the solution and also on the salt concentration. It is least at the isoelectric point and shows a maximum at pH 3.2.[66] In dilute solutions, where micelle formation is reduced, variations of viscosity with

[64b] W. Kuhn, O. Künzle, and A. Katchalsky, *Helv. Chim. Acta*, **31**, 1994 (1948).
[64c] J. J. Hermans and J. Th. G. Overbeek, *Bull. soc. chim. Belge*, **57**, 154 (1948).
[65] W. Kern, *Z. physik. Chem.*, **A181**, 283 (1938).
[65a] R. M. Fuoss, *J. Polymer Sci.*, **3**, 602 (1948).
[66] J. Loeb, *J. Gen. Physiol.*, **3**, 54 (1921).

concentration, temperature, and shearing stress do not differ from those observed for other chain polymers. In more concentrated solutions, however, marked deviations appear, which are due to the formation of fringe micelles. At a certain concentration the viscosity rises sharply and a yield point appears, due to the formation of a network. If the temperature is raised a marked fall in viscosity occurs, due to the micellar breakdown. Since the formation of micelles constitutes a kind of crystallization which takes its course slowly, the end values on cooling are only slowly attained; similar aging effects are observed in the case of other micellar solutions such as methyl cellulose in water (see Chap. H, sect. IV3*c*).

(*c*) *Equations Relating Concentration to Viscosity*

The probability of molecular interaction can no longer be calculated by means of the mole fraction using formula (25). As a rough approximation, the probability can be estimated, if instead of the mole fraction we introduce the volume fraction φ:

$$\ln \eta_{rel} = \varphi_2 k_0 \, 2 \ln \frac{\eta_{12}}{\eta_1} + \varphi_2^2 k_0^2 \ln \frac{\eta_1 \eta_2}{\eta_{12}^2} = \varphi_2 k_1 + \varphi_2^2 k_2$$

It is true that no simple physical meaning can be attributed to the constants k_1 and k_2; nevertheless, those quantities must be closely connected with the intermolecular forces. It will be remembered that η_2 signifies the viscosity of the pure high polymer, and η_{12} the binary viscosity which is due to the exchange effect between polymer and solvent.

The formula has been verified for certain high polymer solutions as shown in Table VI in which measurements by Daneš,[67] and also by van der Wyk and Sievers,[68] are given for two different polystyrenes. Appreciable deviations only appear at concentrations above 20%. The equation:

$$\ln \eta_{rel} = k_1 \varphi + k_2 \varphi^2$$

is valid up to relatively high concentrations for many other chain polymer systems.

The viscosity–concentration curves of many chain polymers are essentially similar; in cases of micellar solution, a more steeply ascending curve is obtained, *e.g.*, for gelatin; this is also true for several branched polymers such as amylopectin acetate. But whatever special form the curve may take, it shows neither maxima nor minima. It is, therefore, not very diffi-

[67] V. Z. Daneš, *Kolloid-Z.*, **68**, 110 (1934).

[68] A. J. A. van der Wyk and J. F. Sievers, *unpublished results.*

TABLE VI

Viscosities of Polystyrene

Polystyrene in toluene[68] at 20°			Polystyrene in benzene[67] at 25°		
	Log η_{rel}			Log η_{rel}	
$w \times 10^2$	Observed	Found	$w \times 10^2$	Observed	Found
0	0	0	0.138	0.0092	0.0091
2 5	0.44	0.41	0.276	0.0182	0.0184
5	0.825	0.80	0.554	0.0361	0.0367
7.5	1.13	1.14	1.106	0.0711	0.0717
10	1.45	1.44	2.260	0.144	0.144
12.5	1.70	1.70	4.474	0.277	0.275
15	1.93	1.93	9.006	0.527	0.515
17.5	2.11	2.11	Log $\eta_{rel} = -10w^2 + 6.6w$		
20	2.31	2.26	Instead of the volume fraction φ		
22.5	2.50	2.36	the weight fraction w was used,		
25.0	2.72	2.42	which here scarcely differs from φ.		
Log $\eta_{rel} = -31.1w^2 + 17.5w$					

cult to suggest fairly simple empirical equations, which by means of one, or better, two constants, necessarily determined for each individual system, conform sufficiently well to the course of the viscosity–concentration curve. Without any attempt at completeness, a list of such equations is given below. A and K are constants to which no physical meaning is attributed; φ signifies the volume of dissolved substance divided by volume of solution.

Arrhenius, Berl, and Duclaux:[69]

$$\log \eta_{rel} = K\varphi$$

Baker:[70]

$$\eta_{rel} = (1 + a\varphi)^K$$

Bingham:[71]

$$\eta_{rel} = \frac{K}{K - \varphi}$$

Fikentscher and Mark:[72]

$$\eta_{rel} = 1 + K\,\frac{b\varphi}{1 - b\varphi}$$

b = "solvation volume" occupied in solution by 1 cc. of dry substance.

[69] S. Arrhenius, *Z. physik. Chem.*, **1**, 285 (1887). E. Berl, *Z. ges. Schiess- u. Sprengstoffw.*, **5**, 197 (1910). J. Duclaux and E. Wollmann, *Bull. soc. chim. biol., IV*, **27**, 414 (1920).

[70] F. Baker, *J. Chem. Soc.*, **103**, 1653 (1913).

[71] E. C. Bingham, *Fluidity and Plasticity*. McGraw-Hill, New York, 1922, page 203.

[72] H. Fikentscher and H. Mark, *Kolloid-Z.*, **49**, 135 (1929).

Fikentscher:[73]

$$\log \eta_{rel} = \left(\frac{0.75K^2}{1 + 1.5K\varphi} + K\right)\varphi$$

Sakurada:[74]

$$\eta_{rel} = 1 + a\frac{b\varphi}{1 - b\varphi}$$

b = specific volume of particles in solution.

Bungenberg de Jong:[75]

$$\log \frac{\eta_{sp}}{\varphi} = a + K\varphi$$

Papkov:[76]

$$\log \eta_{rel} = K\varphi^a$$

Kratky and Saito:[77]

$$\eta_{rel} = 1 + \frac{1}{3}\left(\frac{l}{d}\right)^2 \varphi + K\left(\frac{l}{d}\right)^4 \varphi^2$$

l/d = axial ratio.

Philippoff and Hess:[78]

$$\eta_{rel} = \left(1 + \frac{[\eta]}{8}\varphi\right)^8$$

Bredée and van Bergen:[79]

$$\eta_{rel} = 1 + 2.5V_0\varphi + 3.0V_0^2\varphi^2 + 1.1V_0^4\varphi^4$$

V_0 = "voluminosity."

Bredée and de Booys:[80]

$$\eta_{rel} = 1 + 2.5V_0\varphi + 15\left(\frac{2.5}{6}\right)^2 V_0^2\varphi^2 + \ldots + \left(\frac{2.5}{6}\right)^6 V_0^6\varphi$$

[73] H. Fikentscher, *Cellulosechem.*, **13**, 58 (1932). G. V. Schulz and G. Sing, *J. prakt. Chem.*, **161**, 161 (1943).

[74] I. Sakurada, *Kolloid-Z.*, **63**, 311; **64**, 195 (1933).

[75] H. G. Bungenberg de Jong, H. R. Kruyt, and J. Lens, *Kolloid-Beihefte*, **36**, 429 (1932).

[76] S. Papkov, *Kunststoffe*, **25**, 253 (1935).

[77] O. Kratky and G. Saito, *Cellulosechemie*, **16**, 585 (1935).

[78] W. Philippoff and K. Hess, *Z. physik. Chem.*, **B31**, 237 (1936). W. Philippoff, *Cellulosechemie*, **17**, 57 (1936).

[79] H. L. Bredée, L. A. van Bergen, and J. de Booys, *Chem. Weekblad*, **30**, 223 (1933).

[80] H. L. Bredée and J. de Booys, *Kolloid-Z.*, **79**, 43 (1937).

Bredée and de Booys:[81]

$$\eta_{rel} = \left(1 + \frac{2.5}{6} V_0^1\varphi\right)^6 \qquad V_0\varphi = V_0^1\varphi(1 + V_0^1\varphi)^{a-1}$$

a is a new constant.

Bredée and de Booys:[80,81]

$$\log \eta_{rel} = K'\varphi \qquad K\varphi = K'\varphi(1 + \varphi + 0.1K'\varphi)^{A-1}$$

Houwink, Klaassens, and Veenemans:[80,82]

$$\log \eta_{rel} = \frac{K_1K_2p^6}{1 + p^6}\varphi^{(a_1 + a_2p^6)/(1 + p^6)}$$

K_1, K_2, a_1, a_2, and p are new constants.

Takei and Erbring:[83]

$$\eta_{rel} = \left(1 + \frac{[\eta']}{8}\varphi\right)^8 \qquad \frac{1}{[\eta]'} = a\varphi + \frac{1}{K}$$

a = "stretch factor."

Many attempts have been made to ascribe physical significance to the constants; but all such attempts are based on the erroneous supposition that molecules of dissolved chain polymers behave like relatively large, suspended, compact particles to the surface of which the liquid adheres, so that their effect on the viscosity can be calculated on a purely hydrodynamic basis. This postulate is, however, wholly incompatible with the behavior of chain polymer solutions.

It is not possible, therefore, to obtain from the empirical constants of the viscosity equations physical properties such as solvation volume, degree of coiling, stretch factor, and voluminosity of the solute. Such properties are postulated all too frequently in the colloid-chemical and physicochemical literature. These expressions create a wrong impression to those readers who are not specialists in this field and may even hamper future research.

V. VISCOSITY OF SUSPENSIONS AND EMULSIONS

1. Hydrodynamic Theories

The laminar flow of a liquid is disturbed by suspended particles and this leads to an increase in viscosity. As was first shown by Einstein in 1906, this increase can be calculated on the basis of hydrodynamics. For a sus-

[81] H. L. Bredée and J. de Booys, *Kolloid-Z.*, **91**, 39 (1940).
[82] R. Houwink and K. H. Klaassens, *Kolloid-Z.*, **79**, 138 (1937).
[83] M. Takei and H. Erbring, *Kolloid-Z.*, **95**, 207, 322 (1941).

pension of rigid spheres, which are very small in comparison with the dimensions of the apparatus but large with respect to the molecules of the solvent, and which are completely surrounded by a adherent layer of the latter, the following relationship holds, provided the concentrations are low so that mutual disturbance of the particles can be neglected:

$$\eta_{rel} = 1 + 2.5\varphi \tag{27}$$

$$\eta_{sp} = 2.5\varphi$$

where φ is the volume fraction of the dispersed phase, *i.e.*, the total volume of the spheres divided by the volume of the suspension. According to this only the total volume of the spheres is concerned; whether there are a few large or many small spheres is immaterial.

If the spheres are not rigid but consist of a liquid with viscosity η' and retain their form in the course of flow, then, according to G. I. Taylor:

$$\eta_{sp} = 2.5 \frac{\eta' + 2\eta/5}{\eta' + \eta} \varphi$$

The Einstein postulate has been tested by Eirich,[84] using glass spheres and also fungus spores with radii 3 μ to 160 μ and found valid. On the other hand, in earlier investigations by Bancelin[85] on mastix suspensions, a factor of 2.9 instead of 2.5 was obtained. The sulfur sols investigated by Odén[86] also yielded a larger factor. These deviations were probably due to the fact that the particles in these suspensions were not quite spherical.

Attempts have been made to extend the Einstein relationship which is limited to very dilute suspensions of spheres, to more concentrated suspensions, and to suspensions of more elongated or disklike particles. In more concentrated suspensions the flow disturbances created by the spheres are mutually interacting so that the dissipation increases more rapidly than the concentration. Guth, Gold, and Simha[87] on a hydrodynamic basis arrived at the relationship:

$$\eta_{sp} = 2.5\varphi + 14.1\varphi^2$$

Experimentally, using suspensions of glass spheres and fungus spores, these authors obtained values between 6 and 13 instead of the 14.1 figure.

[84] F. Eirich, *Kolloid-Z.*, **74,** 276 (1936); **81,** 7 (1937).
[85] M. Bancelin, *Compt. rend.*, **152,** 1382 (1911).
[86] S. Odén, *Nova Acta Regiae Soc. Sci. Upsaliensis*, (4) **3,** No. 4 (1913).
[87] E. Guth, O. Gold, and R. Simha, *Kolloid-Z.*, **74,** 266 (1936).

Many other equations have been put forward to relate viscosity to concentration.

W. J. D. van Dyck[88] suggests a semiempirical relationship based on the assumption that the viscosity of a suspension of rigid spheres must approach asymptotically the infinite value if all the spheres are arranged in a close packing, *i.e.*, when $\varphi = 0.74$:

$$\eta_{rel} = \left[1 + \frac{2.5\varphi}{2(1 - \varphi/0.74)}\right]^2 = \left(1 + \frac{1.25\varphi}{1 - 1.35\varphi}\right)^2$$

Experiments by Eilers[88] on the viscosity of aqueous emulsions of an asphalt-free bitumen (particle diameter 1.6 to 6 μ) carried out up to a concentration $\varphi = 0.7$ gave fairly good confirmation of this formula.

Further publications are concerned with the calculation of the friction in suspensions of rod- or disk-shaped particles on a hydrodynamical basis. Such particles are set in rotation in the course of flow; if they are very small the Brownian movement disturbs the directive effect of the flow.

The following formulas have been advanced which claim validity for a particular form of elongated particle and considerable Brownian movement:

According to Peterlin[89] for elongated ellipsoids of revolution:

$$\eta_{rel} = 1 + [1.60 + (p^2 - 2.50)/(15 \ln 2p - 22.50)]\varphi$$

where p is the axial ratio; the formulas hold for $p \gg 1$.

For long rods, Simha gives:[90]

$$\eta_{rel} = 1 + \left[\frac{14}{15} + \frac{p^2}{15(\ln 2p - 3/2)} + \frac{p^2}{5(\ln 2p - 1/2)}\right]\varphi$$

This equation has been found valid in the case of suspensions of fractionated tobacco mosaic virus particles the dimensions of which had been determined by electron microscopy, diffusion and sedimentation, and light scattering.[91]

According to Burgers for elongated cylinders:[92]

$$\eta_{rel} = 1 + \left(\frac{4}{15} \frac{p^2}{\ln p - 1.8}\right)\varphi$$

[88] Cited by H. Eilers, *Kolloid-Z.*, **97**, 313 (1941).

[89] A. Peterlin, *Kolloid-Z.*, **86**, 230 (1939). A. Peterlin and H. A. Stuart, *Z. Physik*, **112**, 1, 129 (1939).

[90] R. Simha, *J. Phys. Chem.*, **44**, 25 (1940).

[91] M. A. Lauffer, *J. Am. Chem. Soc.*, **66**, 1188 (1944). G. Oster, P. M. Doty, and H. Zimm, *J. Am. Chem. Soc.*, **69**, 1193 (1947).

[92] J. M. Burgers, in *Second Report on Viscosity and Plasticity*. Royal Netherlands Academy of Sciences, Noord-Hollandsche Uitg., Amsterdam, 1938.

Further equations have been advanced for special cases *e.g.*, for dumbbell-shaped particles, by Kuhn,[93] Yokoyama,[94] and also by Huggins.[95]

Peterlin and Stuart put forward the following equation for the case of disklike ellipsoids:[96]

$$\eta_{rel} = 1 + \left(\frac{4}{9} + \frac{4}{3\pi p}\right)\varphi$$

Feitknecht, Signer, and Berger[97] have confirmed this formula by a thorough investigation of the viscometric behavior of clear nickel hydroxide suspensions. The size and shape of the particles were determined by use of both x-ray and electron microscope: they were hexagonal plates, average diameter 400–500 Å. and 16 Å. thick.

Another equation for disks has been proposed by Simha:

$$\eta_{rel} = 1 + \left(\frac{16}{15}\frac{p}{\tan^{-1} p}\right)\varphi$$

For a "pearl necklace" consisting of N pearls of radius r and mass m connected mutually by links with the length a between centers, the following equation has been derived by Debye[98] which is based on previous work of Kramers:[99]

$$[\eta] = 0.01\frac{\pi r}{6m} Na^2 \frac{1 + \cos\theta}{1 - \cos\theta}$$

θ being the angle between two successive links.

Other hydrodynamic formulas for flexible long chain molecules have been proposed by Brinkman, Kirkwood, and Riseman, and Debye and Bueche.[99a] They arrive at the conclusion that the intrinsic viscosity is proportional to the molecular weight for rather short chains and becomes asymptotically proportional to the square root of the molecular weight for very large values of the latter. The frequently observed proportionality of $[\eta]$ with $M^{2/3}$ is therefore a transition value for medium chain length. In none of these formulas the energetic interaction of the molecules with the solvent is taken into account; they therefore cannot represent a final solution of the problem.

[93] W. Kuhn, *Kolloid-Z.*, **62**, 269 (1933).

[94] S. Yokoyama, *J. Soc. Chem. Ind. Japan*, **49**, 135 (1946).

[95] M. L. Huggins, *J. Applied Phys.*, **10**, 700 (1939).

[96] A. Peterlin and H. A. Stuart, *Z. Physik*, **112**, 1, 129 (1939).

[97] W. Feitknecht, R. Signer, and A. Berger, *Kolloid-Z.*, **101**, 12 (1942).

[98] P. Debye, *J. Chem. Phys.*, **14**, 636 (1946).

[99] H. A. Kramers, *J. Chem. Phys.*, **14**, 415 (1946).

[99a] H. C. Brinkman, *Applied Sci. Research*, **A1**, 27 (1947); J. G. Kirkwood and J. Riseman, *J. Chem. Phys.*, **16**, 565 (1948); P. Debye and A. M. Bueche, *ibid.*, **16**, 573 (1948).

2. Application to High Polymers

It is not permissible to apply these hydrodynamic relationships to solutions of low molecular polymers. The following example illustrates that the calculation of axial ratios on this basis leads to quite false results. From x-ray data it is known that the molecule of the polymethylene hydrocarbon $C_{28}H_{58}$ is about nine times as long as it is wide. The viscosity of its solution in carbon tetrachloride was found to be in agreement with the equation:[100]

$$\eta_{rel} = 1 + 2.5\varphi$$

and thus, by the hydrodynamic formulas, it should be spherical in shape. On the other hand, if the viscosity is calculated hydrodynamically on the basis of the factual axial ratio of 9, then according to Simha's formula:

$$\eta_{rel} = 1 + 8.5\varphi$$

which cannot be correlated with the experimental value.

The application of hydrodynamics alone seems justified only in the solution of coarse compact molecules. If a high polymer solution is found to follow Einstein's rule, *e.g.*, very high molecular proteins, then it may be concluded that the dissolved particles are spherical in shape, since any deviation from the spherical form results in a factor > 2.5. Only a few proteins give this low value. In the case of several proteins such as albumins and hemoglobin Simha's equation for rodlike molecules gives axial ratios which agree rather well with those deduced from sedimentation and diffusion experiments.[101] Furthermore, it is not adequate to apply purely hydrodynamic equations to solutions of most other chain polymers for, as we have seen in sect. IV4*a* and *b*, the energy exchange effect with the solvent cannot be neglected.

In attempting to explain the observed results solely on a hydrodynamic or solely on an energetic basis, one may arrive at qualitatively correct conclusions; however, a comprehensive theory should take into account both points of view and include the purely hydrodynamic and energetic equations as limiting cases.

VI. VISCOSITY AND CONSTITUTION

1. Viscosity and Constitution of Pure Liquids

Only for individual cases has a more than roughly qualitative relationship between constitution and viscosity of pure liquids been reached. In

[100] K. H. Meyer and A. J. A. van der Wyk, *Kolloid-Z.*, **76**, 278 (1936).

[101] J. W. Mehl, J. L. Oncley, and R. Simha, *Science*, **92**, 132 (1940).

general, the viscosity is found to increase with molecular cohesion; it is especially high for polyalcohols and hydroxy acids, but formamide, for example, does not show especially high viscosity in spite of its very high molecular cohesion. Empirical quantitative relationships have been proposed among others for the normal homologous paraffins and their monosubstituted derivatives. According to Dunstan:[102]

$$\log \eta = MA + B$$

where A is a general constant and B is characteristic for the particular series.

Flory[103] has advanced a different equation for the molten linear polyesters (polydecamethylene sebacate, adipate, and succinate) in which the log of viscosity is a linear function of the square root of the weight average molecular weight $\bar{M}$:

$$\log \eta = A\bar{M}^{1/2} + B$$

and he finds the viscosity–temperature variation as:

$$\log \eta = D + \frac{C}{T} + A\bar{M}^{1/2}$$

where A, B, C, and D are all constants.

2. Viscosity and Constitution for Dissolved Substances

With regard to solutions our knowledge is equally incomplete. In the series of homologous normal paraffins in carbon tetrachloride, $[\eta]$ increases with the number of links z but is not proportional to the molecular weight. From the results obtained for the higher homologues it seems that the quotient $[\eta]/z$ has a maximum value for $z = 40$, *i.e.*, $C_{40}H_{82}$. For compounds other than the homologous series, the relationships are extremely complicated (see Table VII).

It can be seen from Table VII that replacement of a single by a double bond has little effect but that a triple bond causes a quite considerable increase in the viscosity; furthermore, perhydrosqualene (hexamethyltetracosane) has a noticeably higher viscosity than tetracosane of equal length, and cycloheptadecane is considerably more viscous than the analogous chain compound.

For polymeric homologues the facts are as follows: $[\eta]$ is found to in-

[102] A. E. Dunstan, *Z. physik. Chem.*, **51**, 734 (1905).
[103] P. J. Flory, *J. Am. Chem. Soc.*, **62**, 1057 (1940).

TABLE VII

Specific Viscosities of Unsaturated Unbranched, Branched, and Cyclic Hydrocarbons Compared with Normal Analogues
(In carbon tetrachloride, 0.886% at 20°)

Hydrocarbon	$[\eta] \times 10^4$
$C_{10}H_{21}—CH_2—CH_3$	33 ± 5
$C_{10}H_{21}—CH=CH_2$	41 ± 5
$C_{16}H_{33}—CH_2—CH_3$	208 ± 5
$C_{16}H_{33}—CH=CH_2$	201 ± 5
$C_{16}H_{33}—C\equiv CH$	253 ± 5
Cycloheptadecane	237 ± 5
n-Heptadecane	190 ± 5
Perhydrosqualene $H(CH_2CHCH_3CH_2CH_2)_6H$	388 ± 3
Squalene $H(CH_2CCH_3=CHCH_2)_6H$	379 ± 4
n-Tetracosane $H(CH_2CH_2CH_2CH_2)_6H$	327
n-Triacontane (isomeric with perhydrosqualene)	449
16-Ethylhentriacontane	450
n-Hentriacontane (same length as previous example)	464
n-Tritriacontane (isomeric with ethylhentriacontane)	503

crease with molecular weight for unbranched chain polymer homologues; in many cases the relationship already mentioned is found to hold:

$$[\eta] = AM^b$$

where b is usually about two thirds. For different highly branched polymers of equal molecular weight, those with the most branched and compact structure have the lowest viscosities. Undoubtedly here too shape exerts its influence in a similar way to that calculated hydrodynamically for coarse particles.

VII. OPTICAL BEHAVIOR OF STREAMING LIQUIDS AND DEFORMED AMORPHOUS SOLIDS

1. General Remarks Concerning Double Refraction Due to Orientation

It is easily seen that molecules which are not spherosymmetrical are anisotropic to light. This anisotropy may be visualized as an ellipsoid; only in this case, it is not the refractive index which is taken as the radius vector, as in crystals, since this would not have a physical meaning with respect to molecular dimensions. The polarizability α is chosen instead; for nonpolar molecules the axes of the polarizability ellipsoid are closely related to the principal refractive indices of a birefringent medium which we can imagine as consisting of the molecules concerned arranged in

parallel. The polarizability is related to the index n by the following formula, which is strictly valid for gases and approximately so for liquids:

$$\frac{n^2 - 1}{n^2 + 2}\frac{M}{\rho} = \frac{4}{3}\pi N\alpha$$

where ρ is density, M molecular weight, and N Avogadro's number.

With the exception of the so-called crystalline liquids, all fluids at rest, consisting of anisotropic molecules, are optically isotropic, since the molecules take up all possible orientations. If, however, this random state is destroyed, as in a flowing liquid consisting of elongated or flat molecules,or in a deformated elastic body, then birefringence may appear. By the applied forces anisotropic molecules are forced into definite orientation. This orientation can be determined by means of a polarization apparatus in the same way as the axes in a crystal.

The magnitude and sign of the double refraction depend, on the one hand, upon the degree of orientation of the molecule and, on the other hand, on the properties of the molecule itself. To rigid members of flexible chain molecules, *e.g.*, glucose rings in cellulose, a polarization ellipsoid can be ascribed. By this means the double refraction can be referred to the optical anisotropy of the chain members and to their degree of orientation (Signer,[104] Müller[105]).

2. Flow Birefringence of Liquids of Low Molecular Weight

(*a*) *Pure Liquids*

In laminar flow the molecules of a liquid are set in rotation by the frictional forces acting upon them; the velocity of rotation is not uniform. It is slowest if the particles happen to have their greatest extension—rods with the longitudinal axis, plates with their planes—at a definite angle with respect to the direction of flow. This angle is called *the angle of orientation*. During stationary flow, a considerable proportion of the molecules will happen to be in this position; because of this, the angle of orientation χ can be measured by means of optical polarization.

For Newtonian liquids the orientation angle is 45° (see Chap. A, Fig. 17). It is measured not with respect to the direction of flow but to a direction perpendicular to it, so that in the case of negative double refraction the orientation angle is noted as greater than 90°, *i.e.*, 135° for Newtonian liquids.

[104] R. Signer, *Trans. Faraday Soc.*, **32**, 296 (1936).

[105] F. H. Müller, *Kolloid-Z.*, **95**, 138 (1941).

For non-Newtonian liquids, and for suspensions of elongated or disk-shaped particles, the orientation varies progressively with increased stress in the sense corresponding to a disposition of the particles with their greatest extension in the direction of flow. Thus, in cases of positive double refraction the angle $\chi = 45°$ increases to a maximum value 90°, for negative refraction from 135° to 180°.

As far as the magnitude of the birefringence is concerned, it is generally small for low molecular liquids owing to the marked effect of thermal motion; it has been studied first by Vorländer,[106] Sadron,[107] and Buchheim and Stuart.[108]

For laminar flow the magnitude of the double refraction is strictly proportional to the rate of deformation: the quotient $(n_\alpha - n_\gamma)/\dot{S}$ (when $\dot{S}$ is the shear velocity) is a constant, dependent on wave length and temperature and characteristic for each substance; it has been designated the dynamo-optical constant M'. A different constant M, the so-called Maxwell constant, relates the relative double refraction $(n_\alpha - n_\gamma)/n_0$ (n_0 is the mean refractive index) to the stress $P = \dot{S}\eta$, thus:

$$M = \frac{n_\alpha - n_\gamma}{n_0} \frac{1}{\dot{S}\eta} \text{ and therefore } M = \frac{M'}{n_0\eta}$$

The dynamo-optical constants M and M' for a number of compounds are shown in Table VIII for $\lambda = 546$ mμ. The orientation angle in each case was 45° and the double refraction positive.

TABLE VIII

Dynamo-optical Constants[108]

Compound	t, °C.	n_0	η	$M' \times 10^{12}$	$M \times 10^{10}$
o-Dichlorobenzene	15	1.555	0.0139	4.00	1.85
o-Xylene	10	1 502	0.00666	1.84	1.84
Benzene	16	1.512	0.00694	0.67	0.64
Toluene	16	1 503	0.00621	0.97	1 04
Phenylethyl alcohol	16	1 534	0.165	17.00	0.67
n-Heptanol	16	1.436	0.0466	2 72	0.41
Ethyl oleate[109]	25	1.415	0 0703	32.5	3.2

[106] D. Vorlander and R. Walter, *Z. physik. Chem.*, **118**, 1 (1925). D. Vorlander and J. Fischer, *Ber.*, **65**, 1756 (1932).

[107] C. Sadron, *J. phys. radium*, **7**, 263 (1936).

[108] W. Buchheim and H. A. Stuart, *Z. Physik*, **112**, 407 (1939).

(*b*) *Solutions of Low Molecular Compounds*

Solutions of low molecular compounds resemble pure liquids with regard to this phenomenon. If the dissolved molecules are much more strongly anisodiametric than are those of the solvent, then they become oriented to a much greater degree than the pure solvent for which the orientation and double refraction may be neglected. Here too, the favored orientation is at 45° with the direction of flow, as has been established by Signer and Gross[109] for solutions of ethyl oleate in cyclohexanone; also, dilute solutions of a low molecular polystyrene ($M \simeq 5000$, determined by use of the ultracentrifuge) were found to behave similarly. The value for the double refraction increased in proportion to the product of the concentration, the flow gradient, and the viscosity of the solution. The results obtained by Signer and Gross are given in Table IX in which the observed double refraction and the dynamo-optical constants are shown together with the Maxwell constants referred to the pure solute by division with φ (concentration in volume).

TABLE IX

Ethyl Oleate in Cyclohexanone

φ	η	$\frac{n_\alpha - n_\gamma}{\dot{S}} \times 10^{10}$	$\frac{n_\alpha - n_\gamma}{n_0} \times \frac{10^{10}}{\dot{S}\,\eta\varphi}$
1	0.0703	0.325	3 2
0.54	0.038	0.0841	3.0
0.36	0.031	0.0546	3.4
0.01	0.0225	0.0007	2 2

The sign for polystyrene is negative whereas the pure monomer as well as ethylbenzene show positive flow birefringence. Signer concluded from this that in the monomeric compounds the benzene rings, for which the direction of greatest polarizability is in the plane of the ring, lie in the direction of flow; for polystyrene they are perpendicular to the direction of flow, because the polymer is essentially a paraffin chain with benzene rings laterally attached.

The constant M of a low molecular polystyrene (mol. wt. approximately 5000) was about ten times as great as that of oleic ester. This is partly due to the higher inner molecular anisotropy of the polystyrene (benzene rings), and partly to its particularly elongated form which facilitates orientation on flow.

[109] R. Signer and H. Gross, *Z. physik. Chem.*, **A165**, 161 (1933).

Until now, no satisfactory quantitative relationship has been deduced between the magnitude of double refraction, molecular shape, and optical properties of low molecular compounds; the formulas available are founded on a hydrodynamic basis and limited to particles which are very large in all dimensions by comparison with the solvent molecules.

3. Optical Behavior of Streaming Solutions of Chain Polymers

Signer[110,111] and Sadron[112] investigated the streaming birefringence of solutions of high molecular polystyrene and of nitrocellulose. For these high polymers the angle of orientation is much greater than 135° or 45°, respectively, and thus the chain segments responsible for the anisotropy orient themselves more nearly parallel to the direction of flow than small molecules.

For a polystyrene of mol. wt. 100,000, the angle of orientation increases with the concentration of the solution, an indication that the chain molecules mutually drag along and that the solution is not a true liquid but is rather to be regarded as a felt. Whereas molecules of low molecular polystyrene, up to a chain length of about 100 carbon atoms, behave like small anisotropic and strongly anisodiametric, rigid particles, the molecules of high molecular polystyrene have different properties; the birefringence increases more rapidly than proportional to the stress. It may therefore be supposed that the threadlike molecules, which in the liquid at rest are not extended but coiled, become extended as a result of streaming and are forced into the direction of flow. This effect is the more pronounced, the greater the concentration.

The magnitude and sign of the double refraction depend on the solvent. The refraction for an incompletely esterified nitrocellulose is negative in amyl acetate and in cyclohexanone but positive in acetone and in methanol. This phenomenon is reminiscent of the corresponding effect on the birefringence of solid nitrocellulose produced by the oriented adsorption of solvent molecules.

In high polymer solutions, coarser, more anisodiametric—and therefore more readily oriented—particles are frequently present, recognizable by the fact that the birefringence tends to a high limiting value even at low stress values. Signer found such particles in solutions of cellulose acetate or nitrate and succeeded in removing them by careful fractionation But

[110] R. Signer, *Trans. Faraday Soc.*, **32**, 296 (1936).
[111] E. H. Scholten, *Dissertation*, Berne, 1942.
[112] C. Sadron, *J. phys. radium*, **9**, 381 (1938).

according to Signer chain polymers, which in a suitable solvent behave as would be expected for flexible threadlike molecules, can show remarkable features in other solvents. These may well be due to the appearance of larger, more compact particles, micelles, but may perhaps also be explained by a crumpling together and to some extent coiling up of the molecular threads.

4. Birefringence of Deformed Elastoplastic Chain Polymers

An amorphous chain polymeric substance in the equilibrium state does not show birefringence since the optically anisotropic chain segments take up all possible positions. By deformation this state of completely random orientation can be destroyed; a certain orientation of the chain members occurs which is manifested by the birefringence.

For the simple case of elastic extension it can be calculated[113] that, to a first approximation, the double refraction and likewise the elastic stress vary in direct proportion to the relative extension $\Delta l/l$. Experiments, by Farwell[114] on Vinylite (structure, see Chap. C, sect. III3*b*) and by Wittstadt[115] on rubber, show that the birefringence does in fact increase proportionally to $\Delta l/l$ for elastic extension up to about $\Delta l/l = 1$. If plastic flow or relaxation occur, both birefringence and stress decrease as a result of the internal rearrangement of the chains and chain segments.

At higher extensions, for which the proportionality between stress and relative extension no longer holds, the birefringence no longer varies proportionally to the stress; where no crystallization occurs, the stress rises more rapidly than the double refraction; where crystallization does occur, as in rubber, the double refraction increases more rapidly than the stress.

Whereas it is comparatively easy to comprehend what is happening in the course of stretching, it is not at all possible to make precise statements with regard to changes of shape and motion of threadlike molecules under shearing stress, *e.g.*, as in the Couette apparatus. The recoil of an elastoplastic high polymer shows that the chains lie at least partly in the direction of flow. It is not improbable that, as is the case of threads suspended in a steadily sheared liquid, they exhibit a motion similar to that of a conveyor belt, which takes the place of the rotational movement of small rigid rods.

[113] F. H. Müller, *Kolloid-Z.*, **95**, 138 (1941).
[114] H. W. Farwell, *J. Applied Phys.*, **10**, 109 (1939).
[115] W. Wittstadt, *Kautschuk*, **15**, 11 (1939).

5. Flow Birefringence of Suspensions

In cases where the particles are large by comparison with those of the solvent molecules and small with regard to the dimensions of the apparatus, the circular motion undergone by rodlike or disk-shaped particles in a laminar flowing liquid can be treated theoretically on a hydrodynamic basis. Usually the shapes of the particles are then stylized, in so far as they are regarded, for example, as elongated or flattened ellipsoids. The angle of orientation and also the degree of orientation can then be calculated from the stress, the size, and axial ratio of the particles. From the degree of orientation and the optical anisotropy of the particles, the magnitude of the birefringence can also be calculated. Conversely, from measurements of the double refraction conclusions can be drawn with regard to the axial ratio and size of the particles, provided the particles are large compared with the solvent molecules but small with regard to the wave length of the light.

Sadron[116] and also Peterlin and Stuart[117] have derived formulas for the angle of orientation which, when expanded as a series, agree with regard to the first member, although not in the subsequent members. This first member, then, is:

$$\chi = \frac{\pi}{4} + \frac{\dot{S}}{12D}$$

$\dot{S}$ is the velocity gradient, D is the rotation diffusion constant which represents the effect of shape on the Brownian rotary motion. Gans has calculated this constant in the case of elongated and flattened ellipsoidal particles:

$$D = \frac{kT}{\eta_1 v}[p]$$

where v is the volume of the particle, η_1 the viscosity of the solvent, and $[p]$ one of the following functions of the axial ratio p:

For prolate ellipsoids ($p > 1$):

$$[p] = \frac{p^2}{p(4p^4 - 1)}\left[-1 + \frac{2p^2 - 1}{2p\sqrt{p^2 - 1}} \log \frac{p + \sqrt{p^2 - 1}}{p - \sqrt{p^2 - 1}}\right]$$

[116] C. Sadron, *J. phys. radium*, **7**, 263 (1936); **9**, 381 (1938).

[117] A. Peterlin and H. A. Stuart, *Z. Physik*, **112**, 1, 129 (1939). See also O. Snellman and J. Björnstahl, *Kolloid Beihefte*, **52**, 403 (1941).

For oblate ellipsoids ($p' = 1/p > 1$):

$$[p] = \frac{p'^2}{4(p'^4 - 1)}\left[1 + \frac{p'^2 - 2}{\sqrt{p'^2 - 1}} \tan^{-1}\sqrt{p'^2 - 1}\right]$$

The approximate magnitude of the double refraction, expressed in terms of Maxwell's constant M is calculated by Peterlin and Stuart as follows:

$$M = \frac{n_\alpha - n_\gamma}{n_0}\frac{1}{\varphi\eta_1\dot{S}} = \frac{2\pi}{1.5n_0^2}\frac{v}{kT}(g_1 - g_2)\frac{b}{[p]}$$

where $(g_1 - g_2)$ is an expression for the optical anisotropy of the particle, φ the volume concentration, and:

$$b = \frac{p^2 - 1}{p^2 + 1}$$

Satisfactory agreement with the theory is only available for flattened particles, actually the case of clear nickel hydroxide sols for which the particle size and shape were determined by an x-ray method and also by means of electron micrographs.[118]

Other measurements[119] have been made on suspensions of tobacco mosaic virus, which has been shown by electron microscopic investigation to consist of rods of length 3000 Å. and width 150 Å. A quantitative proof of the theory has not, however, been based upon these measurements.

For nonhomogeneous suspensions the position is more complicated. The larger particles are readily oriented in the direction of flow even at low velocity of deformation ($\dot{S}$) while the small particles remain largely unoriented as a result of the predominance of the Brownian movement. They therefore make only a small contribution to the double refraction. Increasing the velocity gradient has therefore little effect upon the orientation of the larger particles and the resulting double refraction. The smaller particles only tend to become oriented at higher values of $\dot{S}$. The superposition of these effects results in peculiar relations between $\dot{S}$ and both the angle χ and the double refraction from which relationships conclusions as to the homo- or heterodispersity of the system can be drawn.[120]

[118] W. Feitknecht, R. Signer, and A. Berger, *Kolloid-Z.*, **101**, 12 (1942).

[119] M. A. Lauffer and W. M. Stanley, *J. Biol. Chem.*, **123**, 507 (1938). G. A. Kausche H. Guggisberg, and A. Wissler, *Naturwissenschaften*, **27**, 303 (1939).

[120] J. R. Robinson, *Proc. Roy. Soc. London*, **A170**, 519 (1939). See also C. Sadron and H. Mosimann, *J. phys. radium*, **9**, 384 (1938).

J. FILMS, FOILS AND MEMBRANES

INTRODUCTION

While in everyday life transparent foils of certain cellulose derivatives are known as films, this term is limited, scientifically speaking, to homogeneous layers of monomolecular or polymolecular thickness, which may be solid, liquid, or gaseous. Laminar structures, the thickness of which is of more than molecular dimensions, are known as foils. If the layer has a definite skeletal structure and consists of a submicroscopic meshwork with liquid filling the cavities, it is known as a membrane.

I. THIN FILMS ON LIQUID SURFACES[1]

1. Apparatus

Many substances form films one molecule thick on the surface of liquids; they are said to spread. The properties of such monomolecular films are best studied in the "Langmuir tray" (Fig. 1).

The rectangular tray, the edges of the sides of which are exactly horizontal, is filled with water to the brim, and the surface cleaned by wiping with the barrier, *X*. The substance to be investigated—a definite quantity of fatty acid, dissolved in pentane, or a weighed albumin crystal, for example—is then placed on the surface; the substance spreads immediately and strikes the sides of the tray, the barrier, *X*, and the float, **AA**. The float is attached to the side by two thin, flexible, finely rolled platinum ribbons or by paraffined silk threads, *B*, so that the float and its ribbons separate the film from the clean water surface These ribbons allow the float sufficient freedom of movement for it to be possible to measure the force acting on it. A mirror, *F*, is attached to the float by a very thin wire, and by means of a beam of light indicates the position of the float on a scale. The spreading force driving the float backward is compensated by an opposing force exerted on the float by rotating the torsion device and the wire, *MM*. In this operation the frame, *PQ*, the lower end of which, *P*, is united with the float by a fine wire, is rotated. The extent to which the torsion device must be twisted, in order to induce the float to return to its position before the film is formed, is a measure of the spreading force. This apparatus is sensitive to changes of about 0.01 dyne

[1] See also N. K. Adam, *The Physics and Chemistry of Surfaces*. 3rd ed., Oxford Univ. Press, London, 1941.

per cm. A very sensitive type of balance has been described by Guastalla.[2]

By displacing the barrier, the surface may be diminished and the pressure exerted on AA observed simultaneously. It is customary to plot surface area as abscissa and force/cm. as ordinate; the curves then correspond to the pressure–volume isotherms of gases and liquids. The surface pressure

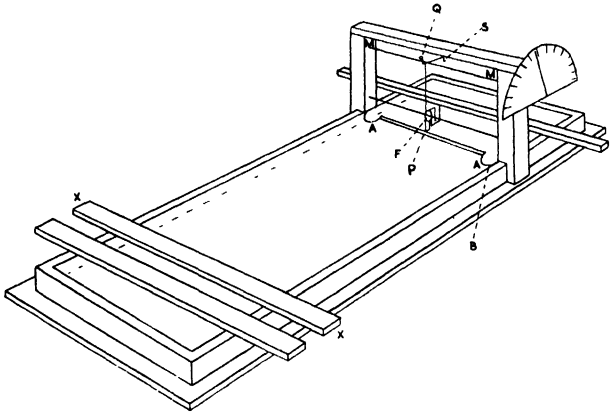


Fig. 1. Langmuir tray for the study of thin films on liquids.

has the dimension of a tension (dyne cm.$^{-1}$); it is equal to the difference in surface tension between the clean surface and that upon which the film is spread.

2. Films of Long-Chain Fatty Acids and Alcohols

Films of long-chain fatty acids and alcohols have been the object of particularly intensive study. The molecules dip their hydrophilic group into the water while the lipophilic residue—the hydrocarbon chain—lies, at great dilution, flat on the water surface. In this region, where each individual fatty acid molecule possesses two-dimensional freedom of movement, the surface pressure, F, is given by the equation:

$$FA = nRT \tag{1}$$

where R is the gas constant and is equal to 8.31×10^7 ergs per degree, n is the number of moles of substance, F is the surface pressure in dynes per

[2] J. Guastalla, *Compt. rend.*, **208**, 973 (1939).

cm., and A is the area of the film in sq. cm. n is evidently equal to W/M where W is the weight of the film in grams and M is the molecular weight of the film molecules. M can thus be deduced: $M = W/n$. This type of film is known as the "gaseous" or expanded film

As the surface area is reduced, the film becomes less compressible, the fatty acid chains are erected and a "liquid," coherent film is formed. Finally, a moment comes when the pressure rises steeply; frequently the film can be displaced as a whole: it is apparently "solid."

A number of chemical reactions can be followed by means of this apparatus. Thus, for example, a liquid film of fatty acid solidifies if baryta is added to the water so that the barium salt is formed; the film can then be pushed about as a whole, as can be seen by blowing on the surface over which talc particles have been scattered.

Oleic acid on permanganate solution is not oxidized if the area per molecule is < 60Å.^{2}, since the double bond is not then in contact with the liquid surface; if the area per molecule is greater, the double bonds come in contact with the reagent and are oxidized.

As Langmuir[3] and Blodgett[4] have shown, a polished, chromium-plated surface becomes covered with a film of fatty acid or barium stearate, if dipped into water on which a film of either of these substances is floating. On withdrawal, a second layer is deposited. While the lipophilic groups are in contact with the chromium surface in the first film, in the second they are directed outward. In this way it is possible to superimpose many layers. If the carboxyl groups are directed outward in the last layer, the film can be wetted by water; if, however, the hydrocarbon residues are exposed, a drop of water will run off the surface. The thickness of such built-up films can be determined by optical methods.

3. Monomolecular Films of High Polymers

From what has been said, it follows that the formation of a monomolecular film (spreading) will occur only for substances containing both hydrophilic and hydrophobic groups, since then the potential energy of the substance is minimal in the film phase. Under these conditions, the former are saturated with water (as in aqueous solution), while the hydrophobic residues reciprocally saturate each other, without separating water molecules, as they would in aqueous solution.

[3] I. Langmuir, *J. Franklin Inst.*, **218**, 143 (1934).
[4] K. B. Blodgett, *J. Am. Chem. Soc.*, **57**, 1007 (1935).

Cellulose derivatives have been investigated by various authors.[5] Such derivatives spread with difficulty; if spreading is achieved, the glucose rings lie flat on the water surface. This is explained by the absence of truly hydrophobic groups.

On the other hand, many proteins are suitable for spreading experiments. In surface films, protein chains are not tangled, even if they were previously in solution in the form of globular proteins, such as egg albumin. In the film phase the molecules are no longer globular; the chains have unrolled and the protein belongs now to the fibrillar type. This change in molecular shape is probably associated with the denaturation of albumin and many other proteins in surface films. As a rule a protein cannot be spread at its isoelectric point: the tangled form is favored. Many nonspreading proteins become capable of spreading after protease attack; presumably, linkages are ruptured which previously hindered unrolling.

For films of high polymers equation (1) must be refined by a correction for the area b occupied by the molecules themselves:

$$F(A - b) = nRT$$
$$FA = nRT + Fb \qquad (1a)$$

When FA is plotted against F then b is the slope of the line obtained and nRT is the intercept of this line on the FA axis. From these data and the weight of the film W the molecular weight M may be calculated: $M = W/n$. Guastalla[6] and Bull[7] have determined M for several proteins in the spread condition. Their values have been computed in Table I, together

TABLE I
Molecular Weights of Proteins

Protein	Solution	Film
Ovalbumin	43,800	44,000
Gliadin	27,000	27,000
Hemoglobin	69,000	12,000
β-Lactoglobulin	41,800	17,100
Zein	40,000	20,100

[5] N. K. Adam, *Kolloid-Z.*, **61**, 168 (1932); *Trans. Faraday Soc.*, **29**, 90 (1933). J. R. Katz and P. J. P. Samwell, *Naturwissenschaften*, **16**, 592 (1928); *Ann.*, **472**, 241 (1929); *ibid.*, **474**, 296 (1929). S. E. Sheppard and R. L. Keenan, *Nature*, **121**, 982 (1928). S. E. Sheppard, A. H. Nietz, and R. L. Keenan, *Ind. Eng. Chem.*, **21**, 126 (1929).

[6] J. Guastalla, *Compt. rend.*, **208**, 1078 (1939).

[7] H. B. Bull, in *Advances in Protein Chemistry*, Vol. III, Academic Press, New York, 1947, page 95.

with the molecular weights obtained by ultracentrifugal or osmotic measurements. Some of the proteins dissociate in the film phase into several fragments.

Protein films become coherent and elastic if the pressure is raised: the film is said to have gelled; when a pressure of 10–15 dynes per cm. is reached the film collapses. Formation of complexes between proteins and various compounds such as cholesterol or detergents has been repeatedly observed.

Langmuir, Schaeffer, and Wrinch[8] have deposited "built-up" films of egg albumin on metal surfaces. The metal plate, of the size of a microscope slide, is first coated with a film of barium stearate and is then dipped in water, on the surface of which egg albumin has been spread, and withdrawn without depositing a second film. The exposed surface of the film is wet; on drying the thickness is 20 Å. If the plate is dipped into pure water and brought out through the film, a film can be deposited in the opposite way. The exposed surface of such a film is dry and nonwettable and a thin layer of water is enclosed between the film and the metal plate. These albumin films are highly impermeable to organic, apolar solvents.

II. FOILS AND MEMBRANES[9]

The properties of foils and membranes are determined by their chemical composition, state of aggregation, and texture. Rigidly cohering, homogeneous foils, consisting of crystallites only, such as are encountered in metal foils, occur only in inorganic high polymers, such as mica. Foils consisting of crystallites recognizable as individual crystallites under the microscope, such as are formed by the evaporation of solutions of guttapercha, chip readily. Amorphous liquid sheets of high polymers can be obtained only at high temperatures, but at ordinary temperatures both amorphous, solid foils, and amorphous, rubberlike foils are known. Foils may also be obtained which contain both amorphous and crystalline regions.

1. Amorphous Foils

Foils in the rubberlike condition can be stretched so that orientation of the chain molecules in the direction of stretching occurs. From the point

[8] I. Langmuir, V. J. Schaeffer, and D. Wrinch, *Science*, **85**, 76 (1937).

[9] A comprehensive survey of membranes and permeability problems will be found in a series of papers by Manegold: E. Manegold, *Kolloid-Z.*, **82**, 269 (1938); **83**, 146, 299 (1938). E. Manegold and K. Kalauch, *ibid.*, **86**, 186, 313 (1939). See also the discussion of the Faraday Society on natural and synthetic membranes, *Trans. Faraday Soc.*, **33**, 411 (1937).

of view of the uptake of foreign substances and hence from the point of view of permeability, they behave like viscous liquids. They are able to dissolve suitable liquids; frequently a limited quantity of liquid is taken up without any perceptible amount of high polymer passing into solution. The plastic and elastic properties of such swollen foils are changed as compared with those of pure foils. The permeability of such swollen foils is very considerably increased since transport now occurs through the absorbed liquid. The foil consists of a network of primary valence chains of the high polymer in which the liquid fills the meshes and, hence, such foils are to be regarded as membranes, in accordance with the introductory definitions.

2. Cellulose Foils

Transparent films of cellulose are generally obtained commercially by squirting a viscose solution through a slit into a precipitating bath. It is customary to use a pre-ripened and somewhat degraded cellulose; in this way, although the mechanical strength is reduced below the limit otherwise attainable, it is easier to obtain an optically homogeneous, nonstreaked film. Commercial viscose foils, such as *cellophane*, contain carboxyl groups—shown by their staining with basic dyes and by their electrokinetic potential, etc. Similar foils can be prepared from solutions of cellulose in ammoniacal copper hydroxide.

X-ray analysis shows that these foils contain very small crystallites along with amorphous material. They are, in fact, structures composed of fringed micelles, the regions in which a regular crystalline arrangement prevails acting as points of linkage between the chains, so that a net structure is formed. X-ray analysis shows a certain degree of orientation: the greater part of the micelles have their long axes in the plane of the foil; in many preparations they also tend to lie in the direction of spinning or rolling. The foil therefore swells anisotropically in water, becoming much thicker, while the area of the film increases only slightly. The birefringence of the foils is in agreement with this structure, as van Iterson[10] showed.

One must suppose that the cellulose solution, which can be regarded as a felt of cellulose chains, retains on coagulation a correspondingly loose structure; as a result of rolling and drying, this felt is then pressed flat. Figures 2 and 3 illustrate diagrammatically the change taking place on pressing.

[10] G. van Iterson, Jr., *Chem. Weekblad*, **30**, 2 (1933).

According to Manegold and Viets,[11] commercial cellulose foils contain about 80% of water when saturated; the framework of cellulose chains is therefore rather spongy when the foil is saturated with water. Damp foils of cellulose are permeable to water and salts of low molecular weight but impermeable to particles 40 Å. and more in diameter. They may therefore be used to eliminate compounds of low molecular weight from solutions of high polymers by dialysis. According to Trillat[12] dry foils are impermeable to air, while damp foils allow air to pass. The air therefore penetrates through the dilated, water-filled pores. Cellulose foils take up water and

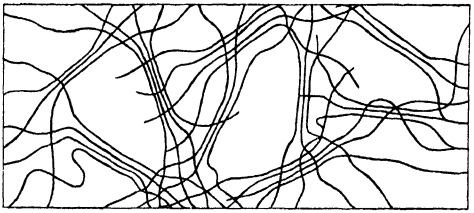


Fig. 2. Structure of a coagulated, swollen cellulose foil (diagrammatic).

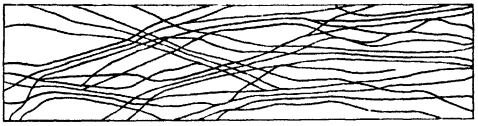


Fig. 3. Structure of a pressed and dried cellulose foil (diagrammatic).

lower alcohols in intermicellar swelling. Commercial cellophane, for example, contains up to 15% of glycerol as a softener. The extensibility in this condition is about 20%. During coagulation, the foil is considerably more plastic; advantage is taken of this fact in the manufacturing of capsules and containers.

If a concentrated viscose solution is poured out in a thin layer on a solid surface and covered with a layer of concentrated salt solution, a much swollen cellulose xanthogenate film is formed. If this is carefully treated with dilute acetic acid, a swollen foil of cellulose II (containing *ca.* 800% of water) is obtained. This displays rubberlike elasticity.

Cellulose foils can be obtained by the saponification of ester foils. The texture and pore diameter correspond to that of the original ester foils.

[11] E. Manegold and K. Viets, *Kolloid-Z.*, **56**, 7 (1931).

[12] J. J. Trillat and M. Matricon, *J. chim. phys.*, **32**, 101 (1935).

Since it is possible to vary the structure of such foils considerably, use is made of them in ultrafiltration when organic solutions are to be filtered—solutions which would attack the nitrocellulose filters commonly used.

3. Foils from Cellulose Esters

Foils of esters—chiefly nitrate and acetate - can be obtained from acetone solutions of the ester in question by pouring this onto a suitable surface and allowing the solvent to evaporate. If the semisolid or solid foil still containing solvent is brought into a suitable precipitant, such as dilute acetic acid, a spongy foil with numerous pores is formed. Coagulation results in the formation of a framework of cellulose ester micelles, the cavities of which are filled with water. By using mixtures of solvents and varying the amount of solvent present at the moment of coagulation, the pore size can be graded and foils obtained letting through particles up to 500 mμ in diameter (Bechhold,[13] Erbe,[14] and Elford[15]). The texture of such porous foils obtained by precipitation is very sensitive to water loss, and they have to be stored in water, since an irreversible diminution in pore size occurs on drying.

Graded ultrafilters are in use for separation and characterization of particles in colloidal suspensions, as well as for the separation of bacteria, virus particles, etc. From the fact that a substance passes through a given filter, one can draw conclusions about the diameter of its particles, if these are spherical. In general the pore diameter is about twice as great as the diameter of the particle; this empirical relation holds for particles up to 500 Å. in diameter.

If the solvent is allowed to evaporate completely from the solution of cellulose ester, foils are obtained which, for all practical purposes, are impermeable to gases and water. The mechanical properties of such "pore-free" filters can be varied by the choice of initial materials, the mode of preparation and the addition of various substances. Degraded material, for example, gives brittle, friable films. The tensile strength of the foils often (but not always) runs parallel to the spec. viscosity of the ester from which the foil was prepared.[16] This is because the viscosity only indicates the presence of constituents of high molecular weight, while the presence

[13] H. Bechhold and K. Silbereisen, *Biochem. Z.*, **199**, 1 (1928). H. Bechhold, *Kolloid-Z.*, **66**, 329 (1934); **67**, 66 (1934).

[14] F. Erbe, *Kolloid-Z.*, **59**, 32 (1932); **63**, 277 (1933).

[15] W. J. Elford, *Trans. Faraday Soc.*, **33**, 1094 (1937).

[16] H. J. Rocha, *Kolloidchem. Beihefte*, **30**, 230 (1930). K. Werner and H. Engelmann. *Angew. Chem.*, **42**, 438 (1928).

of a considerable amount of breakdown products, having a profound influence on the mechanical strength, may be overlooked. Foils from the same nitrocellulose differ in mechanical strength according to the solvent in which they are dissolved (Jones and Miles[17]), indicating that their structures differ. The presence of small quantities of nonvolatile solvent (softeners), such as are used commercially, has a profound influence on the structure and mechanical properties of the foils.[18]

Deformation by stretching, whereby all the chains become oriented in the same direction, has recently been investigated in various ways. As we have seen already, it is possible to determine the degree of orientation by x-ray examination; the necessary formulas have been critically reviewed by Go.[19] Kratky[20] discovered the simple rule that the degree of orientation depends on the degree of stretching. The velocity of stretching and the state of imbibition have no effect on this relationship.

On the other hand, the mechanical properties of the stretched material are very considerably influenced by a number of factors, such as swelling prior to stretching, the velocity of stretching, etc. For this reason, it is impossible to give a stress-strain curve of general validity.

III. PERMEABILITY OF FOILS AND MEMBRANES

1. Permeability of Amorphous Foils

(*a*) *Permeability to Gases*

The quantity of dissolved substance diffusing across unit area in unit time is equal to the product of the concentration gradient and the diffusion coefficient, D. The concentrations in the boundary layers of the foil are equal to the concentrations in the adjoining phases, c_1 and c_2, multiplied by the absorption coefficient, a, provided that equilibration with the environment takes place more rapidly than the diffusion process, which is always true if the environment is gaseous.

As a rule, the term *permeability* refers to the passage of *diffusing* matter;

[17] G. G. Jones and F. D. Miles, *J. Soc. Chem. Ind.*, **52**, 251T (1933).

[18] See, in this connection, A. V. Blom, *Kolloid-Z.*, **61**, 234 (1932); **65**, 223 (1933).

[19] Y. Go, *Bull. Chem. Soc. Japan*, **13**, 198 (1938).

[20] P. H. Hermans, O. Kratky, and P. Platzek, *Kolloid-Z.*, **86**, 245 (1939). O. Kratky, *ibid.*, **64**, 213 (1933); **68**, 347 (1934); **70**, 14 (1935); **84**, 149 (1938). F. Breuer, O. Kratky, and G. Saito, *ibid.*, **80**, 139 (1937). O. Kratky and P. Platzek, *ibid.*, **84**, 268 (1938). Also P. H. Hermans, *ibid.*, **81**, 143 (1937); **83**, 71 (1938). P. H. Hermans and A. J. de Leeuw, *ibid.*, **81**, 300 (1937); **82**, 58 (1938); *Naturwissenschaften*, **25**, 524, 794 (1937). P. H. Hermans, J. de Boovs, and D. Vermaas, *Kolloid-Z.*, **86**, 107 (1939).

as soon as streaming movements are involved the term *porosity* is used. The permeability of a solid or liquid sheet with respect to a gas or a dissolved substance is expressed by the permeability coefficient P, which is the quantity of substance diffusing through unit area and unit thickness in unit time under unit concentration difference between the adjoining phases. The dimensions of the permeability coefficient are cm.^{2} sec.$^{-1}$, like those of the diffusion coefficient, from which it can be obtained by multiplying by the partition or absorption (solubility) coefficient a.

Foils in the rubber state or entirely amorphous (such as rubber foils or polystyrene foils free from cracks) behave like liquids. As in the case of a liquid layer, Fick's diffusion law holds:

$$\frac{dc}{dt} = aD\frac{d^2c}{dx^2} \tag{2}$$

The permeability of such foils to gases is thus determined by the diffusion coefficient, which depends in part on the cross-sectional area of the diffusing molecule and on the viscosity of the foil, and by the absorption coefficient of the gas in the latter. Hence, gases such as ethylene and carbon dioxide, which are soluble in hydrocarbons, diffuse more readily through rubber foils than do hydrogen or oxygen (Daynes[21]).

The quantitative calculation for the stationary state was made by Daynes.[21] Barrer[22] and others[23] have determined P and D for different gases through various polymers (Table II).

TABLE II

Permeability of Polymers to Gases

Material	Gas	Temperature, °C.	P, cm.^{2} sec.$^{-1}$, $\times 10^9$	D, cm.^{2} sec.$^{-1}$, $\times 10^8$	Solubility, cc. of gas/cc.
Rubber 1.7% S[22]	N_2	40	17.3	199	0.066
	CH_4	40	50 1	145	0.262
	n-C_4H_{10}	40	550	34	14.3
Polyvinyl chloride[23]	H_2O	30	149	1.55	
Polystyrene[23]	H_2O	32	840		
Polyethylene[23]	H_2O	32	86		

[21] H. A. Daynes, *Proc. Roy. Soc. London*, **A97**, 286 (1920); *Trans. Inst. Rubber Ind.*, **3**, 428 (1927-28).

[22] R. M. Barrer, *Trans. Faraday Soc.*, **35**, 628, 644 (1939); **36**, 644 (1940): **39**, 237 (1943). R. M. Barrer and G. Skirrow, *J. Polymer Sci*, **3**, 549 (1948); G. J. van Amerongen, *ibid.*, **5**, 307 (1950).

[23] P. M. Doty, *J. Chem. Phys*, **14**, 244 (1946). P. M. Doty, W. H. Aiken, and H. Mark, *Ind. Eng. Chem.*, **38**, 788 (1946). See F. H. Müller, *Kolloid-Z.*, **100**, 355 (1942). P. M. Hauser and A. D. McLaren, *Ind. Eng. Chem.*, **40**, 112 (1948).

(*b*) *Permeability with Respect to Dissolved Substances*

Similar considerations hold for the passage of dissolved substances through a liquid layer immiscible with the solution or through a homogeneous amorphous foil. In place of the solubility, a, the relative solubility, or partition coefficient, of the penetrating substance between foil and surrounding solvent, τ, figures in the equation:

$$\frac{dc}{dt} = \tau D \frac{d^2c}{dx^2} \qquad (2a)$$

where D is the diffusion coefficient of the permeating substance in the foil and $\tau = l_f/l_e$ (l_f and l_e are the solubilities in the foil and in the surrounding medium).

As Overton[24] found, the velocity of penetration of many substances through plant cell membranes runs parallel with the partition coefficient of the substances between oil (olive oil) and water. From this, Overton concluded that the substances penetrate through parts of the cell membranes possessing solubilities similar to vegetable oils and known as lipoids. Later investigations by Collander[25] showed that the velocity of penetration is also dependent on the molecular volume and hence on the diffusion coefficient.

Collander worked with the giant cells of the alga, *Chara ceratophylla*. Each cell contains 10–20 mm.^{3} of cell sap. The cells were left for a given period in aqueous solutions of the nonelectrolytes, and the cell sap was then removed, by piercing and sucking out the contents with a capillary, and analyzed. The permeability coefficients are approximately proportional to the partition coefficients; larger molecules show a smaller permeability coefficient, while very small molecules display a higher permeability coefficient than that corresponding to strict proportionality. The permeability of the living cells to glycerin and sugar is very small but increases more than 1000-fold after death.

The significance of the lipoid nature of certain parts of membranes for their permeability emerges from the following considerations: weaker organic acids penetrate more readily than stronger organic acids since the undissociated, lipoid-soluble fraction is larger than the dissociated, hydrophilic portion in the former. For the same reason, organic acids penetrate more readily than their salts.

[24] S. Overton, *Vierteljahrsschr. naturforsch. Ges. Zürich*, **44**, 88 (1899).
[25] R. Collander, *Trans. Faraday Soc.*, **33**, 985 (1937).

2. Permeability of Membranes to Nonelectrolytes

In membranes (that is, foils) consisting of a more or less compact framework or felt of primary valence chains or micelles, filled with liquid, dialysis takes place through the liquid-filled pores. If the pores are very large, so that the framework offers scarcely any obstacle, the velocity of dialysis is determined by the velocity of diffusion of the dialyzing substance in the liquid filling the pores. The framework only diminishes the area across which diffusion occurs and lengthens the path of the diffusing substance. Taking into consideration these two facts, Manegold[26] was able to calculate the coefficient of free diffusion from measurements of dialysis through collodion membranes rich in water, and found good agreement with the experimentally determined value. If the pores are small, the movements of large molecules are impeded and their dialysis proceeds much more slowly. This phenomenon, discovered by Traube, is known as the sieve effect. In considering cellulose foils, an example of the sieve effect has already been discussed; commercial viscose foils in the moist condition retain particles whose diameter exceeds 40 Å. Ultrafiltration is, of course, a sieve effect.

In addition to the effect of molecular size, dialysis is also influenced by interaction between the substance of the membrane and the diffusing material. If, as in the case of a membrane of nitrocellulose in the presence of nitroaniline, the forces of attraction between these two substances are greater than those between water and nitrocellulose or between water and nitroaniline, nitroaniline will become concentrated around the nitrocellulose framework, and, macroscopically considered, the foil will absorb and dissolve nitroaniline. The nitroaniline molecules are now able to migrate along the nitrocellulose framework, so that under the influence of a nitroaniline concentration gradient across the membrane, nitroaniline diffuses through the nitrocellulose foil as through a liquid layer.

3. Permeability of Membranes to Ions[27]

Permeability with respect to ions and, hence, the electrical conductivity of a membrane, which depends on the transfer of ions through the membrane, is dependent in the first place on the nature of the pore system. In the search for satisfactory insulating varnishes, substances containing hydrophilic groups must be avoided, since they tend to swell and hence the

[26] E. Manegold, *Kolloid-Z.*, **49**, 372 (1929).

[27] Surveys: R. Hober, *Naturwissenschaften*, **24**, 196 (1936); *Physiol. Rev.*, **16**, 52 (1936). E. Manegold, *Kolloid-Z.*, **85**, 186 (1939).

volume of the intermicellar spaces tends to increase; it is also necessary to note carefully whether cracks or fissures appear when the film is formed, as happens frequently in crystallizing substances at the boundaries of the crystallites. From this point of view, the less regularly built copolymers are preferable to the homopolymers—for example, cellulose acetobutyrate is preferable to the triacetate.

(*a*) *Selective Ionic Permeability*

It has long been known to biologists that many biological membranes, such as the envelope of the red blood corpuscle, are selectively permeable to anions, while others, such as apple peel, are selectively permeable to cations. This has generally been determined by experiments on the exchange of ions between two solutions separated by the membrane in question. The literature on this subject is very great, since selectivity has often been regarded as the key to many biological processes. In the attempts made to explain selectivity, the membrane was usually looked upon as a system of positively or negatively charged tubes, the electrostatic properties of which, together with differences in pore diameter, were supposed to explain the phenomenon. This point of view was adopted by Michaelis[28] in his investigations of synthetic selective membranes.

A more fundamental explanation of selectivity first became possible when the chemical composition of membranes and their component primary valence chains was taken into consideration. The view that both synthetic and natural membranes, or parts of organs behaving as membranes, consist of a meshwork of primary valence chains to which lateral, ionizable groups are attached, led the way to the understanding of selectivity. This knowledge has led in turn to conclusions which concern other fields, such as that of bioelectric phenomena. Since these results provide an example of how fruitful the study of high polymers may be, even for the seemingly more remote fields of biology, they will be discussed here, with the conviction that yet other examples of this happy alliance will be added to the list in the future.

Membranes may consist of a network of neutral, acid, basic, or amphoteric primary valence chains. When the chains of the framework bear no lateral ionizable groups, the membrane, or, more correctly, the network, is neutral; if acid groups, such as carboxyl groups, are attached, as in the pectins, the membrane is acidic; if both acidic and basic groups are attached, as in protein chains, the framework is amphoteric and, according to the *p*H of the environment, is acidic or basic.

The indiffusible ions bound to the chains are neutralized by mobile ions of opposite sign, present in the liquid permeating the membrane. If the membrane is bounded by two electrolytes, ions of the sign of the mobile ions of the membrane can change places both with these and with ions of the same sign on the other side of the membrane, that is, the membrane is selectively permeable to these ions. Permeability depends, therefore, on the presence of mobile ions in the membrane. A membrane made from a high polymeric acid will therefore be permeable to cations, while one from a polymeric base will be anion-permeable. In a medium the *p*H of which is on the alkaline side of the isoelectric point of the membrane, an amphoteric membrane will be cation-permeable; the membrane becomes anion-permeable when the *p*H lies on the acid side of the isoelectric point. The

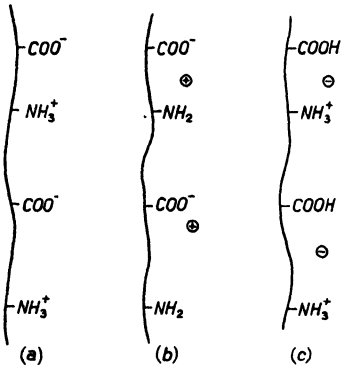


Fig. 4. Amphoteric primary valence chains as constituents of a membrane network: (*a*) at the isoelectric point, no selectivity; (*b*) on the alkaline side of the isoelectric point, cation permeability; and (*c*) on the acid side of isoelectric point, anion permeability.

selectivity is then reversed. Figure 4 shows this relationship clearly. Owing to their acidic character, membranes permeable to cations are stained by basic dyes, while anion-permeable membranes stain with acidic dyes. The basophilic or acidophilic staining properties of a tissue or cell component are therefore an indication of its cation or anion permeability. Conversely, one may conclude from cation selectivity that the high polymer forming the network is an acid, or from anion selectivity, that the polymer in question is a base.

As a result of the deposition or adsorption of acidic or basic substances, the character of a membrane can be made acidic or basic; thus, an acidic membrane can be formed from neutral cellulose by dyeing with substances containing sulfonic groups. Similarly, a membrane of cellulose acetate can be made basic and permeable to anions by the inclusion of a basic dyestuff.[28]

[28] L. Michaelis, *Naturwissenschaften*, **14**, 33 (1926), and later papers in *J. Gen. Physiol.* W. Wilbrandt, *J. Gen. Physiol.*, **18**, 933 (1935); *Trans. Faraday Soc.*, **33**, 956 (1937).

(*b*) *The Quantitative Theory of Selectivity*[29]

The quantitative theory of ionic selectivity was developed simultaneously by Meyer and Sievers[30] and Teorell.[31] The theory depends on the application of the Donnan theory to real membranes. If a membrane is brought in contact with a solution of an electrolyte, a certain quantity of electrolyte will penetrate into the membrane, so that, when equilibrium with the surrounding electrolyte solution is established, the membrane will contain, in addition to the nondiffusible ions and diffusible ions associated with these ("counter ions"), diffusible ions of both signs.

As has been seen already, the permeability depends on these mobile ions. The selectivity is defined by the ratio n_K/n_A where n_K is the number of cations passing through in unit time and n_A the number of anions. This ratio of the permeability numbers is related to the ionic mobilities, U_K and U_A, and to the ratio of the concentrations of mobile cations and anions present in the membrane.

For the sake of simplicity, let us choose a binary electrolyte which is completely dissociated. Let the concentration of the surrounding electrolyte solution be c, the concentration of the penetrated electrolyte, expressed in gram equivalents per liter of imbibed liquid be y and the concentration of the nondiffusible, fixed ions in gram equivalents per liter of imbided liquid be A. A is a constant, characteristic for the membrane at a certain liquid content, and may be called the *selectivity constant*, since it determines the selectivity. A is also equal to the concentration of the counter ions neutralizing the nondiffusible ions. The total concentration of diffusible cations (in a cation-permeable membrane) is $y + A$, that of the diffusible anions, y. According to the Donnan equation, we have the relation:

$$c^2 = y(y + A) \tag{3}$$

and since:

$$\frac{n_K}{n_A} = \frac{U_K(y + A)}{U_A A}$$

[29] Comprehensive treatment of the dialysis potential by E. Manegold and K. Kalauch. *Kolloid-Z.*, **86**, 186 (1939).

[30] K. H. Meyer and J. F. Sievers, *Address to the Assn. Chim. Genève*, 1935; *Helv. Chim. Acta*, **19**, 649, 665, 987 (1936). K. H. Meyer, H. Hauptmann, and J. F. Sievers, *ibid.*, **19**, 948 (1936). K. H. Meyer and P. Bernfeld, *ibid.*, **28**, 962, 972, 980 (1945).

[31] T. Teorell, *Proc. Soc. Exptl. Biol. Med.*, **33**, 282 (1935).

it follows that:

$$\frac{n_K}{n_A} = \frac{U_K \sqrt{4c^2 + A^2} + A}{U_A \sqrt{4c^2 + A^2} - A} \tag{3a}$$

The selectivity, n_K/n_A, is therefore dependent on the concentration, as is shown in figures 5 and 6. At very low external concentration (Fig. 5) the meshwork of the membrane contains practically nothing but diffusible counter ions and the membrane is permeable only to ions of the same sign as the counter ions. At higher concentration (Fig. 6), ions of both signs

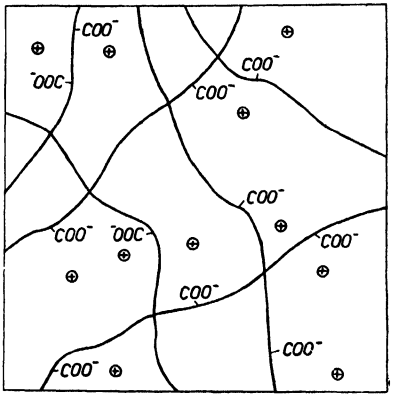


Fig. 5. Cation-permeable membrane in a dilute solution of an electrolyte. Mobile cations only are present.

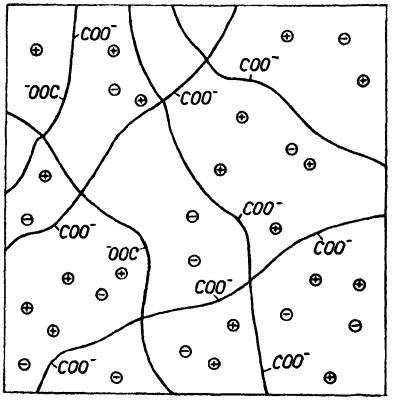


Fig. 6. Cation-permeable membrane in a concentrated solution of an electrolyte. Mobile anions are also present in the membrane

penetrate the membrane; thus, as the external concentration rises, the permeability with respect to the other ion becomes more and more marked.

If the pores of the meshwork are so wide that the ions are not impeded, U_K/U_A remains equal to the ratio of the transport numbers in water If, on the other hand, the movement of the larger ions is hindered by narrow pores, the value of U_K/U_A is influenced by the sieve effect of the particular membrane.

The concentration of the ions inside the pore system of the membrane may be influenced by the solubility in the pore liquid and by forces of attraction exerted by the molecules of the wall on the ions. The influence of this apparent increase or decrease of solubility on the potential has been

studied by Meyer, Sievers, and Bernfeld[32,33]; it can be taken into account by assigning to each ion a partition coefficient L_K or L_A between the pore and external liquids.

Equation (3) then becomes:

$$c^2 L_K L_A = y(y + A)$$

and equation (3a):

$$\frac{n_K}{n_A} = \frac{U_K}{U_A} \frac{[4c^2 + (A/\sqrt{L_K L_A})^2]^{1/2} + A/\sqrt{L_K L_A}}{[4c^2 + (A/\sqrt{L_K L_A})^2]^{1/2} - A/\sqrt{L_K L_A}} \tag{3b}$$

An equation for the membrane potential of a system of two different electrolytes separated by a membrane has been developed by Meyer and Sievers[33] and tested by Meyer and Bernfeld.[32]

In conclusion we may say that selectivity is dependent on the selectivity constant, the ratio U_K/U_A, the concentration of the ions, and their solubilities L_K and L_A, respectively.

(c) *Membrane Potential in the Study of Membranes*

To begin with it is best to make the following simplifying assumptions. (*1*) The membrane is homogeneous, *i.e.*, it does not consist of layers of different properties and is not a mosaic. (*2*) The concentration of the electrolytes in the liquid layers which are in direct contact with the membrane surfaces is constant, so that an unvarying flow of ions is established within the membrane (and a stationary state on both surfaces). (*3*) The partition coefficients L_K and L_A equal unity.

If a membrane, *i.e.*, a layer of any kind, separates two solutions of an electrolyte, an electromotive force E results from diffusion of the two solutions through the layer. According to Nernst its value is determined by the permeability (transport) numbers n_K and n_A:

$$\frac{dE}{dc} = \frac{RT}{F} \frac{n_K - n_A}{n_K + n_A} \frac{1}{c}$$

After substitution of (3a) and integration between the limits of c_1 and c_2 one obtains, for the potential difference between two solutions of a binary electrolyte of concentrations, c_1 and c_2, the equation:

[32] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **28**, 962, 972, 980 (1945).

[33] K. H. Meyer and J. F. Sievers, *Helv. Chim. Acta*, **19**, 649 (1936).

$$E = \frac{RT}{F}\left[u \ln \frac{x_2 + Au}{x_1 + Au} + \tfrac{1}{2} \ln \frac{(x_1 + A)(x_2 - A)}{(x_1 - A)(x_2 + A)}\right] \tag{4}$$

where:

$$u = \frac{U_K - U_A}{U_K + U_A} \text{ and } x = \sqrt{4c^2 + A^2}$$

By measuring potential differences at different concentrations, a number of equations are obtained, from which the two unknowns, A and U_K/U_A = $(1 + u) / (1 - u)$, can be determined. For this purpose the equation can be rewritten:

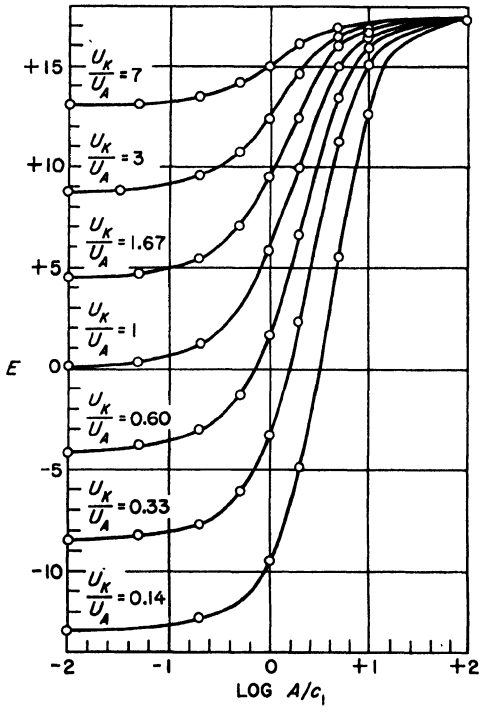


Fig. 7. Potential difference E as a function of log A/c_1 for different values of U_K/U_A.

$$E = \frac{RT}{F}\left[u \ln \frac{x_2/A + u}{x_1/A + u} + \tfrac{1}{2} \ln \frac{(x_1/A + 1)(x_2/A - 1)}{(x_1/A - 1)(x_2/A + 1)}\right] \tag{4a}$$

E is therefore determined by U_K/U_A, x_1/A, and x_2/A (that is, by c_1/A and c_2/A). If we choose a constant concentration ratio (c_1/c_2)—for ex-

ample, $c_1/c_2 = 1/2$—the equation contains two parameters only, U_K/U_A and c_1/A. This enables a simple graphic method of solution to be used.

For a given value of U_K/U_A (*e.g.*, $U_K/U_A = 1$) a curve is drawn of E as a function of $\log A/c_1$. For every other value of U_K/U_A, one obtains another curve (Fig. 7). This graph serves as a basis for the evaluation.

A series of measurements of E are now made keeping the ratio of concentration of electrolytes equal to 1:2, *i.e.*, 0.01/0.02 N; 0.02/0.04 N; 0.04/0.08 N; 0.08/0.16 N; 0.16/0.32 N. These readings of E are entered in the graph as functions $\log 1/c_1$. We then obtain a curve, the "selectivity curve," which is characteristic for the membrane and the electrolytes chosen.

The experimental curve is now compared with the curves of the graph. We ascertain which curve it can be made to fit by displacement parallel to the abscissas. This gives the value of U_K/U_A. We then measure the displacement: it is equal to the difference $\log A/c_1 - \log 1/c_1$ and therefore equal to $\log A$.

(*d*) *Selective Permeability for Hydrogen Ions*

Where several electrolytes are involved, all cations participate in the transport of positive electricity and thus contribute, according to their concentration and migration velocity, to n_K. The same occurs with the anions.

If a sodium phosphate buffer solution of *p*H 6 diffuses directly in a similar solution of *p*H 7, there results practically no electromotive force as the action of hydrogen ions, in spite of their greater velocity, is extremely small with respect to the influence of sodium ions, the concentration of which is 10^5 times greater. If, however, a membrane permeable only to hydrogen ions and impermeable to all other ions is set between the two solutions, there results a membrane potential, such as would be the case, if only the hydrogen ions were present, the others being excluded from carrying electricity and therefore from influencing the membrane potential. The existence of such specific hydrogen ion permeability is thus best revealed by means of buffer solutions.

One cannot, however, distinguish whether a membrane is permeable for hydrogen ions only or hydroxyl ions, or both. One can only say that a membrane is impermeable to all ions except hydrogen or hydroxyl. For, as the product $[H^+]\cdot[OH^-]$ remains constant, the effect of the hydrogen ions in the two solutions equals that of the hydroxyl ions:

$$E = \frac{RT}{F} \ln \frac{[H^+]_1}{[H^+]_2} = \frac{RT}{F} \ln \frac{[OH^-]_2}{[OH^-]_1} = \frac{RT}{F} 2.30(p\mathrm{H}_2 - p\mathrm{H}_1) \qquad (5)$$

at 15° one obtains: $E = 57\ (p\mathrm{H}_2 - p\mathrm{H}_1)$ mv. For greater simplicity, we shall speak of this as hydrogen ion permeability.

(e) *Analysis of a Membrane Consisting of Layers*

In the case of membranes consisting of layers of different permeability, the total potential observed can be considered as the sum of the individual potentials of each single layer. But only the outer layers can be analyzed in the following way. The concentration of the solution on one side of the membrane is kept constant, while on the other side solutions with increasing concentrations are successively brought to bear, each concentration doubling the previous one; *i.e.*, c_1, $c_2 = 2\ c_1$, $c_3 = 2\ c_2$, etc. E is measured immediately after the change of the solution. Only in the outer layer q —

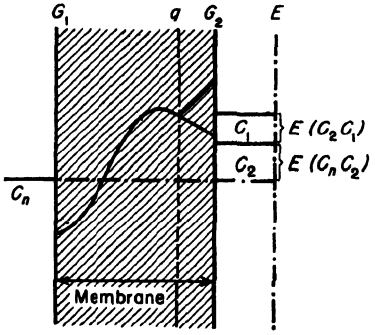


Fig. 8. Analysis of the outer layer qG_2 of a membrane.

G_2 (Fig. 8) is equilibrium immediately obtained, while between G_1 and q the preceding ion distribution remains at first unchanged, so that the sum of individual potentials is kept constant. The potential c_1/outer layer/c_2 is given by the difference $E(c_n/c_2) - E(c_n/c_1)$. Thus, by plotting this number and the following: $E(c_n/c_3) - E(c_n/c_2)$, etc., the selectivity curve can be drawn. The value A and the ratio U_K/U_A for the boundary layer can then be obtained as described above.

Hydrogen ion selectivity can be tested by putting buffer solutions of different pH successively in contact with one surface of the membrane and measuring the difference between the potentials obtained. If the pores of the outer layer are wide, the adjacent solution soon penetrates toward the inner layers. Hence the electromotive force is obviously influenced and may slowly change. If, after a while, the electromotive force reaches a

constant level, it can be assumed that the solution has reached a deeper narrow pored layer through which diffusion is very slow.

If a membrane in layers is bathed by the same solution on either side, no potential will be observed if equilibrium is attained. If this is not the case the membrane presents the phenomenon of *asymmetric potential.* This can be explained in the following way. If the concentration of the ions inside the membrane is different from that of the solution, and if the ions of the inner layers of the membrane, on their way into the adjacent solutions or *vice versa* have to pass through layers of different selectivity, a potential will arise. This asymmetric potential disappears more or less rapidly with death, while living processes maintain the disequilibrium.

If a membrane is of the *mosaic type,* its permeability and selectivity are determined by the areas of greatest permeability.

(*f*) *Examples of Synthetic Selective Membranes*

It should be possible to prepare completely neutral membranes from carboxyl-free cellulose or carboxyl-free cellulose esters; in practice, however, this has not yet been realized.

Examples of cation-permeable membranes are: membranes of partly oxidized cellulose and cellulose dyed with substantive dyes, the sulfonic groups of which make the membrane acid.[33] Nitrocellulose and cellulose acetate membranes are slightly acidic owing to some free carboxyl groups of the cellulose. Polyacrylic acid can be deposited in cellulose acetate membranes by allowing a solution of cellulose acetate and polyacrylic acid in acetic acid to evaporate. Porous collodion membranes become highly selective on oxidation.[34]

A membrane with a very high selectivity constant may be prepared from cellulose glycolic acid by nitrating, dissolving in acetone, precipitating with water, dissolving the dried precipitate in pyridine, and evaporating on a glass plate.

Anion-permeable membranes may be obtained by evaporating a solution of dry Victoria blue base and cellulose acetate in glacial acetic acid.[35] Collodion membranes become highly anion-permeable by adsorption of salmine, a protamine.[36]

A synthetic, amphoteric membrane can be obtained by condensation of triethanolamine with phthalic acid. This membrane, which bears tertiary

[34] K. Sollner, I. Abrams, and C. W. Carr, *J. Gen. Physiol.*, **25**, 7 (1942).
[35] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **28**, 962, 972, 980 (1945).
[36] I. Abrams and K. Sollner, *J. Gen. Physiol.*, **26**, 369 (1943).

amino groups along with carboxyl groups, is anion-permeable in a neutral medium, but becomes cation-permeable when the reaction of the medium is alkaline. The dissociation of the amino groups is suppressed under these conditions, and the carboxyl groups are ionized. If methyl iodide is added to the tertiary amino groups, the membrane remains anion-permeable even in an alkaline medium.

Foils of alkali-containing glass are permeable to cations when in contact with molten sodium nitrate (Manegold[37]); this observation is in agreement with the picture of glass as a primary valence net in which alkali ions neutralize the nondiffusible SiO-ions. Cation-permeable membranes can also be obtained from zeolites.

The best-known example of a membrane permeable only to hydrogen ions is the well-known glass membrane of the glass electrode. Many synthetic organic membranes, *e.g.*, the collodion membrane of Michaelis, exhibit the phenomenon of specific hydrogen ion selectivity in a very imperfect way: the migration velocity of all other ions is only greatly diminished with respect to the velocity of hydrogen ions, but is not reduced to zero.

The membrane potential is suitable for the characterization of very dense systems for which other methods such as gas permeability are unsuitable. This potential can also be used for the characterization of varnishes, etc.; by this means it is possible to say at once whether a varnish contains free carboxyl groups (to be avoided if absolute impermeability to water is desired) or not. Free acidic groups betray their presence by cation selectivity, as has been seen above. Experiments showed that sheets of rubber and of linoxyn, and layers of paraffin, wax, and mastic were distinctly cation-selective.[28,30]

(g) *Examples of Natural Selective Membranes*

Of natural selective membranes, only a few examples will be given. Apple skin is cation-permeable, as are also the bracts of *Iris* leaves. Most vegetable membranes, like plant protoplasm itself, are cation-permeable owing to the content of pectins, acidic hemicelluloses, or higher fatty acids. The membrane of vertebrate erythrocytes is anion-permeable, that of nerve fibers, permeable to potassium but not to sodium. The basal membrane of the skin which is located between the corium and the epithelium is selectively permeable to hydrogen ions; the same is probably true for the membrane of the muscle fiber (sarcolemma).[38]

[37] E. Manegold and C. Stuber, *Z. physik. Chem.*, **A173**, 321 (1935).

[38] K. H. Meyer and P. Bernfeld, *J. Gen. Physiol.*, **29**, 353 (1946).

It may be taken for granted that the structure of most biological membranes is by no means simple. They may be supposed to consist of superposed layers of different materials, either lipoids (permeable to lipoid-soluble substances) or hydrophilic carbohydrate or protein chains (determining ionic permeability and permeability to water). These two types of materials may also occur side by side, forming a mosaic, which may be of molecular dimensions.

(*h*) *Changes in Permeability and Selectivity*

Insight into the structure of selective membranes enables one to predict under what conditions the permeability will change. Change in pore diameter resulting from swelling or shrinking can, within limits, change the permeability. Shrinkage may be due to a change in the salt content of the medium, or to some chemical reaction. Both chemical changes in the substance of the membrane itself—such as the esterification of carboxyl groups—and changes in *p*H of the surrounding liquid may influence the selectivity and even reverse it completely.

IV. PHENOMENA OBSERVED IN THE PASSAGE OF CURRENT THROUGH SELECTIVE MEMBRANES

1. Changes in Concentration

When a direct current is passed through a series of electrolytes and membranes, concentration changes occur. The phenomenon is the reverse

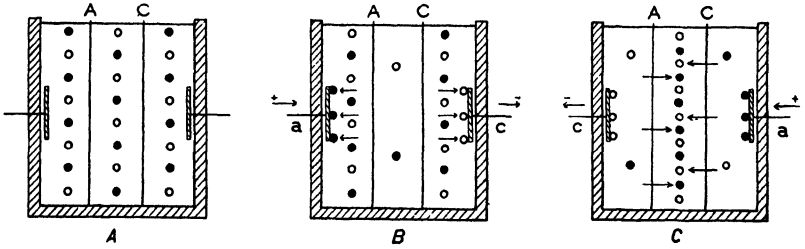


Fig. 9. Migration of ions during the passage of an electric current (○ = cations; ● = anions): A = anion-permeable membrane; C = cation-permeable membrane; a = anode; and c = cathode.

of that just discussed in which an electric current arises as a result of the tendency for equilibrium to be established between electrolytes of different concentration separated by selective membranes. The phenomenon has

been studied by Bethe and Toropoff,[39] and later by Manegold and Kalauch.[40] The nature of the process can be seen from figure 9.

A salt solution is bounded on one side by an acidic cation-permeable membrane C, and on the other by a basic, anion-permeable membrane A. In the spaces on either side, filled with the same solution, electrodes are placed. If the cathode is on the right, cations migrate to the right and anions to the left, leaving the solution in the inner compartment poor in salt. Such a circuit is therefore useful for removing electrolytes from the central compartment by electrodialysis. On reversing the current, salt is concentrated in the central compartment. In both experiments a potential difference opposed to the applied E.M.F., that is, a polarization is set up.

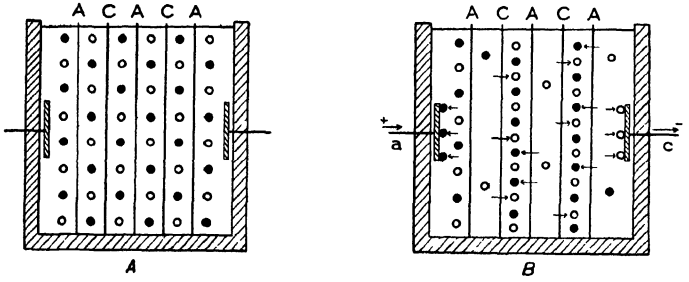


Fig. 10. Polarization during the passage of an electric current through a system containing (A) basic (anion-permeable) and (C) acid (cation-permeable) diaphragms. Lettering as in Figure 9. Distribution of ions: *A* before, *B* after passage of current.

If several cation- and anion-permeable membranes occur in alternation, as in figure 10, concentration will take place in individual compartments, leading to correspondingly increased polarization.

2. Changes in *p*H

The change in concentration mentioned in the last paragraph is always accompanied by a change in *p*H. The latter is caused by the hydrogen and hydroxyl ions participating in carrying electricity, according to their concentration and to their migration velocity. Hydrogen ions migrate with other cations through a cation-permeable membrane; as a result of this displacement hydrogen ions will be found in excess on that side of the membrane which is directed toward the cathode, thus giving rise to an acid

[39] A. Bethe and T. Toropoff, *Z. physik. Chem.*, **88**, 686 (1914); **89**, 597 (1915).
[40] E. Manegold and K. Kalauch, *Kolloid-Z.*, **86**, 313 (1939).

reaction, whereas on its other side hydroxyl ions will appear; through a membrane permeable to anions, hydroxyl ions will migrate together with the other anions, thus giving rise to an alkaline reaction on the anodic side of the membrane and an acid reaction on its other side.

The changes in *p*H will be the more pronounced the greater the difference between the mobility of the hydrogen ions and that of the other cations. An amount of hydrogen ions equivalent to the electricity carried will thus appear on one side of a membrane solely permeable to hydrogen ions and the corresponding amount of hydroxyl ions will be found on the opposite side.

These variations in *p*H occur also in the living organism under the influence of an electric current; it is very probable that acidic and alkaline reactions thus produced play their role in the well-known phenomenon of the electric stimulus.

V. BIOELECTRIC PHENOMENA

The study of the permeability of membranes to ions and of the potentials arising in ionic dialysis leads almost inevitably to the consideration of bioelectric phenomena. The agents responsible for electrical phenomena (injury currents, action currents, or currents produced in special electric organs) in living organisms can only be electrolytic ions, since free charges of electrons or positrons do not occur in aqueous solutions and are therefore excluded from living systems. While it was thought initially that such currents were due to diffusion potentials, it was shown later that this explanation is inadequate, and that membrane potentials must be the source of bioelectric phenomena.

1. Resting Potentials

Differences of potential arising between two solutions of an electrolyte which are brought in contact with two different points of an organ are usually designated as *resting potentials*. If one of the solutions is placed on a cut surface, the potential difference is called the *injury potential* (or even injury "current"). The organ acts as a layered membrane; the measured potential difference is the sum of those at the successive surface layers inside the organ. Quantitative conclusions, therefore, cannot be drawn from the mere observation of the total potential difference; however, it should be possible to analyze the surface layers of the intact surface as well as of the cut, using the method outlined above. Until now such analyses have only been carried out very incompletely.

2. Action Currents as Phenomena Attendant upon Chemical Reactions

The astonishingly rapid changes in potential, known as action currents, which can be observed in all active organs, must be due to rapid changes in the biological system with its fixed and free ions and its membranes influencing ionic exchanges.

If the causes of such variations in potential are sought, it is found that they may be due: (*1*) to changes in the concentration of ions; (*2*) to changes in the permeability of tissues or membranes; or (*3*) to both these effects. Most physiologists favor the theory originally put forward by Bernstein[41] and Tschermak,[41] according to which certain ions in the organ in question are completely separated from the body fluid by a membrane, which, at the moment of activity, suddenly becomes permeable to one of the two types of ions. This ion then diffuses out and therefore charges the external liquid. The opposite case is also conceivable: that the membrane of the resting organ is permeable to a given ion and that at the moment of activity the doors shut, so that once again a change in potential difference results.

As we have seen above, however, such changes in permeability cannot occur except as the result of some underlying chemical change which is associated with changes in ionic concentration. Case (*2*) ist herefore excluded as a possible explanation. On the other hand, change in ionic concentration [case (*1*)] is a phenomenon accompanying all metabolic processes. Ions are formed and disappear in the hydrolysis of creatine phosphoric acid, in the formation of lactic acid from sugar and the oxidation of this acid, in the formation and disappearance of acetylcholine—in short, in many reactions which are known to produce their effects rapidly. If such reactions occur in a medium in which free diffusion is impeded by cation- or anion-permeable elements, they must result in variations in electrical potential.

If the diffusion of all ions except hydrogen ions is impeded by a membrane selectively permeable to hydrogen ions, then all changes in *p*H regardless of the concentration of other ions will give rise to a change in the membrane potential.

Conversely, variations of the membrane potential observed in living cells enveloped in such a membrane indicate variations of the *p*H inside the cell. This is, for instance, the case in muscle fibers: the negative action

[41] J. Bernstein, *Elektrophysiologie*, Vieweg, Braunschweig, 1912. A. von Tschermak, *Allgemeine Physiologie*, Vol. I, Springer, Berlin, 1924.

potential observed during contraction is to be attributed—at least in part—to an increase in *p*H in the fiber.[38]

Since it is certain that membranes exhibiting specific permeability are found in all living cells it can be said that in a living organ variations in the electrical potential are to be expected whenever metabolic processes lead to the production or removal of ions, in particular of hydrogen ions. To give an example: if, as is known from the work of Loewi, Dale, and Nachmansohn, acetylcholine is formed and disappears during the nervous impulse, we can be certain that it is only a question of the way in which the experiment is arranged and of the sensitivity of our measuring instruments, whether or not we can follow this reaction as a variation in electrical potential. Conversely, if the propagation of the nervous impulse leads to the appearance of a negative variation in potential travelling along the nerve, we may conclude that a chemical reaction, involving a change in concentration of ions, is propagated along the nerve and is followed by a second reaction, bringing back the ionic concentration to its normal value.

K. MOLECULAR STRUCTURE OF ANIMAL AND PLANT TISSUES[1]

It has become the task of a special branch of morphology to investigate the structure of living substance down to molecular dimensions. This submicroscopic morphology is therefore intimately linked with the morphology of those structurally important high polymers which constitute a considerable part of the subject of high polymer chemistry.

Objects less than 2000 Å. in any one dimension cannot be resolved by the microscope. Diffraction phenomena, it is true, enable even smaller particles to be seen as points of light, but the form of the particles can no longer be determined. By using ultraviolet light and an optical system transparent to ultraviolet radiation, it is possible to obtain photomicrographs of objects of dimensions down to about 0.1 μ. In such photographs materials which absorb ultraviolet radiation appear dark.

Most proteins absorb ultraviolet light of a wave length of about 275 $\mu\mu$ (Soret's absorption band). Nucleic acids, on the other hand, have a strong absorption band with a maximum at 260 $\mu\mu$ (Dhéré's absorption band). Photomicrographs of an unstained tissue section taken with monochromatic ultraviolet light may reveal the distribution of proteins and nucleic acids. By using the electron microscope one obtains a resolution which approaches molecular dimensions. Objects, however, must withstand the effect of high vacuum which may induce transformations in the organisms. Electron microscopy has given very promising results in the study of collagenous and muscular fibers, in bacterial cytology, and in the investigation of viruses. In spite of these successes the greater part of our knowledge of submicroscopic morphology has been obtained by less direct methods, such as the interpretation of X-ray diffraction patterns, the study of light scattering and of the mechanical, thermal, and electrical properties.

[1] General reviews: W. J. Schmidt, *Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma*, Borntrager, Berlin, 1937. A. Frey-Wyssling, *Submikroskopische Morphologie des Protoplasmas und seiner Derivate*, Borntträger, Berlin, 1938; English edition, Elsevier, New York-Amsterdam, 1948. L. E. R. Picken, "The Fine Structure of Biological Systems," *Biol. Rev.*, **15**, 133 (1940). F. O. Schmitt, *Physiol. Rev.*, **19**, 270 (1939). W. Seifriz, *Structure of Protoplasm*, Iowa State College Press, 1942.

I. MICELLAR THEORY AND CHAIN MOLECULE THEORY

1. Micellar Theory

From the optical properties of starch grains and cellulose fibers as revealed by the polarizing microscope, Nägeli reached the conclusion that they were composed of submicroscopic, elongated, crystalline particles which he called micelles. Later, Ambronn and Frey,[2] making the distinction between intrinsic and structural birefringence, demonstrated the internal anisotropy and, as was then supposed, the crystalline nature of the particles, and described the cellulose micelles as elongated submicroscopic crystallites. By the same method Möhring[3] demonstrated the presence of "crystalline" micelles in chitin, and Stübel,[4] the presence of such micelles in muscle. Consequently, when Scherrer, in cellulose, and Herzog and his collaborators, in chitin and muscle, showed with the aid of x-rays that a latticelike arrangement did in fact exist in these substances, it was generally believed that the crystalline micelle was the structure unit of living substance.

Systematic investigation of the optical properties of animal tissues by Schmidt[5] revealed in many cases the presence of birefringent elements, sometimes persistent, sometimes only appearing in the course of physiological processes. Such birefringent elements, present in sperm tails, in the pseudopodia of *Thalassicola*, etc., were called by him micelles, following Ambronn's views on the crystalline nature of such particles.

None of these investigations[6] attempted to link up the shape and dimensions of molecules (already beginning to be revealed by physical methods) with the study of microscopic morphology, and so to bring about the fusion of morphology and chemistry.

2. Chain Molecule Theory

The first attempt to link biological and molecular morphology was made by Sponsler and Dore,[7] who referred the x-ray diffraction pattern of cellulose fibers, their tensile strength, anisotropic swelling, and anisotropic ex-

[2] H. Ambronn and A. Frey, *Das Polarisationsmikroskop.* Akadem. Verlagsgesellschaft, Leipzig, 1926.

[3] A. Möhring, *Kolloidchem. Beihefte*, **23**, 162 (1926).

[4] H. Stübel, *Arch. ges. Physiol. Pflügers*, **201**, 629 (1923).

[5] W. J. Schmidt, *Naturwissenschaften*, **16**, 900 (1928).

[6] A survey of the aims and methods of submicroscopic morphology by 1926 is given in the *Ambronn-Festschrift*, edited by Wo. Ostwald and A. Frey, Leipzig, 1926.

[7] O. L. Sponsler and W. H. Dore, *Colloid Symposium Monograph*, **4**, 174 (1926).

pansion on heating, to the fact that the fibers consist of parallel chains of glucose units linked together by primary valences. Although both the modes of linkage of the glucose residues, required by Sponsler, and his x-ray analysis were unsatisfactory, nevertheless, for the first time a morphologically conceived molecule, the cellulose chain, was fitted into a macroscopic, morphological picture, thus initiating the profound change in ideas which has resulted from the introduction of chain molecule models into submicroscopic morphology.

Sponsler's cellulose model was replaced by that of Meyer and Mark. According to these authors, chitin micelles also consist of parallel chains, and the micelles of starch, consisting of parallel chains, or segments are arranged radially in starch grains. Silk is similar to cellulose in structure and

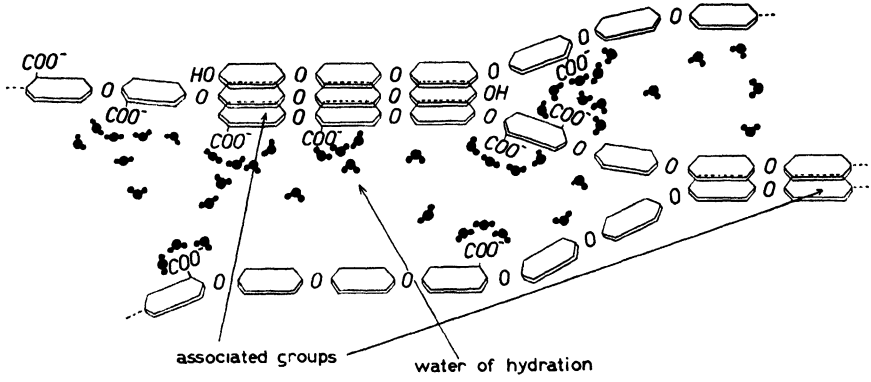


Fig. 1 Diagram showing the arrangement of primary valence chains (polysaccharide chains) in the living plant cell (Meyer, 1930).

formed the first example of a fibrillar protein molecule. All these materials contain long, crystalline micelles, comparable to those pictured by Nägeli and Ambronn.

Subsequent work by Meyer and Mark[8] led, however, in almost all other cases to a different conception of the molecular structure of living matter. Birefringence and anisotropy of mechanical or of swelling properties are not always signs of the presence of crystalline micelles, but are to be referred to the presence of oriented molecules or primary valence chains; the latter may be associated to form crystalline regions, but frequently the chains

[8] K. H. Meyer, *Biochem. Z.*, **214**, 253 (1929). K. H. Meyer and H. Mark, *Der Aufbau der hochpolymeren organischen Naturstoffe*, Akadem. Verlagsgesellschaft, Leipzig, 1930, page 234 *et seq.*

are not associated in this way. In structures composed of proteins in particular, it is exceptional to find such crystalline micelles. Chains of fibrillar proteins are flexible threads which, in living substance, form a loose network. At certain points the chains are linked by chemical bridges or secondary valences, and it is this cohesion which makes possible the preservation of shape. Cell constituents containing pectins are held together in the same way, shown diagrammatically in fig. 1 (p. 827). The three-dimensional network of chain molecules thus replaces the older pictures of the *foam*, *honeycomb*, or *micellar* structure of protoplasm. During the same period similar pictures of the fine structure of living substance were proposed independently by Peters[9] in England and Seifriz[10] in U. S. The same view has been held by Frey-Wyssling[11] since 1938 and has been extended by him to all branches of submicroscopic morphology.

II. INDIRECT METHODS FOR DETERMINING MOLECULAR FINE STRUCTURE OF LIVING SUBSTANCE

It is now proposed to consider observations of all kinds, made on biological objects, in the light of knowledge gained from the physical and chemical study of high polymers. In this way, combining our experience of structurally important high polymers (proteins and polysaccharides) with conclusions drawn from observations on living objects, we shall be able to form a picture of the molecular structure of the individual components of living systems.

1. Points of Linkage in Chain Molecule Framework

The protein chains which constitute the scaffolding of living substance are linked, either by chemical (primary valence) bridges or by molecular cohesion (secondary valence bridges) between certain groups, to form a loose network. These two types of linkage can be distinguished by the action of certain swelling agents, in particular formamide or urea solutions, which rupture secondary valence links only.[12] Linkages of this latter type are present in all reversible protein gels, *e.g.*, gelatin gels; the regions of con-

[9] R. A. Peters, Harden Lecture, *J. State Med.* (*London*), **37**, No. 12, 1 (1930); *Trans. Faraday Soc.*, **26**, 797 (1930).

[10] W. Seifriz, *Am. Naturalist*, **63**, 410 (1928).

[11] A. Frey-Wyssling, *Submikroskopische Morphologie des Protoplasmas und seiner Derivate.* Bornträger, Berlin, 1938; English edition, Elsevier, New York-Amsterdam, 1948.

[12] K. H. Meyer and H. Mark, *Der Aufbau der hochpolyneren organischen Naturstaffe.* Akadem. Verlagsgesellschaft, Leipzig, 1930, page 238.

tact are formed by segments of numerous chains held together by attractive forces into bundles by the action of urea solutions these are separated and liquefaction takes place. Since a considerable fraction of the structure proteins of living cells can also be removed by urea solution we must admit that regions of contact of the same type are also present in living cells.[13] Other molecules may also act as bridges; thus, for example, nucleic acids may link together basic protein chains, or polyvalent metallic ions, such as calcium, can unite with proteins to form insoluble complexes or salts.

In view of the manifold nature of points of linkage, it appears undesirable to attempt a detailed classification such as has been attempted by Frey-Wyssling.[14] The distinction between points of linkage of the primary valence type, associated with high energy and large heat of activation, and linkages of the secondary valence type (with small energy and less heat of activation) may be applied with advantage. For the rest, a detailed study of individual cases will be found to be essential if it is desired to gain any considerable insight into the mechanism of cohesion of the various chain molecules in a tissue or organ.

2. Linkage of Lipoids, Nucleic Acids, and Ions to Molecular Framework

The properties of polyglycine show that the peptide group has no marked tendency to form addition compounds. This property is possessed by the side chains. Roughly speaking, these can be divided into hydrophilic (always polar) and lipophilic, since the latter—that is, the hydrocarbon residues—are related to fatlike substances, and are responsible for linkage with such substances.[15]

Lipoids will therefore be attached with their hydrocarbon residues to the lipophilic groups of the proteins; on the other hand, a linkage between the markedly hydrophilic phosphoric acid or choline residue and acidic or basic protein groups is not excluded.

Many observations suggest that lipoid molecules and protein molecules are associated in the tissue to form a sort of mosaic. This is suggested by the permeability of cell membranes to ions as well as to lipoid-soluble sub-

[13] K. H. Meyer, in W. E. Seifriz, *Symposium on the Structure of Protoplasm*, Iowa State College Press, 1942.

[14] A. Frey-Wyssling, *6th Intern. Bot. Congress*, Amsterdam, 1935; *Submikroskopische Morphologie des Protoplasmas und seiner Derivate*, Bornträger, Berlin, 1938; English edition, Elsevier, New York-Amsterdam, 1948.

[15] K. H. Meyer, *Biochem. Z.*, **208**, 28 (1929).

stances. Such a mosaic may exist on a molecular scale as a protein network in which lipoid molecules are entangled. The intimate association between protein and lipoid is clear from the behavior of living tissues, where lipoids are present in the masked, water-soluble form of lipoproteins.

The relation between proteins and nucleic acids in nucleoproteids is of particular interest. Since the proteins in question are basic histones it is possible that a salt is formed. According to Astbury,[16] the straight molecules of nucleic acid reveal on x-ray analysis a period of 3.3 Å. parallel to the molecular axis. Astbury compares this period with that of the same length in a fully stretched polypeptide chain, and suggests that protein and nucleic acid chains lie parallel in the nucleoproteids and, to a certain extent, interlock. Such double chains of nucleoproteids lie parallel to the long axis of the chromosomes, as is shown by the birefringence of these (Schmidt[17]).

3. Chemical Composition and Fine Structure

If a suitable specimen of a fibrillar protein, or of cellulose or some other polysaccharide (such as pectin or chitin), or of nucleic acid, is stretched, the chain molecules align themselves with their long axis in the direction of stretching. When these materials are spread out in a thin sheet, the axes of the molecules lie in the plane of the film. This suggests the possibility that in fibrils of protein, grown in biological systems, the protein chains lie parallel to the fibril axis. (This holds also for polysaccharides and nucleic acids.) In thin films or membranes the chains lie in the plane of the membrane.

Elongated molecules of fats, fatty acids, or lipoids, on the other hand, are oriented at right angles to the direction of stretching (as Thiessen[18] showed in threads of sodium oleate), since they are oriented at right angles to the longest diameter of their crystallites; in films, such molecules stand approximately normal to the plane of the film.

Up to the present, this regularity has been confirmed in all objects investigated; of these, cellulose fibers and fibrillar proteins have already been discussed. As a further example, the axon sheath of nerve fibers may be mentioned. This is a membrane, composed of lipoids and proteins, surrounding the axis cylinder of the fiber. Meyer,[19] Boehm, [20] Schmidt [21] and

[16] W. T. Astbury and F. O. Bell, *Nature*, **141**, 747 (1938).
[17] W. J. Schmidt, *Naturwissenschaften*, **26**, 413 (1938).
[18] P. A. Thiessen and R. Spychalski, *Z. physik. Chem.*, **A156**, 435 (1931).
[19] K. H. Meyer, *Umschau*, **1932**, No. 20.
[20] G. Boehm, *Kolloid-Z.*, **62**, 22 (1933).
[21] W. J. Schmidt, *Z. Mikroskopie*, **54**, 159 (1937).

Schmitt[22] have shown that the lipoid molecules are, in fact, oriented at right angles to the plane of the membrane, that is, they lie radially.

On the basis of Langmuir's investigation of the behavior of fats in films on the surface of water, it may be argued that wherever lipoids occur, their hydrophilic groups will be directed toward the aqueous phase. The hydrophilic groups occurring in natural lipoids are the alcoholic hydroxyls of cholesterol, the COOH or COONa groups of fatty acids, and, above all, the phosphoric acid group of phosphatides and the quaternary ammonium group of choline-containing lipoids. Where lipoids are in contact with water-filled cavities, they will be oriented in accordance with this principle.

As the apolar, hydrocarbon chains are much more closely related to each other than to water, it is legitimate to suppose that the thinnest lipoid membranes are bimolecular films in which the apolar residues are turned facing each other, while the hydrophilic groups are directed outward and thus establish contact with aqueous or water-containing protein layers.

4. Physical Properties and Fine Structure

Study of the birefringence of microscopically visible objects can give information about the orientation of the molecules if sufficient is known of the chemical composition. X-ray analysis is limited to the study of regions in which a crystal lattice is well established; for the investigation of micellar structures this method is superior to all others.

As early as 1924 Schmidt[23] drew attention to the anisotropy in the mechanical properties of plant and animal fibers, which he interpreted as a sign of the presence of parallel oriented structural units, the nature of which was at that time obscure. It is now known that objects consisting of oriented primary valence chains such as stretched rubber, show typical anisotropy in their mechanical properties; they can be split up into finer and finer fibrils. Conversely, if a biological structure shows a tendency to split in this way, it may be concluded that the object consists of parallel chain molecules.

Similarly, anisotropy in swelling may afford an indication of the structure of the object, as the axis of the chains is at right angles to the direction in which swelling occurs. If swelling takes place in two directions, as in fibrils, the chains lie in that direction in which there is no change in length. Should swelling occur in one direction only, the chains lie in the plane at right angles to the direction of swelling.

[22] F. O. Schmitt, *J. Cellular Comp. Physiol*, **9**, 261 (1937).
[23] W. J. Schmidt, *Naturwissenschaften*, **12**, 269 (1924).

The elastic behavior of biological materials may afford very considerable insight into fine structure, as in muscle, where the relation between the absolute temperature and the elastic force exerted by the stretched muscle has led to conclusions about the molecular structure of muscle fibrils. A positive temperature coefficient of the elastic force indicates that the object is in the rubberlike state. The structure of the object and its ability to withstand deformation are determined by contorted, cross-linked primary valence chains which, on deformation, are forced into a thermodynamically improbable position, from which thermal agitation induces their return as soon as the deforming force is removed.

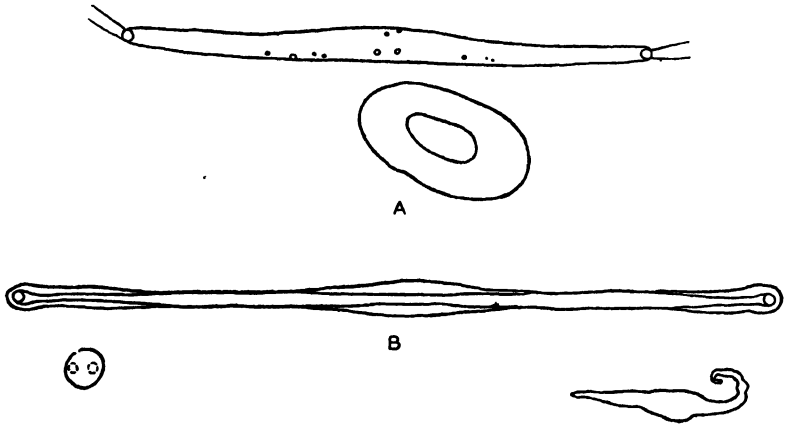


Fig. 2. A, Erythrocyte of the amphibian *Cryptobranchus* stretched between microneedles. Normal erythrocyte below. B, isolated nucleus of an erythrocyte of *Cryptobranchus;* unstretched (diameter = 14 μ), stretched (to 300 μ) and again contracted (W. Seifriz).

As far as the thermoelastic properties of cell components showing high reversible extensibility (that is, extensible to several times their original length) have been studied, they all display this type of elasticity. One can assume, therefore, that an object displaying high reversible extensibility is in the rubberlike state. Twenty years ago Seifriz[24] drew attention to the astonishing elastic extensibility of red blood corpuscles and their nucleus of *Cryptobranchus* which he observed when using the micromanipulator. He reached the conclusion that the ultimate units of the living substance must be elongated in form. At the present time it is to be assumed that the objects in question consist of a loose network of contorted protein chains.

[24] W. Seifriz, *Am. Naturalist,* **63,** 410 (1928).

If such an object shrinks spontaneously on warming, it may be concluded that stretched chain molecules are present, arranged in micelles, and that contraction occurs as a result of thermal agitation of segments. When an object elongates spontaneously on warming, the micelles are placed at right angles to the long axis. A structure of this latter kind has been described by Schmidt[25] in the protein laminae of which the outer portion of the rods and cones of the retina are composed. This work, in which he arrives at a far-reaching analysis of the fine structure of the rods and cones, is a particularly illuminating example of the present-day approach to submicroscopic morphology.

Studies of the electrical properties may also lead to conclusions about fine structure. The detailed investigations of Osterhout[26] on the electrical resistance of the cell wall of the alga, *Valonia*, have revealed the presence of a layer which, in view of its slight conductivity, must consist of lipoids. Then again, selective ionic permeability may indicate the presence of basic or acidic constituents as part of the molecular framework.

Recent work in the field of submicroscopic morphology has shown how science, founded on the study of high polymers, has been able to penetrate successfully to the world of molecular dimensions. But this does not by any means exhaust the contribution of this branch of chemistry to biological problems; we recall the importance of swelling phenomena for the whole field of biological activity, the connection between the elastic properties of high polymers and the biological function of elastic fibers, the fundamental relation between the changes in shape of protein molecules and muscular contraction or protoplasmic movement, the way in which bioelectric phenomena are determined by the peculiar properties of high polymers, and lastly, how the welter of species and individuals is but the realization of the innumerable possibilities of arranging the several amino acids in the giant molecules of the genes. The number of problems in the solution of which biology and the chemistry of high polymers must join forces is inifinite; wherever there is life there is structure, a structure built up from high polymeric molecules.

As research advances, the greater is our perplexity, the greater our veneration and admiration for the true master of our science—living Nature.

[25] W. J. Schmidt, *Kolloid-Z.*, **85**, 137 (1938).

[26] W. J. V. Osterhout, *Trans. Faraday Soc.*, **33**, 995 (1937) and many papers in *J. Gen. Physiol.*

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