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ORGANIC COLLOIDS

by

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Preface

The purpose of this treatise is to give a general survey of organic colloids. The field is growing rapidly and is becoming increasingly important in various industries, as well as in natural science, medicine, agriculture, pharmaceutical technology, etc. The text has been made as elementary as possible as regards the treatment of the various colloid phenomena. Students and research workers who have had basic courses in physics and organic chemistry will find no difficulty in reading this book.

The enormous amount of literature concerning organic polymers and other colloids makes it impossible to include everything in a book of this size. The author has therefore chosen to place the emphasis on basic facts and relationships rather than on theory. Theoretical publications are quoted, but only the most important conclusions of theoretical studies are presented. However, the application of theoretical and empirical laws and rules for solving various practical problems are emphasized. Experimental methods are described, especially those of great practical importance.

The author believes that, in spite of the volume of experimental data and variety of phenomena, the study of organic colloids is not as difficult as it sometimes appears. The size, shape, and chemical constitution of colloidal particles and macromolecules determine largely the properties of colloids. The importance of the influence of chemical composition and constitution on the properties of macromolecular substances is emphasized throughout.

The *Organische Kolloidchemie* of Hermann Staudinger (Nobel Prize Winner 1953) is an outstanding example of clarity and in-

genuity of presentation in the field of colloid chemistry. The author is indebted to Professor Staudinger for introducing him to this field, and for his continuing interest and valuable help during the last twenty years. Staudinger's early conception about the existence of the macromolecules of cellulose, starch, rubber, and other polymers, is now a generally accepted truth. The clear and simple approaches, as brought forward by Staudinger's school, proved to be very useful also for the study of proteins, and in the newly developed fields of synthetic high polymers, however, Staudinger's views proved to be particularly fruitful. It is amazing how much information of practical importance can be gained by a few simple experiments with such complicated systems as resins, jellies, and gums.

Treatment of organic and inorganic colloids separately is justified for two reasons. First, the whole realm of colloid science has grown too enormous. Second, in the case of organic colloids the chemical constitution of the constituent molecules is of paramount importance. Organic colloid chemistry represents a kind of submicroscopic morphology. The methods of organic chemistry and physical views of structure often become equally important. Although many problems have not yet been solved, the practical benefits of the knowledge already acquired is evident in many fields, such as clinical medicine, industrial high polymer research, pharmacy, and food technology. Hundreds of publications appear every week on various topics of organic colloid chemistry and it becomes increasingly difficult to follow up all the important findings in related branches. One of the chief aims of this work is to present a condensed survey of the whole field, as it appears at the present time, in order to facilitate acquaintance with related fields. This is a book for the protein chemist who wants to know the most important facts about rubber and cellulose and for the cellulose chemist who needs an introduction to colloids generally. It is hoped that this work will bring about the broadening of knowledge in related fields and the stimulation of creative thinking.

The book is divided into two parts. The sixteen chapters of Part I present a brief general review of the methods of preparation and study of organic colloids. The eighteen chapters of Part II attempt to give a condensed systematic survey of the most important classes of molecular and micellar colloids.

The author is indebted to Dr. R. Lee Clark, Jr., Director of the M. D. Anderson Hospital and Tumor Institute, and to Dr. A. Clark

Griffin, Head of the Biochemistry Department of our Institute, for their approval and support of this undertaking. Moreover, thanks are due to Dr. R. W. Cumley for linguistic editing of the manuscript, and to the following colleagues, who suggested improvements in several chapters: Dr. Sulo Karjala, Miss M. A. O'Neal, Mrs. Eugenia C. Adams, Mr. John A. Cooper II, and Dr. C. H. Robertson. The names of the many authors who kindly provided copies of illustrations are mentioned in the legends of the illustrations.

Houston, Texas, 1957 B.J.

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PART ONE

METHODS OF PREPARATION
STUDY OF ORGANIC COLLOIDS

CHAPTER 1

Introduction
The Scope of Organic Colloid Chemistry
Basic Terms

Colloid science or colloid chemistry deals mostly with the *sizes* and *shapes* of small particles and large molecules[1]. Most common are the colloidal solutions, such as milk, blood, liquid detergents, glues, collodion, and dispersions of dyes. The colloidal solutions are usually of very complex composition. Milk, for instance, contains droplets of fat, a variety of large protein molecules, sugar, salts, water, etc. A colloid chemist would not analyse the composition of milk fat but he is interested in the homogenization of milk, i.e. splitting of the large fat droplets. He will study how this homogenization retards creaming. The curdling of milk is another colloid chemical phenomenon.

Not all particles which are small are colloidal. A lead shot or a quartz grain are small objects, but they are much larger than colloidal particles. Water molecules, sodium ions, or gold atoms, on the other hand, are much smaller than colloidal particles. An approximate picture of the relative sizes of the various particles in milk is given below (Fig. 1). The objects are presented at approximately ½ million times their actual size.

Some authors have restricted the realm of colloids to particle size limits of 10 angstroms (lower limit) and 1000 angstroms (upper limit), or 0.000,001 mm and 0.0001 mm. However, systems which contain somewhat coarser particles also have some of the very characteristic properties of colloids. Hence, if one wishes to define colloidal particles metrically, the size limits should be extended tc cover a range of 0.000,001 mm to 0.001 mm.

One of the most characteristic properties of colloids is that the

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particles will run through the pores of ordinary filters. These particles, however, are retained by semipermeable membranes, such as cellophane or bladder. Colloidal particles are invisible in ordinary microscopes, but they can be seen under the electron microscope. The chromosomes of cells, the viruses, the very fine particles of clays, and the large molecules of polysaccharides, proteins, and various synthetic high polymers are colloidal.

Another fundamental characteristic of a colloidal particle is its *shape*. The particles of many colloids are asymmetric. Staudinger pointed out that the large molecules of cellulose can be compared with pieces of an elastic steel wire or hair. These cellulose threads are very long and thin. In their length they even surpass the range

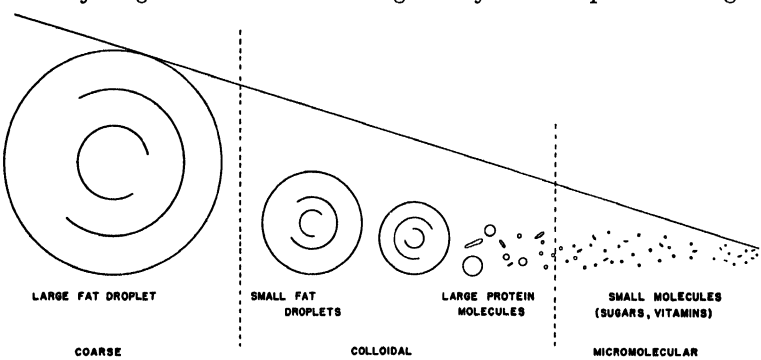

Fig. 1. Objects of various sizes. Magnification scale approx. 500,000.

of colloidal dimensions, but they are as thin as sugar molecules. Solutions of such linear colloids have peculiar mechanical properties. They have a very high resistance to flow, and they set easily. As such asymmetrical particles do not fit into the above metrical classification, Staudinger proposed to define colloidal particles on the basis of number of atoms constituting them. *A colloidal particle is built up of approximately one thousand to one billion atoms.*

The term *disperse system* is even more general than colloid. A disperse system is composed of a *homogeneous medium* and of *particles* dispersed therein. There are no particle size limitations, and both the medium and the particles can be either in solid, liquid, or gaseous state. The formation of clouds, stability of fog, precipitation of dust, foaming, and similar phenomena also belong to the realm of colloid science. Most important, however, are the systems in which the dispersion medium (solvent) is liquid, and the

dispersed particles are either solid or liquid. The solid or semisolid colloidal gels and jellies are also of interest to the colloid chemist. A more thorough classification of various disperse systems will be given in following chapters.

The tremendous growth of colloid science has made it impossible to cover the whole field thoroughly in a single volume. This is one of the reasons why the *organic colloids* were chosen. This separation can be justified also by the importance of *purely chemical* views and methods of organic chemistry in this branch of colloid science[2].

The particles of organic colloids are composed of carbon compounds. The dispersion medium or solvent may be either an inorganic substance or an organic liquid. Water is the most important inorganic dispersion medium or solvent. Benzene, ether, and turpentine are examples of organic solvents.

It proved useful to distinguish between two large groups of organic colloids: (1) *organic dispersions*, and (2) *organic molecular colloids*. The particles of dispersions can be split easily and they also tend to agglomerate and precipitate (or ascend to the top, if the dispersed phase is lighter than the medium). The particles of organic dispersions are composed of many small molecules, or they may be fragments of insoluble solids. Practically any organic substance can be dispersed in the form of a colloid by suitable means. Emulsions of oils in water are examples of this type of colloid. The molecular colloids, on the contrary, are true solutions. The dispersed entities are not aggregates but *single large molecules*.

It has been shown during the last decades that many natural and synthetic polymers, such as starch, serum albumin, rubber, nylon, and many others, are composed of large molecules. In appropriate solvents these substances dissolve easily, for example, glycogen dissolves in water, or rubber in benzene, or nitrocellulose in acetone. The solutions of glycogen in water, or of nitrocellulose in acetone are as "true" as those of sugar in water. The solutions are stable, the molecules usually do not aggregate. Only heating with strong acids or enzymatic action will split the primary valency bonds of the large molecules of the polysaccharides. The dispersed fat droplets in milk, on the contrary, can be split simply by forcing them through narrow clearances.

The existence of molecular colloids makes it expedient to coin a new term for solutions which contain dissolved molecules smaller than colloidal. Such solutions formerly were called molecular solutions. However, molecular colloids also may form true molecular

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solutions. The term *micromolecular* was proposed for the solutions like those of sugar, acetic acid and others in which the dissolved entities are small molecules and (or) ions. This term will be used throughout this treatise. Sugar is a micromolecular substance, and it forms *micro*molecular solutions. Starch is a *macro*molecular substance, and the solutions of starch in water are *macro*molecular.

Macromolecular substances are colloidal by their nature. *A single macromolecule is a colloidal particle.* When a macromolecule is split a new substance results. For example, the macromolecules of starch are converted into dextrines and glucose upon hydrolytic decomposition. Micromolecular substances, on the contrary, do not change chemically when the aggregates of their molecules are split into single molecules, or when they aggregate into colloids. For example, soap is a micromolecular substance. A solution of soap in alcohol is a micromolecular solution, as the dispersed entities in this case are single small molecules, e.g. those of $C_{17}H_{33}COONa$. When such alcoholic soap solution is poured into water, the soap molecules aggregate forming large clusters, because the solubility of soap in water is lower than in alcohol. A colloidal soap solution thereby is formed. The colloidal particles of soap can be split easily, e.g. upon heating the solution or diluting it. Soap is not changed chemically in aggregation and disaggregation. In fact, soap is a somewhat special example of micromolecular substances, since it can be directly dissolved in water, whereby a colloidal solution is obtained. Most micromolecular substances, however, can be dispersed as colloids only by special methods. For example, paraffin oil is a micromolecular substance; an emulsion of oil in water cannot be prepared by simply shaking the oil with water. The oil will disintegrate in droplets but they will flow together again, when the mixture is left alone. In order to prepare stable dispersions of oils in water, certain stabilizers must be added, e.g. a little soap.

There is a great variety of colloidal systems, and many of them are very complex. Colloidal macromolecules often aggregate themselves in larger, complex units, and combine also with other substances, which are present in solutions. Rubber milk (latex), produced by some plants, is an example of such systems. There are no single rubber molecules in latex, as rubber is insoluble in water. The rubber molecules are dispersed in form of microscopic aggregates which stay in solution because the surface of the aggregates is covered with water-soluble substances. Things become even more complicated when several kinds of macromolecules are present. The

task of the colloid chemist is to discover general relationships and rules, in order to predict any possible changes in the properties of such systems.

In the tissues of animals and plants the macromolecules of various proteins and polysaccharides are joined in complex higher order systems. For example, muscle fibrils are composed of several kinds of large protein molecules. Some of them are like strings, while others resemble short rods which may be joined end to end, and become attached to the strings. The fibers of silk, cellulose, nylon, and similar substances are composed of a large number of fibrous macromolecules. They are joined and bound in macroscopic bundles by means of secondary bonds and cohesive forces. One of the great tasks of organic colloid chemistry is to investigate the constitution of these systems. It is important to find out how the mechanical properties of fibers depend on the length, structure and association of the fibrous macromolecules.

The realm of synthetic macromolecules is especially rich and promising. Synthetic polymers, such as organic safety glass, synthetic rubber, nylon, orlon, dacron, polyethylene, and many others have been created on the basis of colloid chemical and organic chemical research. Purely chemical views and data about the reactivity of simple carbon compounds have been important. Synthetic macromolecules are formed by polymerisation (or polycondensation) of small organic molecules, such as ethylene, acetylene, formaldehyde, butadiene, acrylic acid, and others[3].

One of the most challenging branches of organic colloid chemistry is the field of proteins. The reduplication of large molecules of enzymes, viruses, and nucleoproteins of tissues is an example of the unsolved problems. The mechanism of enzyme action, and the importance of the configuration in the specificity of enzymes is another interesting problem.

Characterization of blood proteins in diseases is important for diagnostic purposes. The mechanism of blood clotting, permeability of cell membranes, and formation of antibodies from the globulins of blood are other problems of paramount importance[4].

Some advances have been made in the understanding of all these and similar phenomena in the last decades. And yet, it is only the very beginning. The following chapters will attempt to show that most successful were the endeavors in which the classical methods of organic chemistry were applied hand in hand with the modern physicochemical tools.

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Additional Important Nomenclature

Summary

Colloids – substances composed of colloidal particles.

Colloidal dispersion – a colloid in which the particles are aggregates of many small molecules or fragments of insoluble solids.

Colloidal particle – a particle composed of about 1000 up to 1,000,000,000 atoms. The diameter of such particles, when they are symmetric units, is between 0.000,001 mm and 0.001 mm.

Colloidal solution – any solution containing colloidal particles. Colloidal solutions sometimes are called *sols*.

Degree of dispersion – a quantity varying reciprocally with particle size. The larger the particles the lower the degree of dispersion.

Disperse system – any system in which particles (of any size and state) are dispersed in a homogeneous medium. Colloidal solutions represent a special kind of disperse systems.

Emulsions – colloidal dispersions in which the dispersed particles are in liquid state.

Gel – a solid or semisolid colloid containing considerable quantities of liquid. The particles in a gel are linked in a coherent meshwork which immobilizes the liquid.

Hydrosol – a colloidal solution with water as the dispersion medium.

Linear colloid – any colloid with fibrous particles or macromolecules. The long, asymmetrical particles may be coiled up, or, in some instances, they may be branched.

Macromolecule – large molecule of the size of colloidal particle. The atoms in a macromolecule are joined by primary valency bonds.

Micelle – a colloidal particle composed of many aggregated small molecules.

Micellar colloid – a colloid composed of micelles. Example – a colloidal solution of soap in water.

Micromolecular solution – any solution in which the kinetic units of the solute are small molecules or ions.

Molecular colloid – any colloid in which the colloidal particles are macromolecules.

Organic colloids – colloids whose particles are composed of carbon compounds.

Organosol – colloidal solution with some organic liquid (alcohol, ether, benzene) as dispersion medium.

Particle – any small, discrete entity of matter, regardless of size. A grain of quartz, a microbe, sugar molecule, atom, proton, are examples of particles.

Polymer – (poly = many, mer = part) macromolecular compound; the macromolecules are composed of many *repeating* radicals (mers).

Spherocolloid – a colloid possessing more or less corpuscular, symmetrically shaped, and relatively compact particles.

Other terms will be introduced later.

Common units for the measurement of small particles:

1 angstrom (abbreviated A) = 0.1 mμ = 10^{-8} cm.
1 millimicron (abbrev. mμ) = 0.001 μ = 10^{-7} cm.
1 micron (abbreviated μ) = 0.001 mm = 10^{-4} cm.

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CHAPTER 2

Development of the Modern Organic Colloid Chemistry Classification of Organic Colloids

The realm of organic colloids is boundless. However, it seems that only recently man has learned to apprehend it properly. The early thinker and scientist had no methods available by means of which to discover and investigate this world of small particles and molecules.

The British botanist Robert Brown is one of the early pioneers in studying small particles. It was about 130 years ago when he observed under the microscope small pollen grains which were suspended in water. He noticed that these particles did not stay still, but oscillated and jumped around in a quite chaotic fashion. Extending these observations to many other kinds of small bodies he observed the same phenomenon, if the particles were small enough. He also noticed that the smaller the objects, the more intense was their motion. This important discovery was later named *Brownian movement,* and it was thoroughly investigated by many other scientists. This posed an important question: what was the cause of this chaotic motion?

Thorough research later showed that Brownian movement can be explained neither by external vibration of the microscope nor uneven illumination. It cannot be explained by streaming of water under the influence of capillary forces or evaporation.

The correct explanation of Brownian movement was presented by Wiener in 1863. He came to the conclusion that the motion is due to irregular impacts by the molecules of the liquid in which the particles are suspended. The studies on Brownian movement thus opened a new way of penetration into the realm of molecules.

The German colloid chemist Zsigmondy invented the ultramicroscope in 1905, and with this instrument he could directly detect and observe the motion of colloidal particles. He noticed that these particles, which are much smaller than microscopical pollen grains and similar objects, move even more vigorously than microscopical objects. Moreover, it was found that the motion depends on the temperature, and on the viscosity of the liquid in which the motion occurs. Theoretical relations were developed, and colloidal particles became the object of exact study.

Another milestone in the development of colloid chemistry is the work of the British scientist Thomas Graham who lived in the middle of the 19th century. He investigated the spreading (diffusion) of various substances in pure liquids, and noticed considerable differences in the spreading rate. Glue, for example, spreads very slowly, whereas salt or sugar are much more mobile. According to the diffusion rate, Graham classified all substances into slow spreading glue-like *colloids* ("colla" in Greek means glue) and the fast spreading *crystalloids*. He also observed that glue, albumin, and other "colloids" are unable to penetrate through the pores of bladder, parchment, and similar membranes, while simple inorganic salts, acids and alkalies pass through such membranes easily.

Important chemical work on polysaccharides and proteins was done around the turn of the century by the German organic chemist Emil Fischer and his school. Discoveries concerning the structure of these very complex compounds were made. Moreover, many complicated polypeptides were synthesized. It was shown that the larger and more complex the synthesized molecule, the more solutions of these compounds resemble natural proteins and similar colloids.

Colloid chemistry or colloid science was established as a separate branch of chemistry in the first two decades of our century. The German chemist Wolfgang Ostwald founded a society of colloid chemists, and a magazine specially devoted to colloids (*Kolloid-Zeitschrift*) was issued. The first books about colloids were published. Far reaching generalizations about the application of colloid chemistry in various fields of natural science were made. The very general terms of disperse system, degree of dispersion, and the importance of it, were introduced by Wolfgang Ostwald and the Russian scientist P. P. von Weimarn. The latter author showed that many simple substances (crystalloids according to Graham), such as barium sulfate, sodium chloride, and others, can be pre-

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pared in the form of colloids. The principle was proclaimed that any substance can be prepared in colloidal state by suitable means. The chief condition of this preparation is that the substance to be dispersed must be insoluble in the medium in which it is to be dispersed. For example, sodium chloride can be dispersed as colloid in benzene (in which solvent it is insoluble) but not in water. Many organic substances which are insoluble in water were prepared as aqueous colloidal dispersions by simply intense grinding, especially when certain other substances were added.

At about the same time, many proteins and other natural colloids were crystallized, and it was shown by means of X-ray analysis that these colloids actually possess a crystalline structure. Hence the old classification of Graham into crystalloids and colloids became inadequate. The crystalline state of matter appeared to be the general one. It appeared that there are no colloids as such, but that any substance may exist or can be prepared in a *colloidal state*. It also became evident that the *particle size* is the most characteristic property of the colloidal state.

These views, though they were very useful in the realm of dispersion colloids, brought much confusion about the true nature of the organic molecular colloids. The majority of colloid scientists from 1920–1935 believed that the molecules of proteins, polysaccharides, rubber and similar substances were very small, i.e. that their molecular weight was approximately 500–1000. It was believed that the entities in solutions of proteins or cellulose derivatives are not macromolecules but aggregates of many small molecules.

McBain, for example wrote in 1936: "From the variation in relative viscosity with mere change in temperature or with change in solvent or treatment of nitrocotton and from the occasional enhancement of the viscosity brought about by simple physical means, it is concluded that cellulose derivatives are association colloids like soaps and that association and formation of micelles or more complicated structures appear to be essential features of cellulose and its derivatives"[1].

The erroneous idea that all natural and synthetic polymers are aggregates of small molecules developed partly on the grounds of the generalizations outlined above. Another source of this concept was the so-called micellar theory of Nägeli (1877). According to this author, many natural substances, such as the proteins, starch and cellulose, are composed of small crystalline units, the so-called *micelles*. A micelle is an aggregate of many small molecules bound

together by secondary bonds or cohesive forces. This micellar theory was modernized by K. H. Meyer, Mark[2] and others. They concluded, mostly on the basis of X-ray studies, that cellulose is composed of crystalline micelles built up of approximately 40 to 60 chains of glucose radicals, each chain containing 30 to 50 glucose units. The chains, according to this concept, are held together by cohesive forces, even after dissolution in appropriate solvents. A cellulose fiber was believed to be composed of a large number of elongated bundles or packets, as illustrated in many publications from 1935–1945. Natural rubber, according to Pummerer (1927) and other authors, has the molecular weight of several hundreds only, and formulas like $(C_5H_8)_8$ or $(C_5H_8)_{10}$ were written. They were based on wrongly interpreted molecular weight data obtained with solutions of rubber in menthol and camphor. Later, studies showed that the large molecules of rubber were decomposed in these solutions, and that much higher molecular weights are obtained, if decomposition is avoided.

There were only a few chemists around 1925–1930 who believed that natural and synthetic polymers are composed of large molecules. The majority of chemists believed that polysaccharides, rubber and other polymers were micromolecular, and that the small molecules were joined in micelles by cohesive forces. Staudinger was one of the few who took a different point of view. Being an organic chemist well versed in the matter of polymerization reactions and the reactivity of some simple organic compounds, he assumed that reactive small molecules possibly might form large molecules. The polymerization reactions are exothermic, and true primary bonds usually link the small molecules which polymerize.

Resinous, sticky bodies are often formed in these polymerization reactions. The polymerization products often appeared insoluble, and this led to the assumption that the radicals in such bodies were linked throughout by strong primary valency bonds. When these resinous substances were soluble, their solutions were very thick, viscous. It seemed to Staudinger that the entities in such solutions were *large molecules* of the polymer, *not micelles*. Staudinger expressed this view quite clearly in 1922. Some experimental evidence also was presented[3]. It was shown that upon hydrogenation of rubber a colloidal hydrogenated rubber is formed. This actually proved that there are no rubber micelles built of small molecules. If there were such small molecules (e.g. of the size $(C_5H_8)_8$), the micelles would disintegrate upon hydrogenation, and a low-molecular, volatile hydrorubber would be obtained.

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It seemed very unlikely that a micelle composed of hundreds of small molecules of $(C_5H_8)_8$ could react throughout with hydrogen, and then recombine, forming a colloidal unit. More plausible was the other assumption, namely, that the isoprene radicals C_5H_8 in rubber are linked by means of strong primary bonds in large molecules. Staudinger and associates in the following years presented much more data supporting their views about the existence of macromolecules. It was shown that the low molecular weights obtained earlier for rubber and other polymers were due to oxidative decomposition. The long and thin molecules of rubber can be cleaved easily, especially in the presence of oxygen. When the latter is eliminated, much higher molecular weights for rubber are obtained.

Although there was considerable evidence about the existence of large molecules (*macromolecules* according to Staudinger), the colloid chemists preferred the concept that the particles of all organic colloids are micelles. This was due to wrong generalizations based on the experiences with the colloidal soap solutions and emulsions. A soap particle in an aqueous soap solution is a micelle indeed, since the many small molecules of sodium oleate or a similar compound are aggregated and held together by weak cohesive forces. It seemed to many colloid chemists that rubber, cellulose and other polymers may have a similar constitution. This is an excellent example which shows that theories can be right or wrong, not just more useful or less useful. The micellar theory about the constitution of the colloidal particles of polymers appeared to be wrong. The most convincing evidence of this was presented by Staudinger and his associates. They showed that rubber, cellulose derivatives, and many other polymers can be chemically changed without changing the carbon backbone. If there were micelles, they would disintegrate in such chemical changes. The proof of the existence of macromolecules is the greatest contribution to modern colloid science. This was generally recognized by the awarding of the Nobel Prize to Staudinger in 1953.

In the early period of organic colloid chemistry (1910–1930) the chemical characteristics of the substances were often disregarded. The substances investigated often were impure and ill defined. Little interest was devoted to the question of the dependence of the colloidal properties on chemical constitution. Surface phenomena and the adsorption of electrolytes and solvent molecules on the surfaces and inside the micelles were believed to be of decisive

importance. This was again a wrong generalization. Later studies showed that these phenomena, though very important in cases of dispersions, are much less significant in polymers. The classifications and terms introduced by a few leading colloid chemists also contributed to some confusion in respect to the organic colloids. All colloids were classified into "emulsoids" and "suspensoids". According to this classification, not only the emulsions, but also the solutions of soaps, proteins, nitrocellulose and rubber belong to the emulsoid group. It was believed that the particles resemble droplets. This unfortunate classification was partly due to the concept that all colloids have a micellar structure. It was wrongly assumed that, for instance, a nitrocellulose particle in acetone solution is composed of many small molecules which are held together by cohesive forces (and secondary bonds) and assume the shape of a droplet. Hardy, Zsigmondy and other noted colloid chemists classified all colloids into *reversible* (redissolvable) and *irreversible*. Most of the organic colloids belong to the first class. The soaps, gelatin, and nitrocellulose, can all be redissolved. This classification is of some practical importance, but it also implies that the aforementioned substances are constitutionally related, which is wrong.

Most popular is the classification of all colloids into *lyophilic* and *lyophobic*[4]. Lyophile or lyophilic means 'liking liquid'. Lyophobe or lyophobic means 'fearing or disliking liquid'. In the case of water as solvent or dispersion medium the appropriate terms *hydrophilic* and *hydrophobic* are used (J. Perrin, H. Freundlich). Soaps, gelatin, albumin and starch are hydrophilic colloids. They are directly soluble in water, whereby colloidal solutions are formed spontaneously. This happens because of the high affinity of these substances for water. The colloidal particles in these cases contain large amounts of bound water. In other words: they are strongly hydrated. Affinity for the solvent is the chief reason why such colloids are stable. It is also obvious that the term 'hydrophilic colloids' in most cases has the same meaning as 'emulsoid' or 'reversible colloid'. The term 'lyophile' is more general than 'hydrophile'. Nitrocellulose does not dissolve in water but in acetone, whereby acetone is bound to the nitrocellulose. Rubber dissolves in benzene, as the latter has affinity for rubber. Substances which have no affinity for the solvent cannot be directly dissolved; they can be introduced in such solvents only by special means, and lyophobic dispersions then are obtained. Such lyophobic colloids

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are not stable, they precipitate easily. All hydrocarbons are hydrophobic but they are lyophilic in relation to other hydrocarbons as solvents.

The classification into lyophilic and lyophobic sols is quite useful when the great variety of both organic and inorganic colloids are treated. For the organic colloids alone, however, this classification becomes less important. This is caused chiefly by the very different approach to the study of organic colloids. Most of the organic colloids are not micellar but molecular colloids. The properties of the particular system depend not on surface phenomena but on molecular size, shape, and constitution. According to Staudinger, most useful is the following classification of organic colloids:

(1) Organic Dispersions,
(2) Organic Micellar Colloids,
(3) Molecular Colloids,
(4) Macromolecular Association Colloids.

Re (1): *Organic dispersions* can be classified further into *suspensoids* and *emulsions*. Organic dispersions are lyophobic. They cannot be prepared by direct dissolving of a substance, but only by special methods, e.g. by ultrasonic vibration or intense milling. The particle size in dispersions is relatively large. The particles are grain or bead-shaped. Examples: dispersions of sugar in ether, oil in water.

Re (2): *Organic micellar colloids* are aggregates of small molecules. Contrary to the dispersions, however, these substances can be dissolved spontaneously, and the sols thereby formed are lyophilic. Soaps and synthetic detergents are the most important examples in this group. The size of these micelles can be changed very easily, even by diluting or slightly warming the solutions. The peculiar behavior of these colloids is now well understood. It can be explained by the chemical constitution of the micromolecular compounds. A part of these molecules, e.g. of potassium stearate, $C_{17}H_{35}COOK$, the hydrocarbon radical $C_{17}H_{35}$ is hydrophobic, whereas the —COOK group is polar and hydrophilic.

Re (3): *Molecular colloids* are the most important ones in organic colloid chemistry. Most of the natural and synthetic polymers are molecular colloids. The solids are directly soluble in appropriate solvents, and lyophilic sols or solutions thereby are formed. Contrary to the micellar colloids, the colloidal particles in molecular colloids are not micelles but single large molecules (macromole-

cules). The solutions of glycogen in water, of nitrocellulose in acetone, or of rubber in hexane are examples.

Re (4): *Macromolecular association colloids* comprise another important class of organic colloids. In these systems the macromolecules are linked by means of cohesive forces and secondary bonds into more complex units. Aggregation may occur in any solution of molecular colloids if a non-solvent (for the solute) is added. The associated units may be identical chemically, but different macromolecules can also be joined together. Certain proteins are the most important examples of association colloids. It can be shown in many cases that the colloidal particles in protein solutions are composed of several macromolecular subunits which are linked by relatively weak secondary bonds and Van der Waals (cohesive) forces. The nucleoproteins and viruses are examples of very complex macromolecular association colloids.

Staudinger showed that it is useful to classify these last two groups further, namely, according to the particle (or molecular) shape. He showed that the properties of the colloids, especially the mechanical behavior, differ considerably depending on the molecular asymmetry. The molecules of the fibrous structural tissues of plants and animals are fibrous, like long, thin strings. The particles which are transported in veins, however, have a round shape, since fibrous particles might clog the narrow channels. Long and thin also are the molecules of the elastic polymers, such as rubber. Only those synthetic polymers which possess long, thin molecules proved to be useful for the preparation of artificial fibers. Because of this importance of molecular shape, Staudinger proposed to classify all organic colloids into *linear colloids* and *spherocolloids*. The importance of molecular shape was recognized early by most of the leading colloid chemists (K. H. Meyer, H. Mark, H. R. Kruyt, and others). Astbury proposed that those substances possessing very asymmetric molecules be called *fibrous* colloids, and those with symmetric particles—*globular* colloids. Since the particles in the dispersions and micellar colloids are not very asymmetric, it seems useful to apply the classification into linear and spherocolloids only for the last two groups, i.e. the molecular colloids and macromolecular associations. This is shown in the chart on p. 18.

The variety in the realm of organic colloids is so great that any classification will be incomplete. In the classification just outlined emphasis is placed on the *constitution* of the colloidal particles and macromolecules. The first two groups have in common the fact

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ORGANIC COLLOIDS

Dispersions		*Micellar Colloids*	*Molecular Colloids*		*Macromoleculur Association Colloids*	
Suspensions	*Emulsions*		*Globular*	*Fibrous*	*Globular*	*Fibrous*
Examples: India ink, cocoa	Examples: cream, water in petroleum	Examples: soaps, lecithin sols	Examples: glycogen, albumin	Examples: rubber, cellulose	Example: polio virus	Example: fibrin

that the colloidal entities are rather ill defined aggregates of small molecules (as in emulsions or soaps) or fragments of solid materials (as in cocoa, carbon black, finely ground bakelite). Stable dispersions can be prepared only if certain stabilizing substances also are present in the solution. Most dispersions are lyophobic, most micellar colloids are lyophilic. However, many authors call the particles in dispersions micelles. Further classification could be done in all of the groups; for example, in some dispersions, such as a dispersion of sugar in ether or acetone, the sugar particles are composed of many small individual molecules; in a carbon black suspension, however, the atoms in the whole particle are linked into a consistent mass. Further classification into lyophobe and lyophile colloids also could be done in all four groups, but this would complicate the matter too much. Moreover, with respect to constitution, the globular macromolecular association colloids could be further classified into mere aggregates (such as the opalescent aggregates of partly flocculated albumin) or more definite associations, such as insulin.

Not until 1940 were the former concepts about the micellar structure of natural polymers abandoned by most scientists. The existence of large molecules was further substantiated not only by the work of Staudinger and his school (G. V. Schulz, Signer, Husemann, Heuer, Trommsdorff, Kern, and many others) but also by extensive research in other laboratories. Important studies on polymerization (in 1928–1940) e.g. those of Carothers, Flory, Mark, Mellville, and the study of polymer solutions, gave further proof of the existence of macromolecules[5]. New methods of molecular weight determinations were introduced, and old methods were refined.

Very important among the new methods was the simple viscosimetric technique of Staudinger, and the ingenious method of Svedberg and his associates who studied sedimentation in the ultra-

centrifuge. The latter method gave especially interesting results with proteins. It could be shown that many of the well purified proteins appear to be homogeneous. As most of the dispersions and micellar colloids are inhomogeneous, the proteins in solution appeared to be like any molecular solutions. The conclusion was drawn that the entities in the solutions of homogeneous proteins are large molecules. Although this appeared to be not quite true in the light of later work, the data were of considerable importance. (The Nobel Prize was awarded to Svedberg in 1926.)

Further important information concerning the proteins was obtained by means of the electrochemical studies of Pauli, Tiselius, and others. Proteins, and a large number of other organic colloids possess electrically charged molecules. The colloidal particles of proteins or soaps are large polyvalent ions. They move in direct current, and interesting information can be obtained by the study of this mobility. (Tiselius received the Nobel Prize in 1948.) It will be just briefly mentioned here that the amount of free charge the colloidal particles possess is very important in influencing the stability of a colloid. This is especially important for all lyophobic systems, since for them the charge is the only stability factor. Particles which have a charge of the same sign repel each other, and therefore cannot agglomerate and precipitate.

In the branch of micellar colloids and stability of emulsions very important contributions were rendered by Langmuir, Harkins, Adam and Sir Eric Rideal, to mention only a few[6]. They studied, for example, surface films of organic substances, estimated the film thickness, and investigated the spatial configuration and orientation of the molecules on surfaces. It was shown that the orientation on the surfaces depends chiefly on the chemical constitution and configuration of the molecules. Most of this information was obtained by means of ingenious experimental devices such as the surface balances. It could be shown that many substances can be spread on the surfaces of liquids as monomolecular layers. The data on the orientation of organic molecules composed of both hydrophilic and hydrophobic groups were especially interesting and practically important.

Another characteristic feature in the development of modern organic colloid chemistry was the striving for *purity* of the substance to be investigated. The more physical chemically-minded early colloid chemist gave little concern to the purity of the organic colloids he investigated. Ill defined, impure, partly decomposed

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substances often were studied. The results of such studies, of course, were poorly reproducible, and of very little value in most cases. Some organic chemists, on the contrary, were so thorough in the purification of labile natural organic colloids that the macromolecules were decomposed in the isolation and purification. It must be pointed out that mild yet thorough purification procedures were applied mainly by those who had the right vision about the existence of labile macromolecules. Staudinger was the first who applied such methods for rubber and cellulose derivatives, and the enzyme chemists and virus chemists treated the proteins in the same manner. The Nobel Prize in 1946 was awarded to three American chemists: to J. B. Sumner and J. H. Northrop for the isolation of crystallized enzymes, and to W. M. Stanley for his work on isolation of viruses. Mild methods for the fractionation and isolation of blood serum proteins also were elaborated in the U.S.A. by E. J. Cohn and his associates. New discoveries on the constitution and colloid chemical properties of well-purified proteins are piling up steadily, yet most of the problems are far from being solved. Examples of such unsolved problems are: the shape of protein molecules in solution, the exact configuration of polypeptide chains in the protein molecules, and the number and manner of linkage of these chains in various cases.

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CHAPTER 3

The Properties of Organic Substances as Depending on Chemical Constitution and Molecular Weight

1. Various Types of Molecules

Over half a million organic compounds have been isolated and studied. Most of them are micromolecular substances, i.e. their molecular weight is well below 10,000. In order to solve the problems concerning the constitution of both micellar particles of organic colloids and the macromolecules, some important facts about simpler molecules ought to be considered.

All those organic compounds which are gaseous at ordinary temperatures are composed of very small molecules. Examples are: methane CH_4, ethane C_2H_6, ethylene C_2H_4, acetylene C_2H_2, phosgene $COCl_2$. Organic liquids also are micromolecular, for example, ethyl alcohol C_2H_5OH, chloroform $CHCl_3$, diethyl ether $C_2H_5{\cdot}O{\cdot}C_2H_5$, benzene C_6H_6, acetic acid CH_3COOH, butyl amine $C_4H_9NH_2$. Solid organic substances vary widely in molecular size. For instance, the urea molecules $CO(NH_2)_2$ or glucose molecules $C_6H_{12}O_6$ are small, but those of starch or albumin are large.

It was learned long ago that the properties of organic substances depend on their constitution and molecular weight. In order to find the rules which correlate the properties with the size and constitution of the molecules the substances, first, should be classified. As a result of such classification we have the so-called *homologous series* of various hydrocarbons, of alcohols, acids, and many other groups. The paraffins or saturated open chain hydrocarbons are the simplest compounds. These compounds are very stable, and they are hydrophobic substances. The properties of normal

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paraffins, e.g. their volatility, change rather regularly with the increase of the molecular weight. The same regularity can be stated in other homologous series, for instance, in the alcohols. This is illustrated in Table 1.

TABLE 1

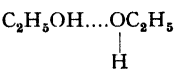
HOMOLOGOUS SERIES OF NORMAL PARAFFINS AND ALIPHATIC ALCOHOLS. THE INCREASE OF BOILING POINTS WITH RISING MOLECULAR SIZE

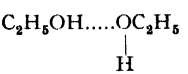
Paraffins		*Boiling point*	*Alcohols (normal)*		*Boiling point*
Methane	CH_4	—164°	Methyl alcohol	CH_3OH	+64.7°
Ethane	C_2H_6	— 93	Ethyl alcohol	C_2H_5OH	78
Propane	C_3H_8	— 45	Propyl alcohol	C_3H_7OH	97
Butane	C_4H_{10}	+ 0.6	Butyl alcohol	C_4H_9OH	117
Pentane	C_5H_{12}	36	Amyl alcohol	$C_5H_{11}OH$	137
Hexane	C_6H_{14}	68.7	Hexyl alcohol	$C_6H_{13}OH$	157
Heptane	C_7H_{16}	98.4	Heptyl alcohol	$C_7H_{15}OH$	176
Octane	C_8H_{18}	125.8	Octyl alcohol	$C_8H_{17}OH$	194
Nonane	C_9H_{20}	150.7	Nonyl alcohol	$C_9H_{19}OH$	213
Decane	$C_{10}H_{22}$	173	Decyl alcohol	$C_{10}H_{21}OH$	231
Undecane	$C_{11}H_{24}$	195	Undecyl alcohol	$C_{11}H_{23}OH$	131 (at 15 mm pressure)
Dodecane	$C_{12}H_{26}$	215	Dodecyl alcohol	$C_{12}H_{25}OH$	143 (at 15 mm pressure)

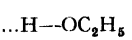
Two important conclusions can be drawn from the Table: (1) the larger the molecules the less volatile is the substance in the same series, and (2) the alcohols are much less volatile than the hydrocarbons which have approximately the same molecular size. The molecular weight of butane is 58, and that of ethyl alcohol 46, yet the former is much more volatile than the latter. This indicates that the intermolecular forces which act among the molecules in alcohols are much more pronounced than in hydrocarbons. Physical chemical studies have shown that the charge distribution in the molecules of alcohols is not symmetric, the molecules, like those of water, are *polar*. The positively charged side of such a polar molecule attracts the negatively charged part of another molecule. Clusters of molecules thereby are formed in polar liquids. Intermolecular association is explained also as a consequence of *hydrogen bond formation*[1]. According to this concept, the hydrogen atoms in water molecules, and the hydrogens in the –OH groups of the

alcohols are able to form weak bonds with strongly electronegative atoms of adjacent molecules. For example, in alcohols this bond will be R·OH....O·R. In water, alcohols, and phenols many molecules

are thus bound in loose clusters, such as in ethanol:







In alcohols such clusters are of indefinite size. In carboxylic acids, however, definite dimers can be formed. Hydrogen bonding occurs also in amides and imides, in which the hydrogen atom of the –NH-groups is able to bind electronegative atoms of other molecules. The dissociation energy of these hydrogen bonds is only about 4–6 kcal. per mole, whereas the dissociation energy of the primary C–O bond is 70–75 kcal.

There is another important property by which polar organic liquids differ from non-polar ones—the internal resistance to flow or viscosity. Intermolecular linking causes a liquid to be relatively thick, viscous. Propyl alcohol, for instance is much more viscous than pentane. In the same homologous series, e.g. in paraffins, the viscosity increases with increasing molecular size. The paraffin oils are viscous, as the molecules in these liquids are of considerable length.

Very interesting and important is the dependence of *solubility* on chemical constitution. The solubility properties are governed by a simple rule: *similar dissolves similar*. Hydrocarbons are soluble in other hydrocarbons, the higher molecular solid alcohols are soluble in simple alcohols. If an organic molecule is composed of a hydrocarbon radical and a polar group, the solubility is determined

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by the relative sizes of both components. Methyl alcohol is soluble in water, since in the CH_3OH molecule the polar OH-group dominates. The solubility of amyl alcohol $C_5H_{11}OH$ in water is low, because in this case the hydrocarbon radical comprises a large part of the molecule. The higher alcohols, such as decyl alcohol $C_{10}H_{21}OH$, are practically insoluble in water. The same rules govern the solubility of other organic compounds. Acetic acid CH_3COOH is soluble in water, as the polar and ionogenic carboxyl group comprises the major part of the molecule. Oleic acid $C_{17}H_{33}COOH$, on the contrary, is insoluble in water, for the hydrocarbon radical is relatively large in this case. However, this acid is soluble in various oils in which acetic acid is insoluble. The polar part of the oleic acid is hydrophilic, the hydrocarbon radical is hydrophobic or *lipophilic* (fat liking). *The compounds in the homologous series of alcohols, acids, esters, ethers, aldehydes, ketones, amines, and other similar compounds become less hydrophilic and more lipophilic with increasing molecular size.*

This conclusion is especially significant for the problems of micelle formation, molecular orientation in monolayers, and stabilization of emulsions. Experience has shown that micellar hydrosols are formed always when molecules possessing long hydrocarbon radicals and polar groups are introduced into water. For example, in the case of sodium palmitate

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2\cdot$ $COONa$ ← polar head

Hydrocarbon tail

there is a long hydrocarbon 'tail' and a polar 'head'. The tail is hydrophobic, and it is pushed out of the water. There is no affinity of water to hydrocarbon, but some affinity exists between the hydrocarbon radicals themselves. Because of this, the molecules aggregate into micelles, the latter having a very interesting constitution: *the hydrocarbon tails all are turned inside the micelle.* The polar and ionogenic heads, on the contrary, have affinity to water, and hence they are located on the surface of the micelle (see Chap. 5).

The solubility of high polymers in various organic liquids also can be predicted on the basis of chemical composition. Rubber, for example, is a hydrocarbon, and it is soluble in hexane or benzene but not in methyl alcohol or acetic acid. Polyvinylalcohol molecules contain many OH-groups, and this polymer is soluble in water and in alcohol. Albumin molecules contain many polar and ionogenic groups on their surfaces, hence albumin is soluble in water, but

insoluble in hydrocarbons or even in alcohol. When both polar and non-polar groups are constituting the macromolecules, the prediction, however, becomes uncertain.

2. The Variety of Isomers in Homologous Series

The properties of organic compounds depend not only on the chemical composition but also on the *structure* of the molecules. The carbon atoms can be linked not only in open chains but they can form branched chains and rings as well. These various chains then can be saturated with hydrogen atoms and other elements to various degrees, and oxygen, nitrogen, sulfur and many other atoms may be placed in various parts of the chain or ring. The more carbon atoms are linked the greater is the number of possible isomers. This is illustrated in Table 2 for several of the simple paraffins[2].

TABLE 2

THE MOLECULAR WEIGHT AND NUMBER OF ISOMERS FOR SOME PARAFFINS

	Molecular weight	*Number of isomers*
CH_4	16	1
C_4H_{10}	58	2
C_6H_{14}	86	5
$C_{10}H_{22}$	142	75
$C_{15}H_{32}$	212	2513
$C_{20}H_{42}$	282	21,585
$C_{100}H_{202}$	1402	about 10^{10}

According to the data presented in Table 2, the number of isomers increases astoundingly with increasing number of carbon atoms in the hydrocarbon molecules. Most of the isomers of the lower molecular hydrocarbons are prepared, purified, and well investigated; all isomers, for instance, of eicosane $C_{20}H_{42}$, however, have not been isolated. The calculated numbers of isomers just show the potential possibilities of variations. The natural hydrocarbons are not yet completely investigated, and it is not known how many of all these complicated hydrocarbons will be found and identified[3].

This example does not cover all the possibilities of variations in hydrocarbons. There are series of homologs of *unsaturated* hydro-

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carbons in which the possibilities of isomerism are even greater than in paraffins. In the ethylene, acetylene and diene series isomers occur not only because of different arrangement of carbons but also because of different locations of the double and triple bonds. When oxygen, nitrogen and other elements are introduced in the molecules, the number of isomers increases even more. Millions of isomers are possible of compounds which have the molecular weights of several thousands. Such compounds, according to our definition, are still micromolecular. In the realm of macromolecular substances the number of possible isomers is infinite.

In spite of these tremendous potential possibilities, there is a *preferential formation* of certain kinds of compounds only. Not all isomers occur in nature with an equal frequency. The reasons for this preferential formation of some isomers are different in different cases. Some combinations of atoms are less stable than others. Usually the most stable or thermodynamically most probable combinations are formed. This is because of the second law of thermodynamics. However, not only a skilled chemist in his laboratory but nature itself is able to form many rather unstable combinations of atoms. Many organic compounds are formed in living cells. This is possible in very narrow temperature limits only. The origin of life is unknown, but it seems that specific conditions, besides the temperature limits and chemical nature of carbon, nitrogen and water have been important. Some organizing factors have been involved which governed the formation of only some carbohydrates and proteins through a long and complicated pattern of reactions. What have these factors been? Have the reactions occurred on the surfaces of inorganic substances (minerals) of specific structural characteristics? We can just guess. Whatever the reasons have been, carbon, hydrogen, and oxygen have combined preferentially to form cellulose and similar carbohydrates, whereas other oxygen-rich compounds in plants are found in insignificant amounts.

The complicated organic compounds are unstable at very high temperatures. Many of them, however, are relatively stable at the moderate ordinary temperatures. If single molecules are considered, they usually have different reactivity in different parts of the molecule. In unsaturated compounds, such as isoprene and rubber, the double bonds are most reactive. In alcohols most reactive are the hydroxy groups (toward most reagents). In proteins most reactive are the carboxyl –COOH, amino $-NH_2$, and the sulfhydryl –SH groups. These facts about the reactivity of both micromolecular

and macromolecular organic compounds are very important in organic colloid chemistry.

3. The Polymer-homologous Series

Some homologous series have been presented in the previous sections. In the paraffin series the molecular size increases regularly by adding one methylene group $-CH_2-$. Only the first twelve hydrocarbons and the first twelve alcohols (without any isomers) were presented in Table 1. However, many more hydrocarbons and alcohols are known. For example, a hydrocarbon pentacontane $C_{50}H_{102}$ is a solid which melts at 92° C. The carbon chains can be extended even longer than that. According to the generally accepted definition, however, all the substances whose molecules are composed of 50 and more carbon atoms are still low-molecular (micromolecular). The term macromolecule is used when about 1000 atoms are linked, and when the molecular weight is about 10,000.

The simplest macromolecular hydrocarbon is polyethylene (also called polymethylene);

$$\ldots CH_2\text{-}CH_2\text{—}CH_2\text{—}CH_2\text{—}CH_2\text{—}CH_2\text{—}CH_2\text{—}CH_2\text{—}CH_2\text{—}CH_2\text{—}CH_2\text{—}\ldots\ldots$$

As the chains are very long, and of not quite definite length, the formula can be abbreviated as follows:

$$\ldots(CH_2)_n\ldots$$

There are two important differences between a micromolecular and a high polymer hydrocarbon: (1) the number of carbon atoms in a *micro*molecular hydrocarbon is relatively *small* and *definite*, whereas in *macro*molecular hydrocarbons it is *large* and *indefinite*; (2) the low molecular substances have definite, known *end groups*, whereas usually little is known about the end groups of macromolecular chains. Therefore the formulas often are written without the end groups. When the chains become extremely long, the end groups become less significant in determining the gross properties of the substance. Their amount is so small, in comparison to the bulk, that it is difficult to determine them.

The indefinite value of n in the presented formula of polyethylene is a very important feature. The significance of this is apparent if one considers the difficulty encountered in separating individual hydrocarbons from paraffin oil, though there the molecules are much smaller than in polymers. *All polymers are mixtures of polymer-*

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homologs, just as paraffin oil is a mixture of difficultly separable micromolecular hydrocarbons.

Considering the amazing multitude of isomers, one might think that molecules of very different constitution appear in the polymers, such as polyethylene, polystyrene, rubber. However, in most natural and synthetic polymers the macromolecules are all of *the same (or very similar) constitution*. In linear polymers the molecules differ in the chain length only. Therefore, it is not incorrect to speak about *polymer-homologous series*. A polymer-homologous series of polyethylene then can be presented as follows:

.....
$(CH_2)_{1000}$
$(CH_2)_{1001}$
$(CH_2)_{1002}$
$(CH_2)_{1003}$
....
$(CH_2)_{1100}$
$(CH_2)_{1101}$
.....

It is quite conspicuous that the individual members in such series, e.g. $(CH_2)_{1100}$ and $(CH_2)_{1101}$ must be practically indistinguishable. In reality polymers are composed of a very large number of different size molecules, though their constitution is uniform.

Any polymer-homologous mixture can be fractionated by suitable means (Chap. 8). However, the separated fractions never are absolutely homogeneous. In such fractionations a polymer may be first liberated from any low-molecular products (mol. weight about 1000–3000). Fractions which have molecular weights of 5000–10,000 can further be separated. It must be pointed out that there is no sharp borderline between micro- and macromolecular molecules. Polymers which have the molecular weights about 3000–10,000 are called *semi-colloids* (also hemi-colloids or 'half-colloids'). The true macromolecular part of the polymer then may be fractionated further, and macromolecular fractions with mol. weights of 10,000–100,000, and very high molecular fractions with mol. weights 200,000–400,000 and more can be separated. Each of these fractions can be fractionated further, and even more homogeneous components can be obtained, for example one with the mol. weight of 60,000–80,000 for the major part of the polymer. However, no polyethylene or polystyrene fractions are known which are absolute-

ly homogeneous, i.e. that *all* molecules have the same relative weight, say, 140,000.

The residue constituting the macromolecules (e.g. $-CH_2-$) is called *mer*. The subscript n indicates the number of mers in a molecule of a polymer, and is called the *degree of polymerization*, For instance, in the case $(CH_2)_{1000}$ the degree of polymerization is 1000. The molecular weight is obtained by multiplying the degree of polymerization (denoted often P instead of n) by the radical weight of the mer:

$$\text{Molecular weight} = n \times (\text{radical weight}).$$

In the case of $(CH_2)_{1000}$ the molecular weight then will be $1000 \times 14 = 14{,}000$.

4. Classification of High Polymers

In Chapter 2 the molecular colloids have been classified into linear colloids and spherocolloids. The molecular *size* and *shape* are very important in determining the properties of a macromolecular substance. However, the properties of organic colloids do not depend solely on molecular size and shape but also on chemical *composition* and *constitution*. Moreover, it can be predicted from the chemical constitution of the micromolecular constituents of the large molecules whether they are linked linearly or spatially. This is especially important in conducting polymerizations, i.e. making polymers from simple reactive organic compounds. Such reactive starting materials are called *monomers*. Ethylene $CH_2=CH_2$, vinyl chloride $CH_2=CHCl$, and styrene $C_6H_5CH=CH_2$ are examples of monomers. Such molecules polymerize linearly, for instance:

$$n\,CH_2{=}CHCl \xrightarrow[\text{activation}]{} n{-}CH_2{-}CHCl{-} \xrightarrow[\text{polymerization}]{} (-CH_2-CHCl-)_n$$

In many natural and synthetic polymers the repeating units (mers) are the same, and the way of linking is linear. Some examples of such polymers are presented in Table 3.

All the mentioned polymers have in common certain mechanical properties, for example, their solutions are thick, viscous. In bulk they are deformable with various ease, and some of them are highly elastic (rubber). However, they differ considerably not only in their molecular weights but also in some mechanical, electrical, optical, and chemical properties. The differences appear even if

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TABLE 3

EXAMPLES OF NATURAL AND SYNTHETIC LINEAR POLYMERS

Material	*Chemical composition*	*Molecular weight*
Polyethylene	$(CH_2)_n$	5000 – 40,000
Polyvinyl chloride	$(CH_2CHCl)_n$	10,000 – 150,000
Polystyrene	$(C_6H_5CHCH_2)_n$	60,000 – 1,600,000
Rubber, natural	$[-CH_2-C(CH_3)=CH-CH_2-]_n$	150,000 – 1,300,000
Cellulose, natural	$(C_6H_{10}O_5)_n$	300,000 – 1,500,000
Cellulose in rayon	$(C_6H_{10}O_5)_n$	70,000 – 100,000
Nylon	$[-NH(CH_2)_6NHCO(CH_2)_4CO-]_n$	10,000 – 32,000

specimens of the same average molecular weights of these linear polymers are compared. For example, rubber is more elastic than cellulose of the same molecular weight. Nylon gives much stronger fibers than polyvinyl chloride of the same molecular weight. The polymer hydrocarbons are all good electrical insulators; cellulose and nylon are inferior in this respect. The solubility of the mentioned linear polymers differs considerably. The polymer hydrocarbons are soluble in hydrocarbons in which solvents, cellulose and nylon are insoluble. The differences in *chemical reactivity* are very great, even between polystyrene and rubber. The former is a saturated hydrocarbon, and hence it is chemically rather inert. The latter is an unsaturated compound, it can be oxidized easily, reacts also with sulfur, whereby its properties are considerably changed. The *chemical differences* are even greater if the hydrocarbons are compared with cellulose. The latter reacts with acids, whereas polyethylene is resistant to acids. These facts show that one must be careful in any generalizations about the dependence of properties on molecular size and shape. For this reason the various classes of organic colloids will be treated separately in Part II of this treatise.

Although all natural and synthetic polymers are mixtures of polymer-homologs, the constitution of their molecules is astoundingly uniform. Constitutional variations, however, are not uncommon. The polysaccharides cellulose, starch and glycogen are well known examples. All of them are built of the same glucose radicals but the arrangement (constitution) is different. In the macromolecules of cellulose the arrangement is linear, in starch there is some ramification, in glycogen the branching is very considerable, and the macromolecular meshwork is rather compact. This is an ex-

ample where viscosity, solubility and other properties are determined largely by the molecular shape and constitution.

So far only simple polymers have been mentioned. The macromolecules of such simple polymers are composed of many repeating units. In many other instances two, three or even more different repeating units (radicals or mers) may occur in the macromolecules. Two different repeating radicals appear, for example, in a synthetic rubber called buna S. It was prepared by polymerizing a mixture of butadiene $CH_2=CH-CH=CH_2$ and styrene. In such *copolymerization* the monomers do not polymerize separately to polybutadiene and polystyrene but chains are formed in which both radicals appear in an irregular sequence. The composition of such chains may be illustrated as follows:

$$\ldots\ -(CH_2CH=CHCH_2)_n-\underset{\displaystyle C_6H_5}{(CHCH_2)_m}-(CH_2CH=CHCH_2)_x-\underset{\displaystyle C_6H_5}{(CHCH_2)_y}-\ldots\ldots$$

Copolymers composed of three or more mers also are known. Moreover, not only linear macromolecules can be formed but ramified structures as well.

Many different constituting units are found in the macromolecules of proteins. These macromolecules are built of about 20 different amino acid radicals (see Chap. 25–28). The long polypeptide chains of these radicals are twisted in various ways, and they usually are cross-linked with the adjacent chains.

Another important class of polymers is that of the so-called *space polymers*. Many substances are known in which the mers are linked by strong primary bonds in all directions in space. An example is bakelite, a phenol-formaldehyde resin. As phenol has three reactive hydrogens, two of them can be used in chain formation and the third for interlinking the chains. Vulcanized rubber is another example of space polymers. The polyisoprene chains of natural rubber have double bonds which react with sulfur in the process called vulcanization. In this reaction sulfur cross-links are formed between the chains. The space polymers are insoluble, because of this chemical interlinking of the chains. The space polymers have no definite molecular weights. Any macroscopic mass of such substance can be considered as a huge macromolecule.

In the following chart are mentioned the chief classes of organic macromolecules.

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ORGANIC MACROMOLECULES

Linear Polymers		*Ramified Macromolecules*	
One repeating mer	Repeating units different	Simple branched macromolecules	Space polymers
Examples: Polyethylene, Cellulose, Nylon	Examples: Linear copolymers, Some proteins	Examples: Starch, Glycogen	Examples: Vulcanized rubber, Bakelite, Some proteins

Denoting by A, B, C, D, the repeating units, the constitution of the four types of macromolecular substances can be illustrated as follows:

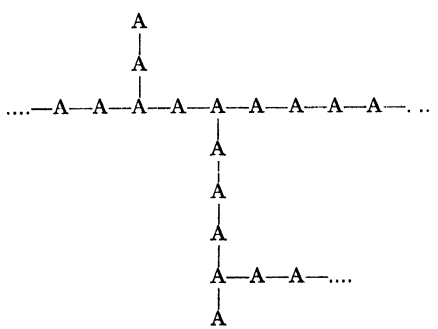
Simple linear polymers:

....—A—A—A—A—A—A—A—A—A—A—A—....

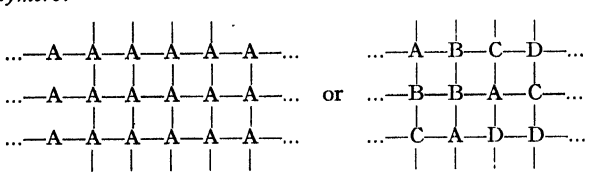
Linear macromolecules with different mers:

....—A—A—B—B—B—B—C—C—C—D—D—A—. .

Simple branched macromolecules



Space polymers:



5. Some Characteristic Properties of High Polymeric Substances

Simple, micromolecular organic compounds, such as ethyl alcohol or naphthalene can be isolated by the classical methods of distillation, and crystallization. They can be characterized by determining the boiling point and melting point. Most of the high polymers cannot be crystallized, and all of them are *non-volatile*. The solid macromolecular substances have *no definite melting points*. Upon heating they become soft or decompose at a certain higher temperature. The *softening point*, i.e. the temperature at which they become soft and plastic cannot be determined with great accuracy. Moreover, many of the high polymers appear resinous and sticky. It is hard to purify them. For this reason, the organic chemist was reluctant to study them in the early period of organic chemistry. Resinous products were simply discarded. Because of this, the development of macromolecular chemistry was slow up to the very recent time. The application of the ingenious new methods of organic and physical chemistry initiated progress in this branch.

We have seen already that all natural and synthetic polymers are *mixtures of polymer-homologs*. Molecules of various sizes are found in any macromolecular substance, whereas the molecules of sugar, alcohol, or naphthalene are all of the same size. Although many macromolecular substances have been obtained in crystalline state, the crystals appear to be composed of not quite identical units. It was shown in several instances of proteins that the crystals contain several chemically different components. The crystals of linear polymers differ from crystals of micromolecular substances mostly in the *variety of length of the building units*. According to Staudinger, a molecular lattice of a micromolecular substance can be illustrated as in Fig. 2 A, whereas a macromolecular lattice is like that in Fig. 2 B. Staudinger[4] investigated the crystallization

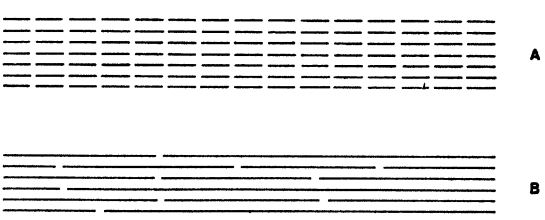


Fig. 2 .Molecular (A) and macromolecular (B) lattice.

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of polyoxymethylene $(CH_2O)_n$ which is a polymerization product of formaldehyde, and possesses rather short chains. It was found that the longest chains crystallize out first, and the shorter are more hard to crystallize. However, no quantitative separation of the polymer-homologs is possible; in the first fractions of the crystallites are included also some of the lower molecular components.

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CHAPTER 4

The Formation of Large Molecules

Large molecules are formed from small molecules by *polymerization* or *polycondensation*. Both terms express the idea that *many* (poly) small molecules unite. In a simple polymerization the small molecules combine into large ones and no side products are formed. In polycondensation some micromolecular side products (mostly water) also are formed. In both polymerization and polycondensation true *chemical reactions* occur, i.e. primary valence forces are involved[1].

The formation of polymers was observed by many chemists long ago, but the explanations of the phenomena were often incorrect. It was, for example, observed that styrene, $C_6H_5CH{=}CH_2$, which is a non-viscous liquid, becomes in time more viscous, and sets finally to a solid glasslike mass. The formation of various aldehyde resins is another example. It was also noticed that the unsaturated compounds, such as the esters of acrylic acid polymerize most readily.

1. Polymerization

Several simple examples of polymerization have already been mentioned in the preceding chapter. A more detailed discussion, however, would be desirable. According to Staudinger, Schulz, and others, the mechanism is as follows: First, the molecules of a monomer must be *activated*; second, the activated molecules *combine*, and, finally, the chain growth is *terminated* by some process[2].

Let us first consider the activation. The ease with which various unsaturated molecules can be activated is different. Energies in the

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range of 35 kcal. have been estimated for a mole of ethylene, and about 25 kcal. per mole of styrene. However, it must be pointed out that, in order to polymerize a mole of styrene, one does not need this whole amount of energy. The polymerization, as mentioned before, can start spontaneously, as only a small fraction of the monomer molecules must be activated. The energy needed for the activations of the rest of the monomer is furnished by the chain growth which is an *exothermic* reaction. For this reason room temperatures may be sufficient for the initiation which for substituted ethylenes can be symbolized as follows:

$$R\cdot CH=CH_2 \longrightarrow R\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\!-\!CH_2$$

Light also can initiate polymerization. Furthermore, the activation can be promoted by impurities present in the monomer, or by certain catalysts, such as BF_3. Organic peroxides are often used as initiators. The mechanism and kinetics of this initiation was studied by Mark, Flory, Schulz, Tobolsky and others[3]. It could be shown that free radicals of the initiators are formed, and these free radicals react with the monomer, for example, benzoyl peroxide $C_6H_5CO\cdot O\cdot O\cdot CO\cdot C_6H_5$ is split into free radicals 2 $C_6H_5CO\cdot O$ which react with styrene as follows:

$$C_6H_5CO\cdot O + C_6H_5CH=CH_2 \longrightarrow C_6H_5CO\cdot O\cdot CH_2\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\!-, \quad \text{and}$$

$$C_6H_5CO\cdot O\cdot CH_2\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\!- + C_6H_5CH=CH_2 \longrightarrow C_6H_5CO\cdot O\cdot CH_2\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\cdot CH_2\!-\!\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\!-$$

and further:

$$C_6H_5CO\cdot O\cdot CH_2\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\cdot CH_2\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\!- + C_6H_5CH=CH_2 \longrightarrow$$

$$C_6H_5CO\cdot O\cdot CH_2\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\cdot CH_2\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\cdot CH_2\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\!-, \quad \text{and so forth.}$$

Thus the chain grows longer and longer in one direction. The peroxide group in the chain remains as one end group, but the other end continues to react, as it actually is a free radical $R\cdot\underset{\displaystyle C_6H_5}{\underset{|}{CH}}\!-$.

With this description of chain propagation we enter the second

phase of polymerization reactions—*the chain growth.* As mentioned, this is an exothermic reaction. The polymerizing material heats up, and must be cooled off in order to conduct the polymerization in the desired way. It ought to be mentioned also that the heats of activation in the cases of peroxides as initiators are relatively low (4–5 kcal. per mole of vinyl monomer), and that the heats of chain growth exceed this amount considerably. Some data are presented in Table 4[4].

TABLE 4

HEATS OF POLYMERIZATION IN kcal. PER MOLE OF MONOMER

Monomer	*Polymer, solid*	*Heat of Polymerization*
Ethylene	Polyethylene	25.9
Styrene	Polystyrene	16.3–18.0
Vinyl acetate	Polyvinyl acetate	21.3
Vinylidene chloride	Polyvinylidene chloride	14.4
Methyl acrylate	Polymethyl acrylate	18.7
Methyl methacrylate	Polymethyl methacrylate	13.0–16.3
1,3-Butadiene	Buna rubber	12.2–38.4
Isoprene	Polyisoprene, rubber	17.9–24.1

The chain growth is a rapid process. The life span of a free radical in the process of polymerization is estimated of the order of a few seconds only. During this time many hundreds of monomer molecules react with the radical, and they are added to the growing chain. This continues until the growth is terminated by some kind of reaction.

The *termination* may occur because of a variety of causes. Firstly, the unsaturated ends of two growing chains can meet and combine. Secondly, the radical may meet some other molecules which are present as impurities or are added intentionally in order to terminate the reaction. Phenol, nitrobenzene, and hydroquinone are examples of substances which are able to terminate chain growth. Hence hydroquinone and other substances are added to monomers in order to stabilize them during storage and transportation. When unwanted polymerization occasionally starts, the stabilizer interrupts it by terminating the chain growth.

Certain *modifiers* are often used in polymerization in order to prepare polymers with some desired properties. The *chain length* is

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one of the most important features which determines many properties of any polymer. Some modifiers act as agents which terminate chain growth, and at the same time initiate the formation of new free radicals. Mercaptans are such modifiers which are able to terminate the growth of a chain, initiating at the same time the formation of new free radicals, for example:

$$\text{.....CH}_2\text{—CH(R)—} + \text{C}_{12}\text{H}_{25}\cdot\text{SH} \longrightarrow \text{....CH}_2\text{—CH}_2\text{(R)} + \text{C}_{12}\text{H}_{25}\cdot\text{S—};$$

growing chain; if polystyrene R will be C_6H_5. — dodecyl mercaptan — terminated chain — free radical

and

$$\text{C}_{12}\text{H}_{25}\cdot\text{S—} + \text{CH}_2\text{=CHR} \longrightarrow \text{C}_{12}\text{H}_{25}\cdot\text{S—CH}_2\text{—CH(R)—}$$

monomer — new growing chain.

Ferrous salts also are used in influencing polymerization reactions. In mixtures with peroxides they accelerate the formation of free radicals from the peroxides, for example:

$$\text{Fe}^{++} + \text{H}_2\text{O}_2 = \text{Fe}^{+++} + \text{OH}^- + \text{OH};$$

free radical

and

$$\text{CH}_2\text{=CHR} + \text{OH} \longrightarrow \text{HO—CH}_2\text{—CH(R)—}$$

monomer — free radical of activated monomer.

In this example the monomer is activated by the free OH group which remains as one of the end groups in the chain. When organic peroxides instead of H_2O_2 are used, the free peroxide radicals R—O— will be formed.

Polymerization has a certain likeness with crystallization. In both instances the size of the entities formed depends on several factors, chiefly on the rate of the formation of nuclei and on the growth rate. If the material is distributed on a few nuclei, the particles formed will be large. If many nuclear centers are formed, the particles can not grow too large. An important factor is temperature. It usually promotes the formation of nuclei more than the growth rate. Hence lowering of temperature will favor the forma-

tion of long chains. The lower the temperature and the purer the monomer the longer chains will be obtained. A high concentration of initiators and modifiers will produce shorter chains.

The polymerization reactions can be carried out in various ways: (1) in *bulk*, (2) in *solution*, (3) in *dispersions* of monomers. Polymerization in bulk of a monomer can occur spontaneously, as already mentioned. On commercial scale this is done in cases when the polymer formed melts without decomposition, in order to apply it for various purposes. Monomers can be polymerized also in suitable solvents. For example, styrene can be dissolved in alcohol, and the polymerization can be initiated by certain catalysts or initiators. The solution warms up on polymerization, and an amorphous mass of polystyrene separates out of the solution. In some cases the solvent itself may participate in the termination of the growing chains. Polymerization of dispersions of monomers is very important, and, depending on the conditions, it may produce either a finely precipitated polymer (bead polymerization) or stable dispersions (emulsion polymerization)[5]. In these instances the monomer is slightly soluble in the solvent which usually is water. Most commonly the monomer is dispersed by means of suitable emulsifying agents, and certain catalysts and modifiers also are added. Depending on the system, the polymerization may occur either in the water phase, at the surface, or inside the dispersed droplets. If soaps or other stabilizing detergents are present, the resulting particles of the polymers formed are kept in a form of dispersion. Such dispersion resembles rubber latex or cream, it is handy for various applications, such as surface coating. The amount of additives is small in comparison with the amount of monomer. It is also important that the best polymers are usually obtained in *copolymerization*. For example, for good protective coatings mixtures of butyl methacrylate and vinyl acetate are emulsified by means of sulfonated castor oil, and polymerization is initiated by adding small amounts of hydrogen peroxide, and other substances. The mixture must be warmed up to 60–70° C and stirred. The beginning of the polymerization can be perceived by observing the spontaneous rise of the temperature, because of the exothermic character of the chain growth. In polymerizing large amounts cooling becomes necessary[6].

It must be pointed out that the dispersions obtained in emulsion polymerization are not molecular solutions but dispersions of limited stability. They are milky and more or less consistent depending

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on the concentration of the dispersed phase. In order to retain the stability after polymerization, these dispersions must contain a certain amount of stabilizing soap or other stabilizing agent which protects the particles from coalescence and precipitation. These particles are aggregates of many macromolecules of the polymer.

2. Polycondensation

Wallace H. Carothers is one of the great pioneers in the branch of polycondensation or condensation polymerization. His excellent review written in 1931 is an example of clarity and stimulating power, and can be read profitably even after more than 20 years of extensive development of this branch[7]. This article is characteristic for the approach an organic chemist has to organic colloids. Carothers, like Staudinger, had not the slightest doubt that synthetic and natural polymers are macromolecular substances. Many new synthetic polymers were developed by Carothers and his associates in the laboratories of E. I. du Pont de Nemours and Company in Wilmington, Delaware. Most useful of these is *nylon* which is produced now in huge quantities, and is used as starting material for various things in everyday life and industry.

Polycondensation or condensation polymerization differs from simple addition polymerization chiefly in that water or some other micromolecular substance is formed beside the polymer. Moreover, polar monomers usually react in a polycondensation. And finally, simple addition polymerization usually can be initiated more easily than polycondensation.

The molecules capable of mutual reaction and chain formation must possess at least two reactive groups, they must be *bifunctional.* Examples of such molecules are: the glycols HO—R—OH, hydroxyacids HO—R—COOH, diamines H_2N—R—NH_2, dicarboxylic acids HOOC—R—COOH, and amino acids HOOC—R—NH_2. *Trifunctional* or *polyfunctional* molecules also may undergo polycondensation, but in such instances branched chains and space polymers are obtained. Examples of trifunctional compounds are glycerin HO—CH_2—CH(OH)—CH_2—OH and glutamic acid HOOC—CH(NH_2)—CH_2—CH_2—COOH.

The simplest polycondensations are those in which only one kind of bifunctional molecules react mutually, and long chains with the same repeating units are formed. Examples are:

$$n\text{HO—CH}_2\text{CH}_2\text{—OH} \longrightarrow$$

ethylene glycol

$$\text{HO—CH}_2\text{CH}_2\text{—O—CH}_2\text{CH}_2\text{—O—CH}_2\text{CH}_2\text{—O—}\ldots\ldots\text{—CH}_2\text{CH}_2\text{—OH} + (n-1)\text{H}_2\text{O};$$

polyethylene glycol

$$n\text{HO—RCOOH} \longrightarrow$$

hydroxy acid

$$\text{HO—R—CO—O—R—CO—O—R—CO—}\ldots\ldots\text{—O— R—COOH} + (n-1)\text{H}_2\text{O}$$

polyester

In the first example ether bonds are formed between pairs of glycol molecules, the first step being:

$$\text{HO—CH}_2\text{CH}_2\text{—OH} + \text{HO—CH}_2\text{CH}_2\text{—OH} \longrightarrow$$
$$\text{HO—CH}_2\text{CH}_2\text{—O—CH}_2\text{CH}_2\text{—OH} + \text{H}_2\text{O};$$

the di-glycol continues to react the same way with more monomers, and the chains become longer and longer. In the second example the hydroxy groups react with the carboxyl groups of another molecule, whereby ester bonds are formed:

$$\text{HO—R—COOH} + \text{HO—R—COOH} \longrightarrow \text{HO—R—CO—O—R—COOH} + \text{H}_2\text{O};$$

the ester reacts with more molecules and the chain length increases:

$$\text{HO—R—CO—O—R—COOH} + \text{HO—R—COOH} \longrightarrow$$
$$\text{HO—R—CO—O—R—CO—O—R—COOH} + \text{H}_2\text{O},$$

and so forth.

Amino acids and their derivatives also can undergo polycondensation[8].

The more complicated polycondensation products which are formed from two or more monomers are very important. Of the synthetic polymers of this kind first developed were the polyesters produced using as starting materials some dicarboxylic acids and glycols. When succinic acid HOOC—CH_2CH_2—COOH is heated with ethylene glycol, esterification begins at about 170° C, whereby water vapor is evolved. After completed esterification and liberation of unreacted glycol by distilling at low pressure a thick liquid mass remains; on cooling it solidifies to a hard, opaque mass, the polyester. The reaction is illustrated as follows:

$$n\text{HOOC—CH}_2\text{CH}_2\text{—COOH} + n\text{HO—CH}_2\text{CH}_2\text{—OH} \longrightarrow$$
$$\text{HOOC—CH}_2\text{CH}_2\text{—CO—O—CH}_2\text{CH}_2\text{—}\ldots\ldots\text{—O—OC—CH}_2\text{CH}_2\text{—CO—O—CH}_2\text{CH}_2\text{—OH}$$
$$+ (2n-1)\text{H}_2\text{O}.$$

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It could be shown that these polyesters have a molecular weight of several thousands, i.e. they are semi-colloidal.

Much more important commercially are the polyamides which are produced by condensing dicarboxylic acids with diamines. The various types of *nylons* are the products of these polycondensations. One of the most important examples in this series of reactions is the polycondensation of adipic acid HOOC—$CH_2CH_2CH_2CH_2$—COOH and hexamethylenediamine H_2N—$(CH_2)_6$—NH_2:

$$n H_2N\text{—}(CH_2)_6\text{—}NH_2 + n HOOC\text{—}(CH_2)_4\text{—}COOH \rightarrow$$
$$H\text{—}[HN(CH_2)_6NH\text{—}OC(CH_2)_4\text{—}CO]_n\text{—}OH + (2n-1)H_2O.$$

Polyamide, nylon

This polymer has excellent mechanical properties, thermal and chemical stability. It can be drawn to fibers and spun and woven to fabrics which compete with natural silk.

The constitution of nylon in fact resembles very much the constitution of silk. Both are polyamides composed of long chains in which the —NH—CO— groups appear in a regular sequence. In both nylon and silk hydrogen bonding between the —CO— and —NH— of adjacent chains occurs along the whole chain length in many places. The result of this is increased mechanical strength of the fiber.

The nylons and also the polyamino acids are simple analogs of proteins. Silk, although its chains are built chiefly of glycine and alanine radicals, is much more complex than nylon. Wool, the fibrous collagens, as well as the myosins and fibrins are even more complex. The mechanism of polymerization of these macromolecular compounds, as it occurs in living cells, is unknown. The same can be said about the formation of such relatively simple natural polymers as cellulose. A more detailed survey of all these macromolecular substances will be given in Part II.

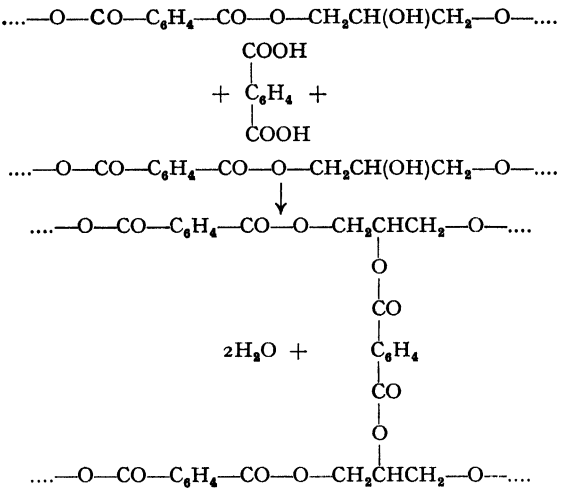
3. The Formation of Space Polymers

The polycondensation of bifunctional molecules is relatively simple, as linear molecules are formed which possess definite end groups. For example, the molecular weight of a polyester can be determined simply by titrating the purified polymer with alkali. The higher the molecular weight the smaller will be the amount of alkali which reacts with the end groups of equal amounts of polymer. Moreover, the viscosity increases gradually with increasing length of the polyester chains.

The reactions are more complex in the polycondensation of tri- and polyfunctional molecules. For example, if the trifunctional glycerin reacts with the bifunctional phthalic acid (or its anhydride), space polymers instead of linear chains are formed. In the initial stage of this reaction chains are formed which are then cross-linked in the next stages as follows:

$$n\text{HO—CH}_2\text{—CH(OH)—CH}_2\text{—OH} + m\text{HOOC—C}_6\text{H}_4\text{—COOH} \rightarrow$$
$$\text{HO-CH}_2\text{-CH(OH)CH}_2\text{-O-CO-C}_6\text{H}_4\text{-CO-O-CH}_2\text{CH(OH)CH}_2\text{-O-....CO-C}_6\text{H}_4\text{-COOH} + p\text{H}_2\text{O},$$

and the —OH groups of two chains can react with one molecule of dicarboxylic acid forming a bridge:



As the chains are long and many hydroxy groups are hanging on them, the interlinking may occur between many chains. In fact, the interlinking may take place in the whole mass between all chains. As a result an insoluble resin is formed which can be regarded as a single giant molecule. In the course of polycondensation of such polyfunctional molecules the bridging and cross-linking are perceivable by a sudden increase of the consistency of the system and gelation.

Space polymers can be formed of any linear polymers which contain active atomic groupings on the chains. One of the most important examples is the vulcanization of natural or synthetic rubbers. For example, the buna rubber contains double bonds in

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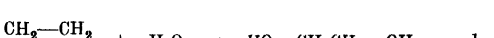
the chains, and these unsaturated spots react with sulfur. The originally soluble, sticky, and chemically rather unstable polymer thereby is transformed into an insoluble, more stable, and mechanically improved space polymer.

Sulfur bridges are found also in proteins. In wool, hair, and other keratins the polypeptide chains are linked through many sulfur bridges, and the keratins are insoluble space polymers of a very complex structure.

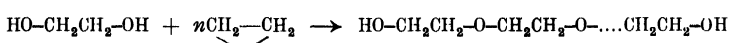
4. Ring Formation and Ring Scission in Polymerization and Polycondensation

Bifunctional molecules containing several active groups do not always combine in long chains. For example, the hydroxy acids may react intramolecularly, and micromolecular lactones or cyclic esters can be formed. Similar reactions occur with the amino acids. They do not react intermolecularly as easily as it may seem, or intermolecular reactions occur between two molecules only, whereby cyclic molecules are formed. For example, glycine does not polycondense readily on heating, but is converted mostly to an anhydrized dimer—diketopiperazine.

Whether chain formation occurs or not, depends on the properties of the starting material and on the conditions of the reaction. In some cases cyclic anhydrides may be formed as intermediates in a reaction of polycondensation. Reactive cyclic molecules may be even used as starting materials. An example is the polymerization of ethylene oxide. The polymerization in this case is initiated by a trace of water:

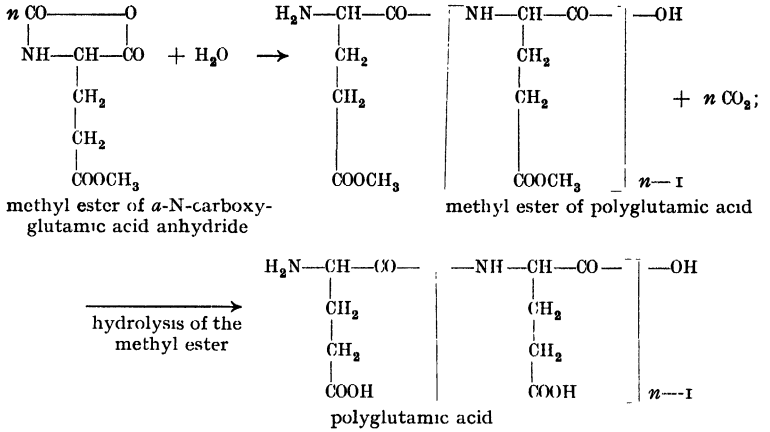


$$\mathrm{CH_2{-}CH_2} \;(\mathrm{O}) + \mathrm{H_2O} \longrightarrow \mathrm{HO{-}CH_2CH_2{-}OH}, \quad \text{and}$$



$$\mathrm{HO{-}CH_2CH_2{-}OH} + n\,\mathrm{CH_2{-}CH_2}\;(\mathrm{O}) \longrightarrow \mathrm{HO{-}CH_2CH_2{-}O{-}CH_2CH_2{-}O{-}\ldots.CH_2CH_2{-}OH}$$

The preparation of some polyamino acids by using cyclic derivatives of amino acids is of interest (*l.c.*[8]). For instance, glutamic acid does not form chains under ordinary conditions (heating, catalysts), but its derivative the γ-methyl ester of α-N-carboxyglutamic acid anhydride undergoes polycondensation easily, when dissolved in cold pyridine:



Like the case of ethylene oxide, traces of water initiate the reaction. The activated molecules of the carboxyglutamic acid ester then line up in chains. The polymeric ester can be saponified by means of alkali which will produce a salt of the polyglutamic acid, which by ordinary methods can be converted to pure acid. This reaction is an example where carbon dioxide is formed as by-product instead of water.

5. Dependence of the Properties of Polymers on Chain Length

The properties of polymers, as already indicated, differ very much depending on chemical composition and constitution. However, in the same polymer-homologous series the properties change regularly with the changes in molecular weight or with the length of the chain. Although all polymers are mixtures of molecules which are of similar constitution but various size, it is possible to characterize any polymeric product by determining the mean or average molecular weight (see Chap. 7, 8, and 9).

Many semicolloidal polymers are microcrystalline powders, they are not plastic, and in molten state or in concentrated solutions they can not be drawn into fibres and spun. The viscosity of their solutions is low. The very high molecular polymers are not microcrystalline powders but more or less completely amorphous plastic masses. They can be drawn into fibers and spun. The solutions of such polymers are very viscous.

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In Table 5 are presented data about the polycondensation products obtained from hydroxydecanoic acid[9].

TABLE 5

POLYESTERS OF HYDROXYDECANOIC ACID

Average molecular weight	*Melting (softening) point*	*Spinnability*	*Tensile strength*
1,720	72–74°C	Absent	
3,190	74–75	Absent	
5,670	73–75	Very short fibers	Very weak
7,330	74–75	Long fibers, but will not cold-draw	Very weak
9,330	75–76	Long fibers that cold-draw	Weak
16,900	77–78	Spins and cold-draws easily	Moderate

The softened polymer can be extruded and oriented in the shape of fibers only when the molecules are long enough. Moreover, the strength of the fibers increases considerably when the average molecular weight approaches 10,000. Calculations show that such chains are about 800 angstrom units long, i.e. they are colloidal in their length. About 60 residues (mers) of the hydroxydecanoic acid are lined up in such macromolecules. Many other examples of the dependence of mechanical properties on molecular size and structure will be shown in Chapters 16–27.

6. Degradation of Large Molecules[10]

All macromolecular substances are relatively stable when in bulk, although the stability depends on chemical composition and molecular structure. The cross-linked space polymers are more stable than linear polymers, when exposed to high temperatures or chemical reagents. All organic substances, of course, are decomposed at sufficiently high temperatures, and in the case of macromolecular solids the lower limits of temperature at which the decomposition begins are usually about 200–400° C. This decomposition on heating may be *irreversible* or *reversible*, depending on the polymer and the temperature. Irreversible decomposition is most common, and the chemical reactions which thereby occur are most complex and little

investigated. Decomposition of proteins and polysaccharides on frying and baking are examples. New macromolecular substances rich in carbon may be formed from the molecular fragments in such high-temperature decompositions or "cracking" of organic substances. The synthetic linear polymers, however, often yield the original monomers on heating, and the formation and degradation of the macromolecules may be more or less reversible. The polyesters and polyamides are degraded by heating with acids or alkalies. Macromolecular solids are degraded and decomposed not only by heat and chemicals but also by high energy radiations (such as from nuclear reactors), as well as by electrical and mechanical treatment. For example, the molecular weight of linear polymers decreases on intense milling of the solids.

The stability of macromolecules in solutions is even less than in solid state, and the stability varies considerably depending on the type of the large molecules. Heating, radiations, oxygen, and many other agents may cause degradation of large molecules in solutions. Linear macromolecules thereby can be split more readily than the compact particles of the molecular spherocolloids. This important fact can be explained by simple geometric and mechanical considerations. It is obvious that nothing much will happen to a spherical macromolecule when one valency bond will be split somewhere on the surface of the sphere; if, however, a bond in the middle of a linear macromolecule will be ruptured, two short chains will be formed. Such cleavage is always associated with profound changes in the properties of the solution of the polymer[11]. This instability of linear macromolecules was not considered by some early investigators of these polymers, as already mentioned in Chap. 2.

7. Synthesis of Graft and Block Copolymers[12]

The constituting radicals (mers) appear in the chains of an ordinary copolymer in an irregular sequence. When, for example, the copolymer is prepared from two monomers A and B the macromolecules of a copolymer can be symbolized as follows:

.....AABABBAABABBBBAABAAAABBABBBBABAA.....

However, the polymer chemists know how to prepare mixed polymers which have a more regular structure than in the ordinary copolymers. These studies have been initiated not only by mere curiosity but also because of the great practical interest and

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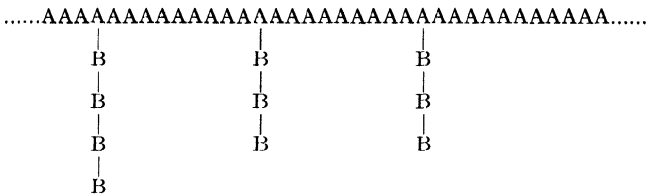
commercial importance of such substances. Since the ordinary copolymers have disorderly built chains, there are limitations in changing the properties of the polymers by means of rational planning. A more rational modification of properties is possible with the graft and block copolymers. The *graft copolymers* are composed of branched chains; side chains of another component ...BBBBBBB... are "grafted" on the chains of an ordinary simple polymer ...AAAAAAAAAAA... The constitution of the resulting graft copolymer then can be presented thus:



Another possibility of relatively orderly built mixed polymers is the case of the so-called *block copolymers* which are composed of more or less uniform "blocks" of two or more components. The constitution of these polymers can be illustrated as follows:

...AAAAAAAAAAAABBBBBBBBBB... or
...AAAAAAAAAABBBBBBBBBBBBAAAAAAAAAAA...

The grafting of the side chains can be accomplished in various ways. It is a relatively simple problem when the macromolecules which have to be modified contain some active groups which then may be made to react with some monomer (B). Natural and synthetic rubbers can be modified by grafting polyvinyl side chains on the rubber molecules. The chemically inert polytetrafluoroethylene (Teflon) can be made adhesive by grafting branches of polyvinyl acetate on the linear macromolecules of this polymer. A method by means of which graft or block copolymers can be synthesized is to activate or partially split the components by ultrasonic, ultraviolet or some other radiations. For such treatment, the polymer ...AAAAAAAAAA... is usually dissolved in the monomer B. The long chains of ...AAAAAAAAAA... are either activated or split under the influence of the radiation. If they are only activated, the polymerization of B is initiated at the activated sites, and a graft polymer will result. (The radiation energy activates also the monomer B, whereby the lining-up of the B-units at the activated

spots of the A-chain is granted.) If, however, the A-chains are cleaved, the growth of B will occur at the freshly formed ends of the A-chains, and a block copolymer will be obtained. Very convenient is the manufacture of mixed block polymers by means of emulsion polymerization. A monomeric oily liquid A is emulsified in an aqueous solution of another monomer B, and the polymerization of B is initiated by some added catalyst. The growing chains of ...BBBBBBBB... come in contact with the oil phase, initiate the polymerization of A, and a mixed block copolymer is finally produced. Many other methods have been proposed for the preparation of graft and block copolymers, and some of them have already found important applications in the technology of polymers[13].

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CHAPTER 5

Preparation of Dispersions and Micellar Colloids

Macromolecules are formed in chemical reactions of polymerization or polycondensation. The resulting substances are molecular colloids. Dispersions and micellar colloids are prepared either by means of simple mechanical disintegration or by spontaneous aggregation. Any micromolecular substance can be ground to fine particles and dispersed in a liquid. A colloidal dispersion will result when such fine particles are insoluble in the particular liquid. Hydrophobic oils can be homogenized by means of emulsifying machines, and dispersed in water. Spontaneous formation of micelles by aggregation occurs with organic molecules which are composed of hydrophobic and hydrophilic parts. Dispersions are coarser and less stable than spontaneously formed micellar colloids. However, dispersions can be stabilized by various means, especially by adding a stable micellar colloid, such as a soap solution.

1. Mechanical Dispersion Methods

Solids and liquids can be dispersed by milling and grinding. Ordinary grinding in a mortar or milling in a ball mill, however, will not produce colloidal dispersions. For example, the finest flour or powdered sugar appears on microscopic examination to be composed of rather coarse particles. Much finer dispersions can be obtained by means of colloid mills and homogenizers. They differ from ordinary mills in several ways: (1) the disintegration occurs in an indifferent medium, (2) the shear stresses are very great, and (3) the substance is forced through extremely narrow clearances.

Fig. 3 illustrates these points. The substance to be dispersed is suspended in a liquid, and the mixture is sheared between two surfaces. Both solid and liquid particles (droplets) are disintegrated under the influence of the shearing force, and the process can be repeated in order to get very fine dispersions. However, experience has shown that it is impossible to obtain truly colloidal dispersions in this way. Although the particles are smaller than those obtained in ordinary milling, they are larger than most macromolecules. Moreover, the dispersions contain particles of various sizes. This pertains also to homogenization of emulsions. Although the droplets after homogenization are smaller than before and also more uniform, their sizes still show considerable variation. The dimensions of the particles and droplets coming out of a colloid mill are about 0.0002 mm–0.005 mm.

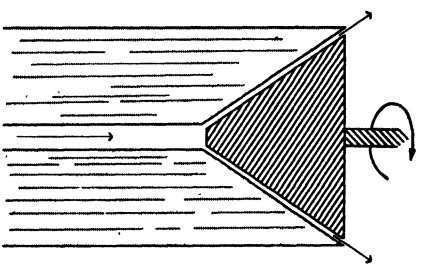

Fig. 3. Emulsifier.

The liquids or solids to be dispersed in a particular liquid must be insoluble in it; if this condition is not met, the material will simply dissolve. It is impossible to prepare dispersions of sugar in water, but sugar can be dispersed in various organic solvents which are non-solvents for sugar. Lipophilic substances, such as oleic acid, on the contrary, cannot form colloidal solutions in ether or alcohol, as oleic acid is completely soluble in these solvents, and the dissolved molecules are much smaller than colloidal particles. Oleic acid, however, can be colloidally dispersed in water.

Space polymers and other macromolecular substances, which are insoluble in a particular liquid, can also be dispersed with the aid of mechanical disintegration. For example, a piece of bakelite (a phenol-formaldehyde space polymer) can be ground and dispersed in water. By rubbing fingers in water it is possible to produce a fine dispersion of skin epidermis. Organic tissues and cells are

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disintegrated in various types of tissue homogenizers. Cellulose, polystyrene, and other macromolecular substances become more easily dispersible after long milling. The disintegration of high polymeric materials on milling was studied by Staudinger and associates[1]. It can be shown that the molecular weight of macromolecular substances, such as cellulose or polystyrene, is decreased upon long milling, and the materials thereby become more readily dispersible. Some of the very long chains may be disrupted by such treatment. An increase in temperature and the presence of oxygen are important factors in such chain cleavage. It is obvious that cleavage of primary bonds must also occur on disintegrating the masses of space polymers.

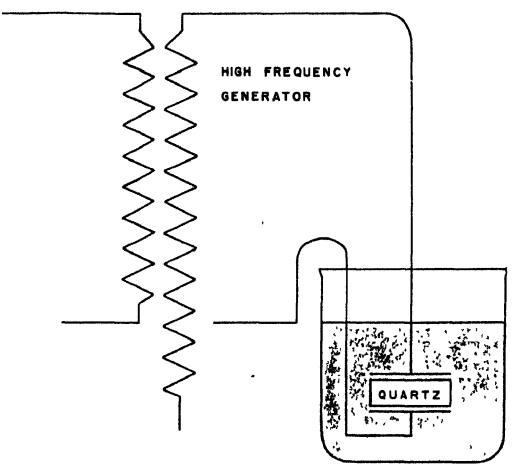

Fig. 4. Ultrasonic radiator.

Mechanical dispersion of solids and liquids may also be accomplished by high frequency sound waves. The lowest frequency of audible sound is 16 cycles per second, and that of the highest pitch sound which is still audible is about 15,000 cycles per second. The wavelength of this high pitch sound is 2.2 cm in air, and about 8 cm in water. If any vibrator produces vibrations of a still higher frequency, the sound is inaudible to humans. These ultrasonic vibrations can be produced by quartz oscillators induced by high frequency generators (Fig. 4). Langevin showed (in 1918) that quartz crystal discs, if connected with a high frequency electrical

generator, produce mechanical oscillations having the character of sound waves. Ultrasonic vibrations with a frequency of 25,000–1,000,000 cycles per second can be produced by such devices. (For lower frequencies of 3,000–50,000 cycles per sec. magnetostrictive sound generators are used.) It has been shown by many investigators that oils and many solids can be dispersed as colloids by irradiation with high frequency ultrasonic waves[2]. Such a high frequency ultrasonic beam seems to act like a bullet. The impact of such a "sound bullet" is determined by the frequency. The intensity of the vibration is also important. This can be illustrated by the number of bullets hitting a target. The intensities of the vibrations are high, reaching approximately 10 watts/per cm^2, which is many million times higher an intensity than that produced by an average radio. Not only are loosely built solid aggregates dispersed, but macromolecules can be split into shorter fragments[3]. Microbes are killed easily, the cells being torn to pieces.

Dispersion by ultrasonic radiation is used extensively for industrial purposes, for instance, in the preparation of emulsions, finely dispersed paints, dyes, and graphite sols. Sound waves can also be used for the opposite purpose—precipitation of dispersed material. For this purpose vibrations of relatively lower frequency are used.

2. Preparation of Dispersions by Change of Solvent

Dispersions can be prepared not only by mechanical disintegration of solid or liquid masses but also by the opposite procedure: by aggregating small molecules upon addition of a non-solvent to a solution. Any organic substance can be dispersed in this way. For example, the higher alcohol homologs are practically insoluble in water but soluble in methyl alcohol. When such a solution is poured into water, a dispersion of the substance in the water-methanol mixture will result, if the amount of water is large enough. Similar experiments can be carried out with the water-insoluble higher fatty acids, fats, terpenes, carotenoids, and many other substances. If the particular substance is insoluble in alcohol, another solvent can be found for it; then, when the substance is dissolved, the solution must be mixed with a liquid which is a non-solvent for the particular solute, but is miscible with the solvent. Upon sufficient dilution with this non-solvent the solute will be pushed out of the solution. It will appear either in the form of macroscopic molecular aggregates or in a highly dispersed state. Most of the dispersions

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produced in this way are coarse and unstable. If the solute is heavier than the medium, the dispersed particles precipitate; they float on top if the dispersed phase is lighter than the liquid used as the medium. However, some of the organic dispersions prepared in this simple way possess astounding stability. They are the micellar colloids, such as soaps. For instance, sodium oleate, $C_{17}H_{33}COONa$, is soluble in alcohol, and this alcoholic solution is micromolecular. Single small molecules of soap are dispersed in alcoholic solution, as can be shown by means of osmotic or cryoscopic measurements. When such a solution is poured into water, a colloidal soap solution is formed, and this colloid is stable. The question then is: why are the soap colloids stable; why are the aqueous dispersions of fats, carotenoids, naphthalene, and similar substances not stable? These questions will be discussed in the following section.

3. Colloid Stability Factors

One obvious reason that coarse dispersions are not stable is the relatively large size of the particles. As there is usually some difference in density between the particles and the dispersion medium, they either sink or float to the top. The separation of the phases may be slowed down by increasing the viscosity of the medium. It is obvious that the movement of the particles will be slowed down if they have to move in a thick medium. Glycerin may be added to aqueous dispersions in order to stablize them. It is also self-evident that the separation will be hampered if the density of the medium is adjusted as closely as possible to the density of the particles.

It can be shown, however, that particle size, density differences, and viscosity are not the decisive factors in stability. Aggregation occurs even if the particles are very small, if the viscosity is high, and if there is no density difference at all. The smaller particles have even a greater tendency to aggregate than the larger ones. What is the reason for this aggregation? There are two reasons: *Brownian movement,* and the *attraction between surfaces* of the particles.

If the dispersion is coarse, there is no Brownian movement, as the particles are too large to be influenced by molecular bombardment. Hence the following discussion pertains to very fine dispersions only, and to true colloids as well. In such systems the particles are steadily displaced by the uneven molecular impacts; they are

in Brownian motion. They neither sink nor float, even if their density differs much from that of the medium. However, because of this motion they may *collide*, and these collisions may result in *aggregation*. The latter occurs because of the *attraction* which always exists between similar surfaces (Van der Waals forces, cohesion). Hence, a fine dispersion will not necessarily be stable. The original small particles will not separate out in this case but their aggregates will. Moreover, it can be expected from the above that the finer the dispersion the faster will be the aggregation. This is substantiated by the facts that: (1) the *Brownian movement increases* in intensity with *increasing temperature* and *decreasing particle size*, and (2) *the surface area increases* with *increasing subdivision*. The latter statement can be proven by a simple calculation of the regular splitting of a compact cube. Each split will result in the formation of new surfaces. If we start with a cube with an edge length of 1 cm, the total surface of the cube will be 6 cm^2. If the cube could be ground to smaller cubes with an edge length of 1 mm, the one original cube will produce 1000 small cubes with a total surface of 60 cm^2. On further subdivision the particle size decreases further, and the number of particles and the total surface area increases, as shown in Table 6.

TABLE 6

INCREASE OF TOTAL SURFACE ON SUBDIVISION

Length of a side of the cube	*Number of cubes*	*Total surface of the cubes*
1 cm	1	6 cm^2
1 mm	10^3	60 cm^2
0.1 mm	10^6	600 cm^2
0.01 mm	10^9	6000 cm^2
0.001 mm (1 μ)	10^{12}	6 m^2
0.000,1 mm (0.1 μ)	10^{15}	60 m^2
0.000,01 mm (100 A)	10^{18}	600 m^2
0.000,001 mm (10 A)	10^{21}	6000 m^2

The Table shows that enormous surface areas are formed on an extensive subdivision. The larger the surface the greater will be the surface forces or interaction among surfaces. The surface area can be considered as the capacity factor of surface energy. The larger the surface the greater will be the surface energy. According to the second law of thermodynamics, the free energy of any system tends

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to decrease. Hence, the particles should have the *tendency to decrease their surface.* That this is true is confirmed by the well known fact that liquids, when in small enough quantities, assume the shape of a sphere, which possesses the smallest possible surface. When many small beads collide, they flow together into a larger bead, because in this way the free surface energy will be decreased. This is why droplets in dispersions flow together and phases separate.

The enormous increase in free surface and surface energy is also the reason why it is so difficult to prepare very fine dispersions by milling. The finer the material is ground the greater become the attractive forces which tend to reunite the particles. This recombining can be partly prevented by using an indifferent material as diluent, or by milling the substance directly in the liquid in which it is to be dispersed. However, even then the most efficient colloid mills produce suspensions of only moderate degrees of dispersion.

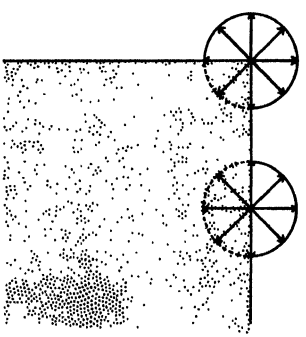

Fig. 5. Attractive forces of molecules on the surface of a crystal.

The mutual attraction of particles can also be looked at from a different point of view. Let us consider the molecules sitting on the surface of a dispersed particle (Fig. 5). These molecules differ considerably from those in the bulk, as the intermolecular forces on the surface are not mutally saturated. Hence attractive forces will be exhibited on the surface. When two particles are pushed together by means of Brownian motion, the surfaces will interact, and the particles will tend to stick together. It is obvious that in the case of crystalline particles, the molecules which are in the corners and on the edges, will be most active, as there the field of mutually unsaturated valency forces is greater than on a flat surface.

This also clarifies the variable action of ultrasonic radiation on emulsions and similar systems. If the radiation energy is very high, the droplets are smashed and dispersed. The attractive forces are overcome. If, however, the sound waves are of only moderate energy, they merely produce a kind of stirring effect which increases the number of collisions, but is too weak to smash the droplets.

Now, when the instability of simple dispersions has been clarified, we can proceed to the next question: why then are the aqueous

soap colloids stable? Why do the soap micelles not aggregate and precipitate or float out? What prevents the aggregation? It is the *electrical charge of the particles*, and the interaction of the particles with the medium. The electrical charge in soaps is much more important than the interaction with the solvent. This can be illustrated by simple experiments. Olive oil is composed of glycerin esters of fatty acids, and it produces coarse unstable emulsions, if shaken with pure water. However, stable emulsions are obtained if a little alkali is added before shaking. Similar experiments can be done, e.g. with oleic acid. Coarse, unstable emulsions of oleic acid are obtained on shaking with water; a stable colloid results on adding some alkali and stirring. What the alkali does is to saponify a small amount of the olive oil, and react with the fatty acid liberated by saponification, whereby *strongly dissociated salts* of the acids are formed. *The anions of the fatty acids confer an electrical charge on the particles.* There was no charge on the droplets of olive oil, as the fatty acid esters are non-electrolytes; hence the emulsion was not stable. There is only a very small charge on the surface of the oleic acid droplets, as this is a weak acid, and only a few molecules are dissociated. The alkali metal salts of the fatty acids, however, are completely dissociated. The particles or micelles of the resulting soaps all have a *negative charge*, hence *they repel each other if collision happens to occur* (see Fig. 6).

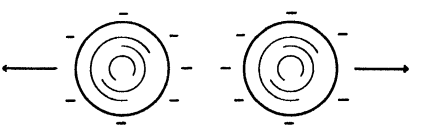

Fig. 6. Mutual repulsion of two negatively charged particles.

This is further substantiated by the peculiar *constitution* of soap micelles. The long hydrocarbon "tails" of the soap molecules are hydrophobic, i.e. they try to escape water. When they happen to arrive at the surface of the water, they stick out in the air. Those which are inside the liquid will contract, and stick together. Consequently, when an alcoholic soap solution is poured into water, the hydrocarbon tails of the soap molecules will associate, and micelles will be formed. The *hydrophobic* hydrocarbon part will be turned *inside* the micelle, whereas the *ionized carboxyl groups* will be turned *toward the water* phase. These ionized carboxyls confer the *charge and stability* to the micelles. The reason for the formation of stable micelles and stable hydrosols of soap is the peculiar constitution of the soap molecules.

Soaps and detergents are not the only substances able to form

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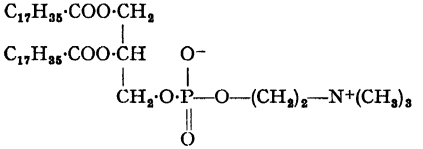
micelles and stable hydrosols. Many dyes, lecithins, bile acids and their salts, and many other micromolecular substances have similar properties. The chief condition for micelle formation is the presence of a large *hydrophobic part* and a strongly *polar group* in the *same molecule.* Some examples are listed in Table 7.

TABLE 7

MICROMOLECULAR COMPOUNDS CAPABLE OF MICELLE FORMATION

Compound	*Formula*	*Sign of charge of micelles*
Sodium oleate	$C_{17}H_{33}\cdot COO^-Na^+$	minus
Sodium dodecyl sulfate	$C_{12}H_{25}\cdot OSO_3^-Na^+$	minus
Sodium dodecyl benzene sulfonate	$C_{12}H_{25}\cdot C_6H_4\cdot SO_3^-Na^+$	minus
Heptadecyl amine hydrochloride	$C_{17}H_{35}\cdot NH_3^+Cl^-$	plus
Lecithin		depends on pH
Sodium cholate	$C_{23}H_{36}(OH)_3COO^-Na^+$	minus
Benzopurpurin	$Na^+{}^-O_3S—C_{10}H_5(NH_2)—N=N—C_6H_3(CH_3)—$ $C_6H_3(CH_3)—N=N—C_{10}H_5(NH_2)—SO_3^-Na^+$	minus

Soaps and other micelle-forming substances are also used as stabilizing agents in the preparation of emulsions of oils or dispersions of solids. The soap covers the dispersed particle, and the ionogenic groups thus confer a charge to the particle to which the soap adheres.

4. Charge and Solvation of Colloidal Particles

It was mentioned before that the stability of a colloid depends on the charge on the particles and on the interaction of the particles with the solvent. Many stable colloids are known whose particles are not charged at all. Their stability depends solely on the interaction with the solvent, the so called *solvation.* In the case of water as solvent this interaction is called *hydration.* Solvation is a broader

term for any liquid. Rubber dissolves in benzene and a stable colloid is formed, as the macromolecules of rubber become solvated by the benzene. No electrical charges are involved in this case.

The stability of a hydrosol of soap depends on both charge and solvation (or hydration in this case). The polar water molecules are bound through dipole attraction and hydrogen bonds to the carboxyl ions of the soap. As the water molecules themselves are interlinked in loose clusters, the soap *micelle is interlinked with the solvent.* It is also assumed that around each ion of the micelle a certain number of water molecules is bound relatively tightly. This tightly bound water can be considered as a kind of *protecting layer* which may interfere with the tendency to agglomerate, if the particles collide.

It is important that relatively stable micellar colloids can be obtained of compounds which do not possess ionogenic groups in their molecules. Long chain esters of sugars or other molecules with several OH groups in the polar head, such as $C_{12}H_{25}COO\cdot CH_2CH(OH)\cdot CH_2\cdot O\cdot CH_2CH(OH)CH_2\cdot OH$ are capable of micelle formation.

Many micellar as well as molecular colloids are known which are *amphoteric* electrolytes. Lecithin is an example of an amphoteric micellar colloid (see Table 7). The phosphate ion carries a negative charge and the quaternary amino nitrogen a positive charge. The net charge on a molecule or micelle in this case depends on the acidity of the solvent. If the solution is acid, the dissociation at the phosphate group will be suppressed, but at the basic nitrogen it will be promoted. Hence the net charge will be positive. In alkaline solutions the dissociation at the nitrogen will be suppressed and at the acidic phosphate it will be increased. In this case the net charge will be negative. It can be shown that the stability of micellar colloids increases with increasing net charge on the micelles. This stability behavior has been very well investigated with proteins. There are both positive and negative charges on the surface of protein molecules. The first come from the ionization of the amino groups and the second from the carboxyl groups. The higher the net charge the more stable is a protein solution to precipitation. If the number of positive and negative ions on a molecule is the same, there is no net charge. Some proteins then precipitate (e.g. casein), some others do not. Gelatin is an example of a protein which does not precipitate when the net charge is zero (*isoelectric* protein). This indicates that the molecules are hydrated and kept

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and the pressure exerted on the float is measured by compensating the torsion by rotating the torsion head. (This torsion balance is calibrated by putting weights on a pan attached to the wire.) This is the Langmuir-Adam film balance; it was modified and developed further in various ways, e.g. a kymograph can be attached to the torsion wire in order to obtain continuous registration of the spreading[12].

The chief variables measured in film spreading are: the *film pressure* (force), and the *area* covered by the film. Suppose that an extremely small amount of a film-forming substance is spread

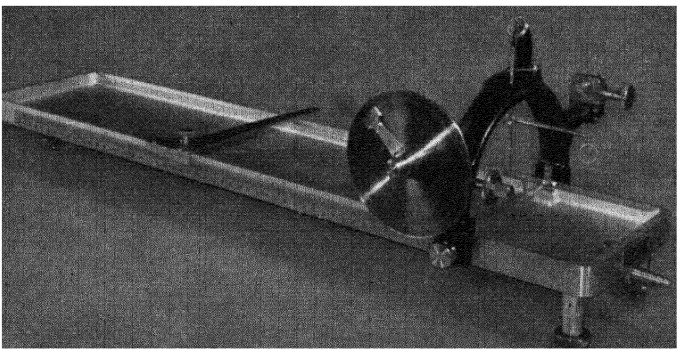

Fig. 16. A surface balance.
(Manufactured by the Central Scientific Company, Chicago.)

on a relatively large surface. When the amount of the substance is so small that it is not enough for even a monomolecular layer, the pressure will not increase upon reducing the area, i.e. pushing the movable slide toward the float. This is because in very large areas the substance forms islands or patches which do not influence the surface tension (and the surface pressure) appreciably. However, the pressure is strongly increased (the surface tension decreased) when the molecules are compressed by the moving slide to a monomolecular film which covers the entire surface area. The force exerted on the float then increases strongly. This continues until the film is compressed to a limit, when the molecules begin to pile up into polymolecular layers, in which case the pressure changes are small. This is illustrated by the curve of Fig. 17. On the abscissa is plotted the area (per molecule) and on the ordinate the pressure

only on the *number* of individual entities (molecules, ions, micelles). For example, the freezing point depression of a solution can be measured easily, and it depends solely on the number of the molecules and ions formed by dissociation of these molecules. These measurements showed that soaps behaved as normal electrolytes only in extremely dilute solutions. These are the solutions in which the molecules are free, not associated. Dissociation occurs according to the equation $R{\cdot}COONa = R{\cdot}COO^- + Na^+$, and it is practically complete; (in addition, hydrolysis of the soap occurs in relatively insignificant amounts: $RCOO^- + H_2O \rightleftarrows R{\cdot}COOH + OH^-$). In solutions below the critical concentration the freezing point depressions are exactly those to be expected in solutions of electrolytes. However, at the critical concentration or above it, *abnormally low* depressions are obtained. This indicates that the *number of ions and molecules has decreased.* There could be only two causes to consider for this phenomenon: (1) the degree of dissociation decreased abnormally, or, (2) the soap anions have aggregated. According to McBain[5] and others, the conductivity changes did not suggest suppression of dissociation, and aggregation into micelles is the reason for these anomalies (see Fig. 7).

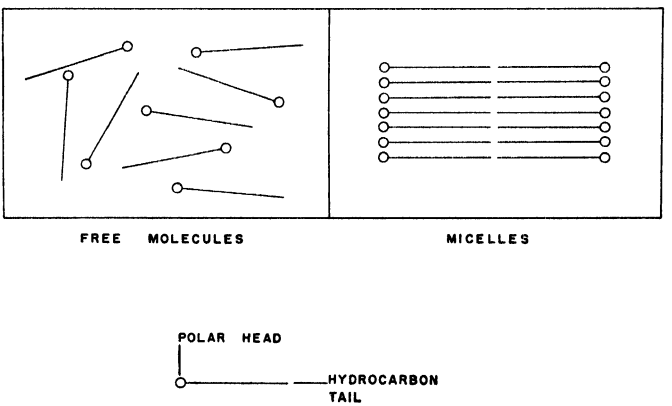

Fig. 7. Schematic illustration of free molecules of soap (in solution), and of soap micelles.

The decrease in critical concentration with increasing length of the lyophobic hydrocarbon chain can be understood easily. When there are only a few carbon atoms in the chain, the cohesive forces

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between the hydrocarbon radicals of two chains are much weaker than they would be between long chains. One has to keep in mind that these forces of attraction have to counteract the repulsive forces of the polar heads of the ions. In the homologous series of fatty acid anions the polar head remains the same (—COO^-), but the length of the hydrophobic radical increases in the series. Hence the repulsive force, in relation to the attractive forces between the similar radicals, decreases as the chain length increases.

6. Micelles in Non-polar Liquids

Soaps are molecularly dissolved in alcohol. There are two reasons for this: (1) dissociation, although not as complete as in water, still persists, and the alcohol molecules have some affinity for the polar head of the soap ions and molecules (or partly dissociated molecules), and, (2) the hydrocarbon radicals of the alcohol are also able to solvate the hydrocarbon chain. In a non-polar solvent, such as mineral oil, the conditions are different. In this case there is rather strong solvation of the chains, but no dissociation, and no interaction of the polar "heads" with the solvent molecules. Consequently, the polar heads in this case are pushed out of the solution. Micelles are formed in non-polar solutions of soaps, but the micelles have a different structure from those in aqueous solutions: the polar heads are turned inside the micelle, and the non-polar tails are turned toward the solvent. This is illustrated in Fig. 8.

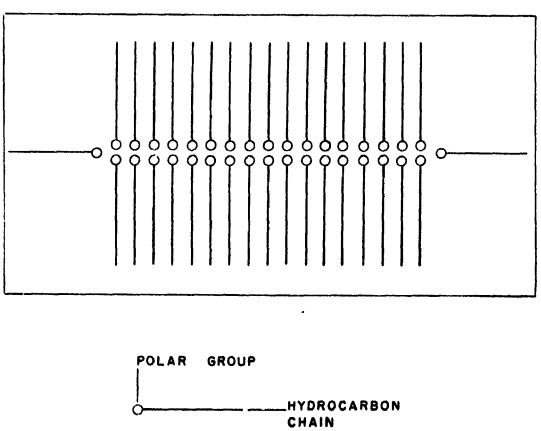

Fig. 8. Soap micelle in a non-polar solvent.

The aluminum soaps, and to a lesser degree the calcium, lithium, and other salts of acids with long hydrocarbon chains are important as additives to lubricating oils and greases. It can be shown that single molecules are present only in extremely dilute solutions, e.g. of aluminum soaps in benzene[6]. In some cases of long chain calcium salts of arylstearates in benzene critical concentrations as low as 0.000,001 moles per liter were found[7].

7. The Formation of Super-micelles

Concentration and temperature are the two chief factors which determine the micelle formation of certain substances in a certain liquid. Micelles can be formed only of substances whose molecules have a hydrophobic part and a relatively small polar "head". Moreover, micelles are formed only above a certain critical concentration at a certain temperature. Low temperatures are favorable to micelle formation. At high concentrations of the solute, and at sufficiently low temperatures, further changes in the organization of the micellar colloid may occur. This can be investigated by studying certain physical and chemical properties of the system (e.g. osmotic acitivity, specific molal volume, degree of hydrolysis) in wide concentration limits at constant temperature. Ekwall[8] noticed that some properties change abruptly at different concentrations. Assuming that the change at the lowest limit may be due to initial micelle formation, the changes at the higher concentrations may be caused by further transformation of the micelles. McBain strongly suggested the possibility that micelles of several kinds may be present in the same system. The possibility of different micellar sizes and shapes must be considered if various concentation limits are compared. Lamellar and needle-shaped structures have been observed in concentrated soap solutions.

The profound difference between micelles and macromolecules has been pointed out before. However, macromolecules may associate into larger and more complex entities which may be called *super-micelles*. Some polymers tend to associate in certain solvents, for example polyvinyl chloride macromolecules in dioxane[9]. In fact, any of the slightly turbid solutions of macromolecular substances to which a precipitant is added can be regarded as super-micellar. Such systems containing aggregated macromolecules can also be designated as macromolecular association colloids.

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CHAPTER 6

Surface Phenomena

1. Surface Tension

The molecules on the surface of liquids and solids differ from those in the interior, as indicated in the previous chapter. In the interior, the forces of attraction are mutually saturated in all directions, whereas on the surface the attraction is one-sided. An inward pull is therefore produced. Because of this pull, small droplets of liquids assume the shape of a sphere, resulting in a minimum volume and a minimum surface area. To increase the surface, work must be done, and the work needed to increase the surface area by 1 cm^2 is called a measure of the *free surface energy*. The free surface energy tends to decrease to a minimum, hence droplets, if allowed to come together, will form one single larger drop. The same is true for small solid particles.

The surface of water has some of the characteristics of an elastic membrane. It holds small objects whose density is far greater than that of water, if no wetting occurs. A needle coated with a film of fat or oil can be floated on water. This is due to the peculiar condition of the surface layer of the water, which is under tension, because of the inward pull. This surface tension is the force (in dynes) which acts at right angles to any line of 1 cm in the surface. The surface tension (in dynes per cm) is numerically equal to the surface energy (in ergs per cm^2).

The surface tension is the result of the mutual attraction between the molecules of the liquid. Hence the greater these intermolecular attractive forces the greater the surface tension. Polar liquids, such

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as water, have a high surface tension, whereas non-polar liquids have a much lower surface tension. Surface tension decreases with increasing temperature, as the intermolecular attraction and clustering is hampered by thermal motion. Some data about surface tension of pure liquids and solutions are compiled in Tables 9 and 10[1].

TABLE 9

SURFACE TENSION OF LIQUIDS AGAINST AIR (in dynes/cm) AT TWO TEMPERATURES

Substance	°C	*Surface tension*	°C	*Surface tension*
Acetic acid	20	27.6	50	24.7
Acetone	20	23.7	60	18.6
Benzene	20	28.8	50	25.0
n-Butyric acid	20	26.8	50	24.0
Chloroform	20	27.1	60	21.7
Ethyl alcohol	20	22.3	50	19.8
n-Hexane	20	18.4	40	16.3
Methyl alcohol	20	22.6	50	20.1
Phenol	20	40.9	50	37.7
n-Propylamine	20	22.4	45	19 4
Water	20	72 7	50	67.9

TABLE 10

THE SURFACE TENSION OF AQUEOUS SOLUTIONS

Substance	*Temp.°C*	*Surface tension for concentrations (%) indicated*				
Acetic acid	30	1.0 %	5.0 %	30.0 %	49.9%	100 %
		γ = 68.0	60.1	43 6	38.4	26.6
Acetone	25		5 0	25.0	75.0	100
		γ =	55.5	38.3	26.8	23.0
n-Butyric acid	25	0.14	1.05	3.83	8.6	100
		γ = 69	56	42	33	26
Ethyl alcohol	30	0.98	4.99	25.0	50.0	100
		γ = 66.1	54.2	34.1	27.5	21.5
Sucrose	25	10.0	20.0	30.0	40.0	55 0
		γ = 72.5	73.0	73.4	74.1	75.7

The surface tension can be measured by various methods. The classical and most important method of capillary rise is described in textbooks of physics and physical chemistry[2]. Measurement of the maximum bubble pressure, and the drop weight are also import-

ant methods. In colloid chemistry, however, the most popular are the ring detachment method of du Noüy, and the stalagmometric method of Traube.

The ring detachment method was improved recently by Harkins and others, and instruments are commercially available for the application of this method[3]. The instrument, the so-called 'tensio-

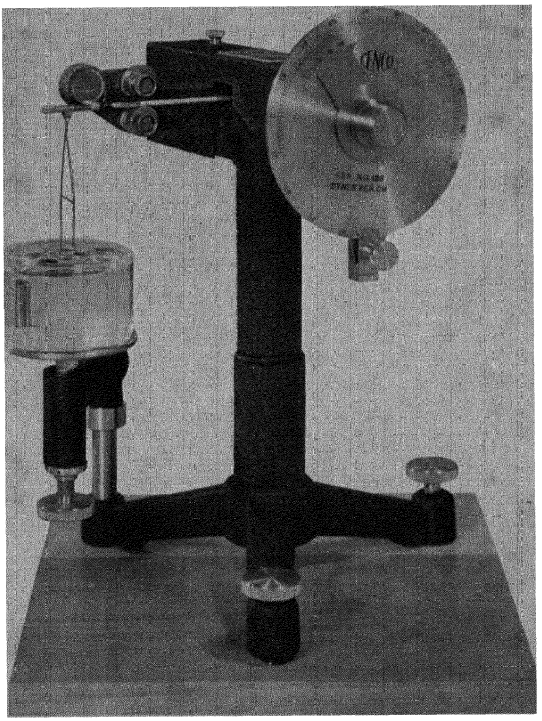

Fig. 9. Tensiometer according to du Nouy.
(Manufactured by the Central Scientific Company, Chicago.)

meter', is composed of a platinum-iridium ring hanging on one arm of a sensitive balance (Fig. 9). The ring is immersed in the liquid, and the force is determined (by increasing the weight of the other arm of the balance) which is needed for detachment of the ring from the surface. If γ is the surface tension, f the force (it equals

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the counterweight m times acceleration of gravity g) and r the radius of the ring,

$$\gamma = \beta \cdot f/4\pi r = \beta \cdot mg/4\pi r.$$

In this equation, β is a correction factor depending on the dimensions of the ring and some other factors. The instrument is first calibrated, and graphs showing the dependence of the surface tension γ on f (or m) can be prepared, and the values of γ can then be read from the measured values of weights m.

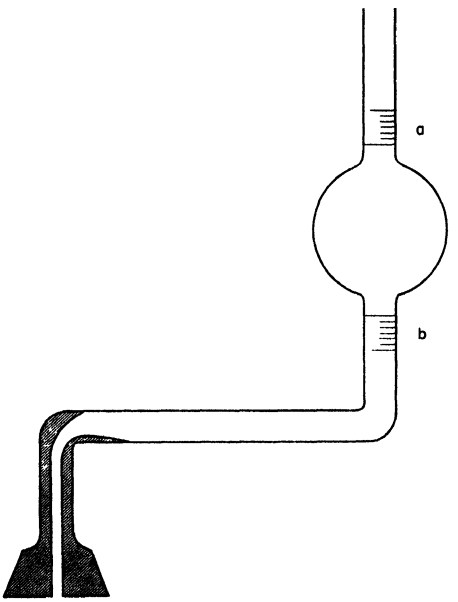

Fig. 10. The stalagmometer of Traube.

The stalagmometric method (meaning drop measurer) is very simple. In this method the number of drops formed by allowing a certain volume of the liquid to drop from a capillary is counted. In this case the work required for the formation of a fresh surface of drops is measured. The instrument is a bent calibrated glass tube (Fig. 10), the lower end of which is a capillary with a smoothly ground, flattened end. The liquid is pulled by a syringe over the upper mark, and the number of drops is determined by counting

while the known volume flows out. The unknown surface tension is determined by the equation:

$$\gamma = 73 Z_w \cdot d/Z,$$

in which Z is the number of drops of the liquid with unknown surface tension, Z_w is the number of drops of the same volume of water, d the density of the liquid, and 73 is the surface tension of water at room temperature[4].

2. Surface-active Organic Substances

It has been known for a time that the surface tension values for water are decreased considerably by some organic substances. If the capillary end of the stalagmometer is touched by a greasy finger, the drops become smaller. Extreme cleanliness therefore should be observed in such measurements. The influence of organic substances on the surface tension of water is demonstrated also by Table 10. All the substances mentioned there, except sucrose, decrease the surface tension of water. Such substances are called *surface-active* or *capillary-active* substances. Sucrose, on the contrary, increases the surface tension of water slightly, and sucrose belongs to the group of *surface-inactive* substances. Glycerin and inorganic salts are examples of other surface-inactive compounds.

When the influence of various organic capillary-active substances is compared, the differences appear quite striking. For example, acetic acid lowers the surface tension of water in much lesser amount than butyric acid (Table 10). The same was found in comparing other organic acids, e.g. valeric acid lowers the surface tension of water more than propionic acid, and even more than butyric acid. It appears that the same is true for other series of homologs, for example, amyl alcohol lowers the surface tension more than methanol, and hexyl amine more than methyl amine. The differences are even more pronounced, if molar concentrations instead of per cent concentrations are compared. Traube formulated a rule according to which the *surface activity increases with increasing chain length in a homologous series.* Some results with the fatty acids are presented in Fig. 11.

The lowering of the surface tension by surface-active substances is understandable when the chemical nature of the molecules is considered. According to the rule of Traube, the larger the hydrophobic part of the molecule the more surface active are the sub-

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stances. As we know, the hydrophobic part is pushed out of water, and this may result in collecting of these molecules on the surface of the water or micelle formation or both. *The concentration of a surface-active substance at the water surface is greater than inside the water.* The hydrophobic radicals stick out on the surface, and they interfere with the intermolecular forces acting between water molecules for purely geometric reasons. The inward pull thus is de-

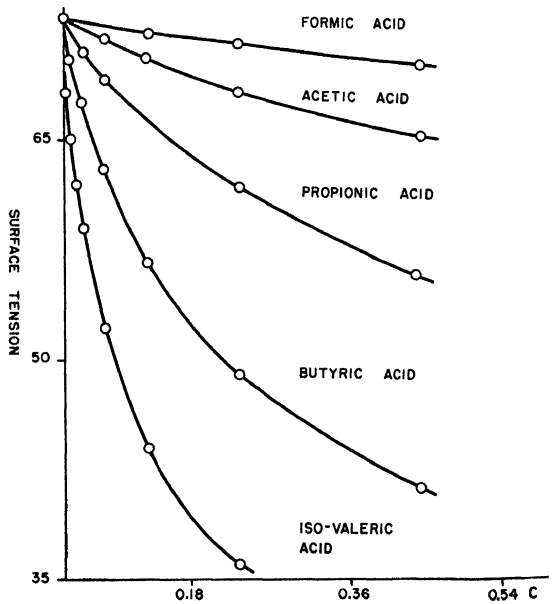

Fig. 11. Decrease of surface tension of the aqueous solutions of various fatty acids with increasing concentration c.

creased, the surface layer of water molecules is 'weakened', the drops on the end of a stalagmometer tube will become smaller. For the same reason, surface-inactive substances are found in the surface layer in a slightly lower concentration than inside.

So far the surface tension of liquids against air has been considered. The *interfacial tension between two liquids* is also important. Most common are the cases where water is one phase and the other component is a hydrophobic liquid. The interfacial tension can be measured, for instance, by the ring adhesion method. It has been found that the surface tension of water against hydrophobic liquids

is smaller than against air. First, even if the liquid has a very low solubility in water, it spreads in it somewhat and lowers its surface tension. Second, the molecules of the other liquid are able to compensate the inward pull somewhat more than air can. The surface tension of water against benzene is 35.0, against *n*-octane it is 50.8, and the interfacial tension of water against *n*-octyl alcohol is only 8.5 (at 20° C). The interfacial tension is lowered even more by adding a surface-active agent to the two liquids, e.g. ether, oleic acid, or soap. A result of such lowering will be the dispersion (emulsification) of one liquid into the other. Hence, the *lowering of interfacial tension promotes droplet formation.*

It is obvious from the aforesaid that the surface tension of micellar colloids must be lower than that of water. This has been confirmed by many observations. All those micromolecular substances whose molecules are composed of a large hydrophobic part and polar 'head' are pushed out of water; they associate into micelles, and at the same time they concentrate on the surface. The reason for this is the hydrophobic character of the large hydrocarbon radicals, their "fear of water".

3. Wetting of Surfaces[5]

The wetting of solid surfaces is another important surface phenomenon. Polar liquids wet solids composed of polar molecules, and non-polar liquids stick to hydrophobic surfaces. Wetting occurs if the attraction between the liquid and the solid surface is greater than between the molecules of the liquid.

The wetting of originally hydrophilic substances by water may be obscured by invisible thin layers of hydrophobic material. For example, clean fibers of cotton or wool are wetted by water; but in textile processing they may be covered with greasy, hydrophobic substances which repel water. The binding of dyes is thereby reduced, and in order to produce well dyed fiber, the material must be washed, i.e. liberated from the hydrophobic layer. If, on the contrary, water-repelling fabrics are desired, they are impregnated with some waxy, hydrophobic polymer. The surface of plant leaves is not sufficiently wettable, the morning dew being the well known indicator of this. The chemical agents used for the protection of plants from insects and virus diseases should adhere to the leaves, and wet them well, in order to obtain maximum benefit. For this reason surface active substances are added to sprays. Surface-active

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substances decrease the surface tension of the aqueous droplets of the spray, whereby the spray comes in better contact with the leaves.

4. Adsorption Phenomena

Adsorption or *sorption* is a loose non-stoichiometric binding of substances on surfaces. The wetting of hydrophilic surfaces occurs through adsorption of water. Solvation of colloidal particles or macromolecules by the molecules of solvent is also adsorption. The names *'wetting'*, *'solvation'*, and *'adsorption'* thus express things which are all of the same nature. The forces involved in adsorption are the physical Van der Waals forces, including hydrogen bonding and other secondary bonds. Some authors distinguish weak adsorption (cohesion) for *chemisorption*. The term 'absorption' is used mostly for swallowing up of gas by solids, e.g. hydrogen by palladium. The binding of phosgene gas by activated carbon, however, is now recognized as *ad*sorption, not *ab*sorption, as the porous mass of carbon possesses *internal* surfaces on which the gas is bound. In this treatise the adsorption and absorption of gasous substances will not be discussed. The problem will be restricted to adsorption in solutions.

As indicated before, hydrophobic molecules tend to pull out of aqueous solution, and their concentration at the water surface must be greater than in the body of the solution. By the same reasoning, hydrophilic molecules will be pushed out of a lipophilic liquid, such as oil.

If any liquid or solid particles are dispersed in water, and if any surface active substance is introduced as a third component, this surface active compound will collect chiefly on all the available surfaces. Hence, its concentration will be high not only on the surface of the solution but it also will be high on the surfaces of the dispersed particles. (And likewise, in an oily medium hydrophilic molecules will assemble not only on the surface but also on the particles dispersed therein.)

In respect to the adsorbability of various surface-active agents we may expect that the more they decrease the surface tension the better they will be adsorbed. In other words: the higher the surface activity the greater the amount which will be collected on all surfaces available. This appears to be true for all aqueous solutions investigated. It is interesting that physical chemists arrived at

similar conclusions on the basis of theoretical calculations and speculations in thermodynamics. The great American scientist J. Willard Gibbs (1878), and independently J. J. Thomson in England (1888), derived equations which correlate distribution of a solute on surfaces with changes in surface tension. For a simplified case of a dilute solution in a state of equilibrium, the excess of solute a (in moles) at the surfaces depends on the concentration c (moles per liter) and on the surface tension change $d\gamma/dc$ as follows:

$$a = -(d\gamma/dc)\cdot(c/RT),$$

where R is the gas constant and T is the absolute temperature. When $d\gamma/dc$ is positive, i.e. when the surface tension γ increases with concentration c of the solute, a must be negative. This would mean that the body of the solution is richer in solute than are the surfaces, which is the case with many simple salts, sugars, and other surface inactive compounds. However, when $d\gamma/dc$ is negative, as in the above equation, a is positive, i.e. the solute is concentrated on the surfaces. (The values of R, T, and c never can be negative.) A negative $d\gamma/dc$ indicates that the surface tension γ is decreased upon the increase of concentration c. This is the case with all surface-active substances (see Table 10), as found experimentally. Gibbs' equation thus states that *the adsorbed amount of solute will be the greater the more the surface tension of the solution is changed by changing the concentration of the solute.*

That the concentration of surface-active substances on the surface layer of water is higher than in the body of the solution was confirmed by several series of experiments[6].

There is another important dependence presented in the above equation of Gibbs: *the amount adsorbed is inversely proportional to the temperature.* This is evident also from molecular kinetic considerations: increased thermal motion will disturb any inhomogeneous distribution. The weakly bound molecules at the surfaces may be knocked off easily by the impacts of the molecules of the solvent.

The above mentioned Gibbs' equation holds only for some simple cases, and it cannot be generalized and applied to any system. Other factors are involved, and it appears to be impossible to predict everything theoretically. For this reason, various experimentally found correlations, chiefly between the amount adsorbed and the concentration, have been presented in the literature[7]. In the adsorption experiments, the *amount adsorbed* is correlated

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directly with the *concentration* of the solute. Furthermore, the amount of the adsorbent (which is usually a porous material or a colloid) must be considered. The temperature influence should also be investigated. Most adsorption studies, however, are carried out at constant temperature. Charcoal was one of the most popular adsorbents in the early studies on adsorbtion. The experiments were conducted as follows. Equal amounts of charcoal were weighed out and introduced into series of solutions of varying concentrations. After stirring for a short time, equilibrium is attained, the carbon is filtered off, and the concentration of the solute which remained in solution is determined by chemical or physical means. If adsorption has occurred, the concentration after the treatment with the charcoal will be smaller than the original concentration. The amount adsorbed will be the difference between these concentrations before and after the treatment. The same series then can be run with another constant amount of charcoal, thus investigating the importance of the amount of the adsorbent, or equal amounts of another adsorbent, e.g. a synthetic resin can be taken.

It was found some 60 years ago that the following empirical relation holds between the adsorbed amount a, concentration c and the amount of adsorbent m:

$$a/m = kc^{1/n}, \quad \text{or} \quad \log a/m = \log k + 1/n \log c,$$

k and n being constants. The logarithmic form of the equation represents a straight line relationship, in which the value a/m represents the amount adsorbed per gram of adsorbent; $\log k$ will be the intercept, and $1/n$ the slope (Fig. 12). The exponential function gives a curve. The shapes of the curves obtained differ quite

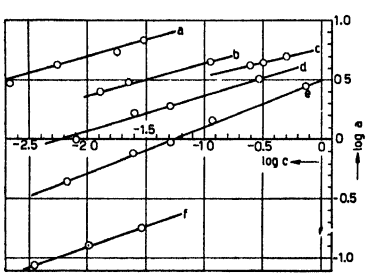

Fig. 12.
Straight line relationships between the logarithm of adsorbed amount and the logarithm of concentration.

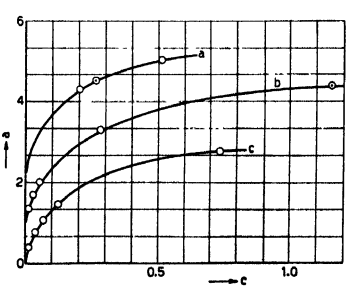

Fig. 13.
The change of amount adsorbed (a) with increasing concentration (c) for three different systems.

considerably (Fig. 13), the exponent $1/n$ having values usually between 0.1 and 0.5 for various systems. The constant k will be different for various solutions and various adsorbents. Since the dependence of adsorbtion on concentration is correlated at a constant temperature, the curves are called *adsorption isotherms*. These isotherms differ chiefly in the higher concentration regions. At low concentrations the general rule holds that the *amount adsorbed increases strongly with concentration up to a certain limit* of c; above this limit the relative amount of solute bound at the surfaces remains constant or may even decrease.

Langmuir (1916) derived an adsorption isotherm theoretically on a molecular kinetic consideration of the adsorption of gas on surfaces. He assumed that the molecules are bound at discrete points, the so-called active centers. He also assumed that the forces of adsorption are short range attractive forces, and the films produced are not thicker than the diameter of one molecule. It could be shown that the classical empirical isotherm is one special case of the Langmuir equation.

Fig. 14. Bands of substances adsorbed in a column.

5. Chromatographic Analysis

Another practically important and convenient method of studying adsorption is to place the adsorbent in a vertical glass tube, and to pass the solution through the column of adsorbent (Fig. 14). For quantitative studies of adsorption the solid plug in the tube is only about 1 cm thick, and a known volume of solution is allowed to rise slowly through the plug. The amount adsorbed then is determined by examining the liquid above the plug. However, of much more importance is the application of adsorption in columns for the *quantitative separation* of organic and inorganic substances from mixtures. For this purpose a long column of adsorbent is used, and the solution percolates down from the top to the bottom. The solute with the highest affinity for the adsorbent will be adsorbed first, and it will stay in the top layers of the column. The other components will move farther, some of them eventually being adsorbed in the lower layers, while others will pass through the column without being adsorbed at all. Such

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columns were used first by the Russian botanist Tswett for the separation of colored substances in plants. The differently colored substances separated into colored bands, hence the method acquired the name of 'chromatographic analysis'. In many cases the components adsorbed are not colored, yet the method can be used for the quantitative determination and preparative separation of colorless substances, such as amino acids. The separation is carried out by continuous fractional *elution*, and collection of the eluted material in a series of tubes[8]. The most weakly adsorbed components in the bottom sections of the column will be eluted first, and they will be followed by the other components in the order in which they are present in the layers of the column. The substances eluted then can be identified by chemical or physical methods.

The chromatographic method has been developed in the last two decades to an indispensible analytical and preparative tool. Since adsorption occurs even from the most dilute solution, small amounts of organic compounds present in tissues in very high dilutions have been separated by this method. The specificity of adsorption is also remarkable. Small differences in the molecular structure (e.g. *cis* and *trans* stereoisomers) result in a difference in adsorption. The method can be used on a micro scale, as well as for industrial purposes with large columns, and with a highly mechanized procedure.

Many different materials, such as powdered cellulose, starch, silica gel, aluminum hydroxide, and various synthetic space polymers are used as adsorbents in the columns. The chemical constitution of these space polymers can be correlated with their capacity to adsorb certain compounds.

A very important branch of chromatography is *filter paper chromatography*. This was developed in England by Martin and Synge[9] and their associates around 1940–44. It is now one of the best semiquantitative micro-methods in organic and especially biochemical analysis. In paper chromatography both adsorption and partition of the solute between solvents plays a part. The method is very simple. A single drop of the solution to be tested is placed on a section of filter paper near the edge, and the edge of the strip (or sheet) is suspended in a mixture of solvents. The latter ascend, carrying with them the substance under test. (Fig. 15). The substance is carried along the paper strip or sheet to a certain height, and remains fixed. When many similar components are present in the specimen to be examined, they each travel a definite distance

depending on the characteristics of each individual component. After the ascent is completed the paper is dried, and the positions of the substances are revealed by means of suitable chemical reactions. For example, the positions of amino acids are found by spraying with a ninhydrin solution. The substances are identified by comparing the chromatogram of the unknown with the chro-

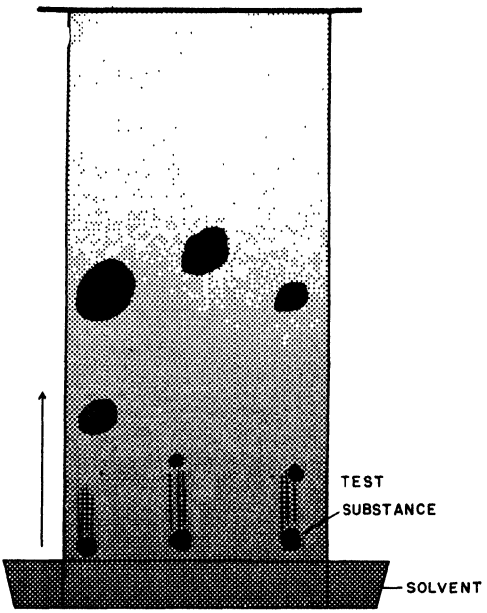

Fig. 15. The ascent of test substances in a sheet of paper immersed in a solvent.

matograms of known substances. Fractions of micrograms of various substances can be detected in this way, and complicated mixtures can be analyzed. New compounds have been discovered by this method in extracts of animal and plant tissues. This happened in several cases when unexpected new spots appeared after development of the sheet, and the spots could not be identified with any of the known substances.

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6. Adsorption and Ion Exchange

In chromatographic adsorption on columns the substances are bound to the adsorbent by very weak forces. This is indicated by the fact that usually they can be eluted easily by means of neutral organic solvents or water. However, in some instances chemical reactions may occur on the surface of the adsorbent. This happens when both the adsorbent and adsorbed substance contain reactive groups, such as carboxyl groups in the adsorbent and some basic groups in the molecules of the adsorbed substance. In such cases the substance may be chemically bound to the material of the column. Another possibility is the *exchange of micromolecular ions.* Sulfonated polystyrene is an example of a synthetic cation exchanger. Space polymers of organic amines on the other hand are able to bind and exchange anions[10]. The following exchange reactions may occur:

Cation exchange:

$$\begin{aligned}
&R{\cdot}SO_3H + Cat.^+ = R{\cdot}SO_3Cat + H^+\\
&R{\cdot}SO_3Na + Cat.^+ = R{\cdot}SO_3Cat + Na^+\\
&R{\cdot}COOH + Cat.^+ = R{\cdot}COOCat + H^+\\
&R{\cdot}COONa + Cat.^+ = R{\cdot}COOCat + Na^+
\end{aligned}$$

Anion exchange:

$$\begin{aligned}
&R{\cdot}NH_2 + H_2O = R{\cdot}NH_3{\cdot}OH\\
&R{\cdot}NH_3{\cdot}OH + An.^- = R{\cdot}NH_3{\cdot}An + OH^-\\
&R{\cdot}NH_3{\cdot}Cl + An.^- = R{\cdot}NH_3{\cdot}An + Cl^-
\end{aligned}$$

The ion exchange resins thus are able to bind ions from the solution, thereby releasing other ions into the solution. Of practical importance are the cation exchange reactions used for removing the cations of calcium and other heavy metals from hard water.

Dowex 50 and the Nalcites are examples of cation exchange resins which are commercially available. Dowex 50 is a sulfonated and cross-linked copolymer of styrene and divinylbenzene. Amberlite ICR-50 is an example of a carboxylic cation exchange resin; it is prepared by copolymerization of methacrylic acid and divinylbenzene, the latter again being responsible for the cross linking of the chains and the formation of a space polymer. These space polymers all have a very loose network so that small molecules penetrate inside the porous solid and react in many places with the active groups of the polymer. Dowex 2 is an example of an

anion exchange resin. The exchange with this space polymer occurs at the benzyl-dimethyl-ethanolammonium ion.

7. Monomolecular Films on the Surfaces of Liquids

The spreading of oily materials on the surface of water was observed long ago. Benjamin Franklin was probably the first who tried to estimate the thickness of the oily layer (in 1765). He spread olive oil on water and estimated the film thickness to be only one ten-millionth of an inch, or about 25 A. More detailed studies about such films were made only in the first decades of this century. Langmuir, Harkins, Adam, Sir Eric Rideal, and many others developed this branch, and showed the usefulness of such studies for the clarification of numerous problems in colloid science. Several comprehensive sources are available in the literature for a more detailed study of this subject[11].

Thin films on liquids are formed by many substances, when small amounts are spread on the surface of liquids. One condition is that the substance to be spread must be either insoluble (more exactly —of very low solubility) or it must be very slowly soluble. Neither glycerin nor sugar forms a film on water, but thin layers of soluble proteins can be obtained. The most interesting and important results, however, have been obtained with the films of hydrophobic organic substances, especially those whose molecules are composed of a large hydrocarbon radical and a polar group.

The spreading experiments are carried out mostly in a rectangular flat trough (Fig. 16). It is filled with water to the brim, and the surface can be swept with a movable glass slide (barrier). This enables one to clean the surface, and also to exert pressure on the film formed on the surface. The best way of spreading oily substances is to dissolve them first in a highly volatile solvent, such as petroleum ether (proteins are dissolved in water-miscible solvents, e.g. 60% propanol); this solution then is spread on the surface by means of a micrometer syringe with a fine needle tip. The most important condition is that the substance to be tested must spread completely, and the solvent must either evaporate or dissolve in the water. The spreading then is observed by means of a float which moves because of the pressure exerted by the spreading film. Various devices are used for accurate measurement of this pressure and the film itself. Fig. 16 illustrates one of the most commonly used instruments. The float is attached to a torsion wire,

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and the pressure exerted on the float is measured by compensating the torsion by rotating the torsion head. (This torsion balance is calibrated by putting weights on a pan attached to the wire.) This is the Langmuir-Adam film balance; it was modified and developed further in various ways, e.g. a kymograph can be attached to the torsion wire in order to obtain continuous registration of the spreading[12].

The chief variables measured in film spreading are: the *film pressure* (force), and the *area* covered by the film. Suppose that an extremely small amount of a film-forming substance is spread

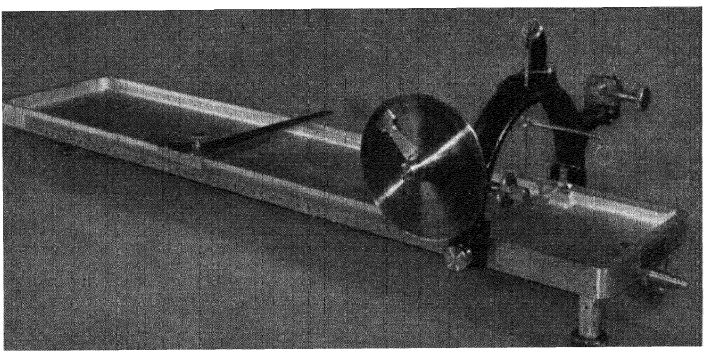

Fig. 16. A surface balance.
(Manufactured by the Central Scientific Company, Chicago.)

on a relatively large surface. When the amount of the substance is so small that it is not enough for even a monomolecular layer, the pressure will not increase upon reducing the area, i.e. pushing the movable slide toward the float. This is because in very large areas the substance forms islands or patches which do not influence the surface tension (and the surface pressure) appreciably. However, the pressure is strongly increased (the surface tension decreased) when the molecules are compressed by the moving slide to a monomolecular film which covers the entire surface area. The force exerted on the float then increases strongly. This continues until the film is compressed to a limit, when the molecules begin to pile up into polymolecular layers, in which case the pressure changes are small. This is illustrated by the curve of Fig. 17. On the abscissa is plotted the area (per molecule) and on the ordinate the pressure

(dyne/cm). In section 1 of the curve there is so little substance on the surface that it does not suffice to form a film. At the point A_0 we have a rather loosely built (expanded) film which remains

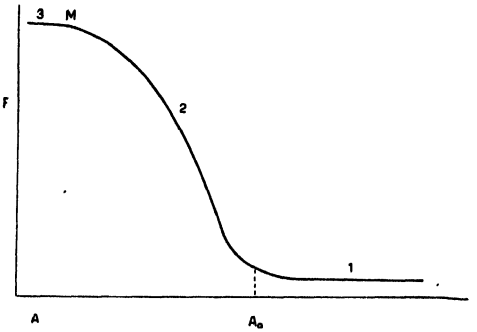

Fig. 17. A common force (F) — area (A) curve.

monomolecular up to the point M, when the molecules begin to pile up. When the total area of the monomolecular layer is known, the area occupied by one molecule can be calculated. Denoting the total area by S, the area of a single molecule by A, the volume of the substance spread by V, and its density by d, A is given by

$$A = SM/VNd,$$

where M is the molecular weight of the substance and N is Avogadro's number.

The fact that the surface area values for single molecules of various aliphatic compounds are practically the same is indeed remarkable. In the expanded film (at point A_0) this area appears to be about 25 square angstroms or $25 \cdot 10^{-16}$ cm^2, and for the compressed films it is near 20.5 sq. angstroms. This is the same area which was obtained for the cross section of a hydrocarbon chain as estimated by X-ray analysis and other accurate methods. Consequently, one must assume that the molecules of stearic acid, dodecyl alcohol, and similar compounds are all arranged in the films with the *hydrocarbon chains upward and the polar 'heads' immersed in the water*. This is illustrated in Fig. 18.

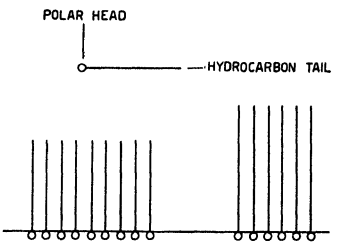

Fig. 18. Orientation of long chain hydrocarbon derivatives on the surface of water.

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The study of monomolecular films has yielded information which is of interest not only in colloid chemistry but also in clarifying structural problems of several organic compounds. For example, the tightly packed monolayers of cholesterol and other steroids indicate that the cross section of a single molecule of these substances must be 37–44 square angstroms, whereas according to the old structural formula about 54 sq. A could be expected. The studies on monomolecular layers supported the now generally accepted formula.

Clowes has done very interesting studies on *carcinogenic substances*, such as benzpyrene, methyl-benzanthracene, and others (*l.c.*[11]). These polycyclic hydrocarbons did not form coherent films by themselves on water, but they could be included in *mixed films* with sterols and other cellular constituents. It was shown that the carcinogenic agents interact with the steroids in mixed films in two ways: first, the hydrocarbon is dissolved in the steroid, and, second, molecular association between the hydrocarbon and the steroid can occur. The strongest binding of the polycyclic hydrocarbons was found in mixtures with certain sterols and certain fatty acids and phosphatides, but the hydrocarbons did not combine with proteins. Also of interest is the finding of Clowes that the alkyl-benzanthracenes and other polycyclic hydrocarbons could be protected from the photochemical influence of ultraviolet light, if they were between sterol molecules in the films. They were affected by the radiation when irradiated in a bulk suspension.

Very interesting studies with monolayers have been made by Sir Eric Rideal, Schulman and their associates[13]. For example, it was found that the oxidation of oleic acid spread on a solution of potassium permanganate in water occurs only when the acid is floating in isolated patches, or when the film is not firmly compressed. Upon compression to a tight monolayer the oxidation stops. This is explained as follows. The double bond of oleic acid is the part of the molecule which reacts with the permanganate, and this bond is in the middle of the hydrocarbon chain. When the oleic acid molecules lie loosely scattered on the surface, the double bond can touch the aqueous surface and react with the permanganate (Fig. 19). When the film is compressed, the hydrocarbon radical is forced out of the surface.

Another example is the reaction of lecithin with snake venom on an aqueous surface. Lecithin reacts only if it is spread loosely, and the reaction is slowed down strongly upon compression of the

film. It was also found that in some instances the molecules of the film, reacting with some substances dissolved in the water, form a 'complex film'. An example of this kind is the interaction of cholesterol spread on solutions of sodium cetyl sulfate and digitonin[14].

So far only the spreading of micromolecular compounds was mentioned. However, monomolecular films of large molecules also are known. Protein monolayers have been known for some time, and a wealth of information has been collected on this subject[15]. The interpretation of these observations, however, is not as unequivocal as in the case of fatty acids and other simpler substances.

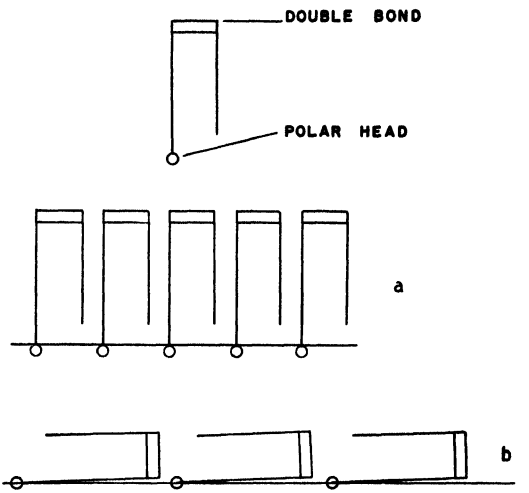

Fig. 19. Orientation of oleic acid molecules on the surface of water in (1) a compressed film (a), and in (2) a loosely scattered film (b).

The protein molecules seem to be flattened out on the surface in most cases, their flexibility is increased, and the unique configuration of the native spheroproteins is changed to a considerable degree. These changes vary from protein to protein, and they also depend to a great extent on the pH of the aqueous solution on which the protein is spread.

The films of simpler polymers are also of considerable interest. Rosano showed in a recent publication[16] that the surface area occupied by one molecule of a semicolloidal polyester molecule depends on the spreading solvent. This indicates that the polyester

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molecules are solvated on the aqueous surface to various degrees, depending on the solvent. The molecular weight of the polymer appeared to be the same on the surface film as in solutions, and it was independent of the solvents used for spreading.

It is interesting that surface monolayers at great dilution can be considered as surface gases, and the gas law $PV = RT$ can be applied. Instead of pressure P the film pressure F is taken and instead of volume V the surface S, so $FS = RT$ (for one mole). Such a 'surface gas' cannot be ideal and corrections in analogy to those of the Van der Waals factors must be taken into account, especially the volume or surface of the spread molecules themselves. According to Rosano then

$$F(S - b) = RT \quad \text{for one mole, or}$$
$$F(S - b) = RT/M \quad \text{for 1 gram, } M \text{ being the molecular weight.}$$

In these equations b is the cosurface of the molecules, and it can be evaluated if the concentration of the molecules is known. Since the other values also are known (R is the gas constant, T the absolute temperature), the molecular weight M can be calculated. The molecular weight obtained by this method agrees with values obtained by other methods[17].

Monomolecular films can be produced not only on water but on other liquids as well. The chief condition is that the substance to be spread must not be soluble in the liquid on which it is placed, although other factors also are involved. Monolayers can also be formed between two liquids, e.g. between oil and water. Of course, it is impossible to use the common technique of movable barriers for the study of such films; the pressure of the film is simply changed by introducing more of the substance between the layers. It is interesting that the cohesion between the hydrophobic parts of the spreading molecules is eliminated in such spreading, since the hydrophobic radicals will be solvated by the oil. Consequently, the flexibility of various macromolecules at a water-oil interface is usually greater than at a water-air interface[18].

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CHAPTER 7

The Size and Shape of Macromolecules and Micelles

Determination of the size and shape of colloidal particles is one of the chief objectives of colloid science. A variety of methods have been elaborated during the last few decades, and many of these methods are applicable to the study of organic colloids. The methods can be divided into two groups: (1) accurate quantitative methods, and (2) qualitative or semiquantitative methods, for approximate evaluation of size and shape. Although the accurate methods are usually preferred, the semiquantitative methods are much simpler and sometimes adequate for practical purposes. The following quantitative methods will be discussed in this chapter: (1) sedimentation in the ultracentrifuge, (2) diffusion, (3) osmotic pressure, (4) light scattering, (5) double refraction of flow, (6) electron microscopy, and (7) the chemical methods. The viscosimetric method, which will be discussed in Chapter 9, is also very important for the study of organic colloids. The surface balance technique was mentioned in the previous chapter. The most important qualitative and semiquantitative methods are: qualitative optical examination, dialysis, ultrafiltration, and precipitation titration. Some of the classical methods of molecular weight determination, such as the freezing temperature depression, are not sensitive enough for colloids, and have rendered erroneous results.

1. Ultrafiltration, Dialysis, and other Semiquantitative Methods

A rough estimate of the particle size of a colloid is sometimes possible by gross observation. If a solution appears turbid, the

particles are relatively coarse. Their dimensions may be around 0.001 mm. A closer estimate is possible by learning to distinguish simple reflection turbidity from opalescence. The reflection turbidity of colorless dispersions is white, whereas opalescent systems appear to be bluish by their reflected light, and yellow or orange when viewed against the light source. This is explained as follows: In the case of 'white turbidity' the particles are so large that all the waves of visible light are reflected and scattered from the surfaces of the particles. Since many kinds of waves are affected, the system appears white from all directions. In opalescent colloids,

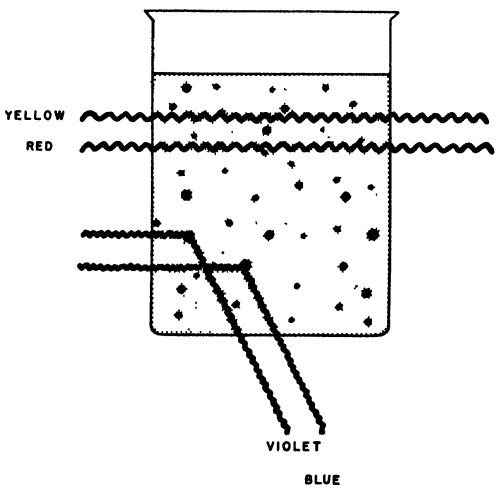

Fig. 20. Opalescence of a colorless colloid. The long-wave red and yellow light passes almost undisturbed through the solution, whereas the short-wave blue and violet light is strongly scattered.

however, the particles are so small that only the short waves are scattered, whereas the long waves of yellow, orange, and red pass through the colloid undisturbed. Consequently, the colloid appears bluish by its reflected light and orange against the light source. (see Fig. 20). The size range of the particles in opalescent colloids is about 500–5000 A. If a solution is clear and shows no opalescence, the particles must be smaller than 500 A. Whether a particular system is colloidal or not can be answered by means of the following simple experiments. The solution is poured into a cellophane tube,

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which is then placed in a container of pure solvent. Cellophane is a semipermeable membrane composed of regenerated cellulose. The pores and cracks between the solid cellulose fibers are so small that colloidal particles are unable to penetrate the membrane. After a certain period of time the initially pure solvent can be tested chemically for the presence of the solute. Since only micromolecular solutes may pass through cellophane, the colloid will remain inside the tube. This method is called *dialysis*. (Fig. 21). It is a very useful and important technique, especially for the purification of colloids, which, by this means, can be liberated from all micromolecular impurities, such as inorganic salts, alcohol, or glucose.

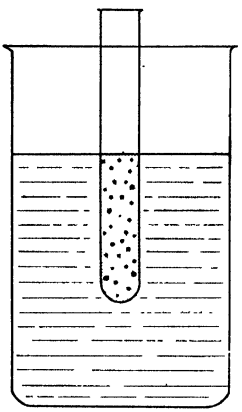

Fig. 21. Dialysis. Colloidal particles remain inside the tube, whereas micromolecular ions and molecules diffuse through the semipermeable membrane into the solution (or pure solvent).

Another simple test for a semiquantitative estimation of particle size is *ultrafiltration*. Colloidal particles will pass through the pores of ordinary filter paper, as well as through the finest (UF) sintered glass filters. However, the semipermeable membranes used in ultrafiltration will retain colloidal particles. Cellophane or nitrocellulose filters are used for this purpose, and a sintered glass support is used for filtration under pressure. Cellulose may also be used as supporting material, e.g. the extraction socks. Ultrafilters of various pore size can be prepared by impregnating these socks with a solution of nitrocellulose, and drying to various degrees. The ordinary collodion, a solution of nitrocellulose, in an alcohol-ether mixture, is suitable for this purpose. These solvents evaporate easily, and the more the remaining film dries out, the smaller the pores will be. Colloids of known particle size can then be used for the estimation of the pore size. By filtering an unknown solution through a series of ultrafilters with various pore diameters, and testing the filtrate, the particle size can be roughly estimated.

The ultrafiltration phenomena and penetration of particles through semipermeable membranes was thoroughly investigated by Bechhold (1907), Elford[1], Ferry[2], and others. Elford studied the virus proteins by this method, and pointed out some of its limitations. The colloidal material may be adsorbed at the surfaces of

the capillaries. Because of this clogging effect, the colloidal particles may appear smaller than they really are. According to Elford, only in the case of coarse ultrafilters is the maximum diameter of the passing particles the same as the average pore diameter. It should also be pointed out that the pore size in a membrane is not uniform. Such a membrane must be considered, not as a punched sieve with holes of equal diameter, but rather as a meshwork of fibers (see Chapter 15).

The choice of a membrane for the purpose of dialysis or ultrafiltration depends on the properties of the solvent. Cellulose membranes can be used for practically all solvents, whereas nitrocellulose cannot be used for acetone or several other organic solvents. Defatted animal membranes also are very useful for dialysis and ultrafiltration, since they are composed chiefly of fibrous proteins which are insoluble in water and organic solvents.

Another simple method for semiquantitative estimation of particle size is the *precipitation titration.* This method is used only for the comparison of polymers of similar constitution, i.e. of polymer-homologs[3]. For example, the degree of polymerization of a series of nitrocellulose fractions, or the approximate degree of hydrolytic decomposition of gelatin samples may be determined by this technique. A known volume of the colloid is titrated with a liquid which is miscible with the colloid but will cause aggregation of the particles. For example, a nitrocellulose solution in acetone can be titrated with water, or better, with a water-acetone mixture; a gelatin solution can be titrated with alcohol. The amount of precipitant added will depend on the molecular weight (particle size) of the high polymer; the smaller the molecules the more of a precipitant is required to produce aggregation. This relation is based on the fact that the solubility of high polymers having the same constitution depends on the molecular weight of the polymer (see Chapter 12).

The simplest method for obtaining information concerning the particle shape is viscometry. A diluted colloid (about 1%) which shows a high resistance to flow has asymmetric particles. The methods for a quantitative measurement of viscosity will be described in Chapter 9.

2. Sedimentation in the Ultracentrifuge

Coarse, filterable particles may be separated by centrifugation in the usual laboratory centrifuges which attain speeds of approxi-

mately 4000–6000 revolutions per minute. Colloidal particles cannot be sedimented at such low speeds. Being very small and light they are pushed about by the thermal motion of the solvent molecules and their motion is little influenced by gravitation. The smaller the particles or macromolecules the more like ordinary small molecules they become, and the more powerful gravitational force is required in order to displace them.

T. Svedberg and his associates (1920–1935 in Uppsala, Sweden and in the University of Wisconsin, U.S.A.) developed the first analytical ultracentrifuges by means of which the sedimentation of colloids could be studied. Many difficult problems were involved in the development of these instruments, especially in the construction of suitable rotors and cells, as well as in adapting suitable optical means for measuring particle displacement. The first ultracentrifuges rotated at a speed of about 15,000 r.p.m., and later this speed was increased to 80,000 r.p.m. and higher. The classical ultracentrifuges of the Svedberg type are driven by oil turbines. The cell is placed in a rotor built of the highest quality steel, and it revolves in an atmosphere of cooled hydrogen at low pressure. The displacement of large molecules in the cell is observed during centrifugation through quartz windows by suitable optical methods[4]. Due to the expense and complexity of construction only a few such instruments are in use.

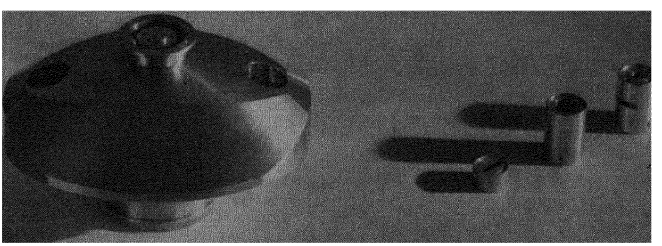

Fig. 22. The rotor and cells of the ultracentrifuge.
(Courtesy Spinco Division, Beckman Corp., Belmont, Calif.).

Ultracentrifuges of different design were developed later in the U.S.A. and in Germany; several of these are now available commercially. Pickels has designed an electrically driven ultracentrifuge which is now a standard tool in many laboratories[5]. This instrument

has many advantages over the Svedberg type machines since it is less expensive, more compact, easily operated, and the accuracy of its results is at least comparable with the oil turbine centrifuge. The rotor is fashioned of aluminum alloy, and the cell resembles that of the Svedberg ultracentrifuge (Fig. 22). The rotor is attached

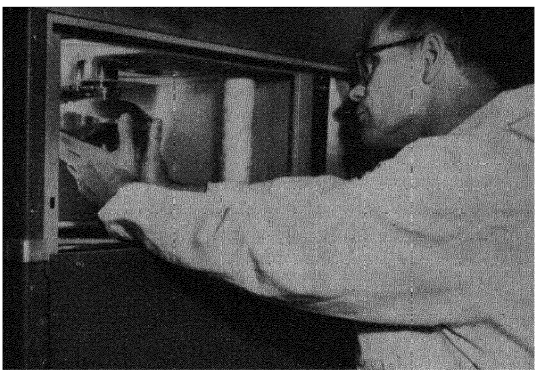

Fig 23 The Spinco ultracentrifuge. Placement of the rotor in the chamber.

to the drive by a vertical flexible shaft which makes the rotor self-balancing, and minimizes the danger of rotor disintegration (Fig. 23). The rotor spins in a high vacuum, and the chamber may be cooled by refrigeration. The displacement of the colloid is followed by an optical system which permits accurate photographic registration of the boundary during the run.

By following the boundary shift, the sedimentation method makes it possible, not only to estimate molecular weight but also to determine the relative homogeneity of the system with respect to the molecular weight. By means of the cylindrical lens system, the different mass species will appear as peaks, the position of which can be accurately measured. When *two* peaks appear in the sedimentation diagram, the colloid contains *at least two* different species of macromolecules (or other kind of particles)(Fig. 24).

The molecular weight of macromolecular substances may be determined by means of an ultracentrifuge in two ways. Most important is the *sedimentation velocity* method. It is based on the fact that the sedimentation velocity depends upon the molecular

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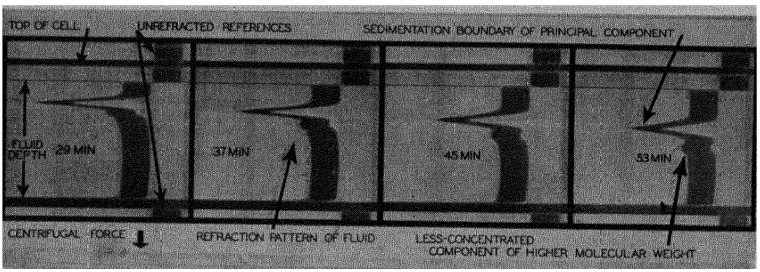

Fig. 24. A sedimentation diagram: Ultracentrifugation of commercially prepared gamma-globulin (1%). (Courtesy Spinco Div., Beckman Corporation)

weight and shape of the sedimenting entities. The centrifugal force acting on a mole of particles at a distance x from the axis of rotation equals the difference between the centrifugal weight

$$Mx\omega^2$$

and the buoyancy exerted by the displaced liquid

$$MxVd\omega^2, \text{ i.e.}$$

$$Mx\omega^2 - MxVd\omega^2, \quad \text{or} \quad M(1 - dV)x\omega^2$$

In this expression M is the molecular weight, d the density of the solution, V the partial specific volume of the particles (inversely proportional to the density of the particles), and ω is the angular velocity (radians per sec). In a steady state of sedimentation this centrifugal force must be equal to a resisting force $f \cdot dx/dt$, where f is the frictional force per mole. Hence,

$$M(1 - dV)x\omega^2 = f \cdot dx/dt \tag{1}$$

From this equation the unknown M can be calculated, if the other variables are known. Density and partial specific volume may be determined by pycnometric methods, and the angular velocity can also be measured with suitable devices. There then remain the parameters x, dx/dt, and f. According to Svedberg, it is advantageous to consider, instead of the sedimentation velocity dx/dt, the *sedimentation constant* s, the latter being defined as follows:

$$s = \frac{dx/dt}{x\omega^2} \quad \text{or (in integrated form)}$$

$$s = \frac{\ln x_2/x_1}{(t_2 - t_1)\omega^2} \tag{2}$$

The sedimentation constant is defined as the sedimentation velocity per unit centrifugal force, and the absolute unit for it is the second. It is obvious that the sedimentation constant can be calculated, if the positions of the moving boundary x_1 and x_2 can be measured at the time intervals t_1 and t_2. A colloid is characterized to a reasonable degree if its sedimentation constant is known (providing it contains only one sedimenting component). The sedimentation constants of colloids usually vary between $2 \cdot 10^{-13}$ and $150 \cdot 10^{-13}$ second. The factor 10^{-13} ordinarily is omitted, and the constants are stated in S units (called svedbergs). For example, the sedimentation constant of serum albumin is found to be 4.0–4.6 S.

A precise evaluation of the molecular weight M is more difficult since one more unknown is involved—the frictional force f. The latter can be calculated, if the diffusion coefficient D of the material is known, as $f = RT/D$ (where R is the gas constant and T absolute temperature). Combining equations (1) and (2), and replacing f by RT/D the following expression for M is obtained:

$$M = \frac{RT \ln x_2/x_1}{D(1 - \bar{V}d)(t_2 - t_1)\omega^2} \tag{3}$$

Detailed information concerning the calculation of sedimentation constants and molecular weights can be found in the previously mentioned book of Svedberg and Pedersen, and from other sources[6].

The molecular weight of macromolecular substances can also be determined by the ultracentrifuge without knowing the diffusion constants. The solution is centrifuged at a relatively low speed (15,000–30,000 r.p.m.) until equilibrium between sedimentation and diffusion is reached. The concentration of the colloid is then determined by optical means in several levels of the cell. The molecular weight may be calculated from

$$M = \frac{2RT \ln c_2/c_1}{(1 - \bar{V}d)(x^2_2 - x^2_1)\omega^2} \tag{4}$$

c_2 and c_1 being the concentrations at x_2 and x_1. The chief disadvantage of this method is that a long time is required in order to achieve the equilibrium.

It should be pointed out that the above mentioned relations are valid only for more or less idealized cases. The degree of dilution is very important. Equation (4) is valid only for very dilute solutions, and for more concentrated solutions certain corrections must be made[7]. The same is true for the sedimentation constants. These

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are usually determined for a series of concentrations, the s values are plotted versus concentration and extrapolated to zero concentration. Moreover, the s values are recalculated for conditions at 20° C, in pure water and such *reduced* s values are denoted by $s°_{20}$.

The best sedimentation results have been achieved with the spherocolloids, especially with the proteins. The molecular weights, as determined by the sedimentation velocity method agree well with those obtained by the sedimentation equilibrium method, as shown in Table 11. Noteworthy is the fact that many proteins appeared to be homogeneous in respect to the molecular size. Results with the linear polymers, however, have been less satisfactory, since these substances are polymolecular (chains of various length). The frictional force is also much greater than in spherocolloids, causing the molecules to sediment slowly, and making evaluation of the boundary shifts difficult[8].

TABLE 11

THE MOLECULAR WEIGHT OF SEVERAL PROTEINS FOUND BY THE SEDIMENTATION VELOCITY AND BY THE SEDIMENTATION EQUILIBRIUM METHODS

Protein	M *from sedimentation velocity*	M *from sedimentation equilibrium*
Myoglobin (from muscle)	16,900	17,500
Pepsin (from stomach juice)	35,500	39,000
Insulin (from pancreas)	41,000	35,000
Lactoglobulin (from milk)	41,500	38,000
Ovalbumin (from eggs)	44,000	40,500
Serum albumin (horse blood serum)	70,000	68,000
Amandin (from almonds)	330,000	330,000

There have been important contributions to the field of sedimentation recently. Beams and colleagues have developed a new, magnetically suspended equilibrium ultracentrifuge[9]. This instrument has certain unique features. The rotor is accelerated to operating speed by an air turbine seated below the vacuum chamber. A thin, flexible shaft passing through vacuum-tight oil glands connects the turbine to the rotor during the acceleration period but is disconnected during the operating period. The rotor coasts freely during the observation of the sedimentation in the vacuum chamber. It has been estimated that the rotor loses only one revolution per second per three days when running at a speed of 18,000 r.p.m. The sedimentation in the cell during the run is

observed by means of an interferometer which gives accurate information about the concentrations of the sedimenting substance in different levels of the cell.

Air driven and air floated rotors have been developed in various ways. Ford and associates[10] have demonstrated recently a relatively inexpensive and efficient instrument which could be used in any laboratory. The rotor is constructed with opposed radial holes in which glass capillary tubes are inserted. The rotor is driven by compressed air, and it floats steadily, rotating with a reasonably constant speed. The sedimentation in this simple instrument can not be observed during the rotation, but the centrifuge is stopped at appropriate time intervals and the capillaries are examined under a microscope. The results were shown to be satisfactory when compared with those obtained from more accurate instruments. This instrument is especially important for its use as a micromethod.

Recently, there has been important progress in the improvement of cell construction and evaluation of the boundary shift. Kegeles has developed a new type of boundary forming cell by means of which the molecular weights and sedimentation constants can be evaluated at the same time, even for relatively low-molecular substances[11].

3. Molecular Size and Diffusion

Diffusion is the spreading of the solute into the solvent. If a dense and concentrated solution is carefully layered with the solvent, the solute diffuses, and after a certain time the solvent will be distributed evenly throughout the solution. Diffusion of colloidal particles or macromolecules is due to the Brownian movement. The diffusion rate, however, is much less than the velocity of oscillations in the Brownian movement; the particles in the spreading layer collide, moving in various directions. Moreover, the movement is "statistical", i.e. the boundary of the spreading substance is not absolutely sharp but some particles, by mere chance, will spread farther, even if they are of exactly the same size. Diffusion occurs as illustrated in Fig. 25. This behavior of the particles is due to the chaotic nature of thermal motion. Some particles are out of the boundary, not because the original boundary was imperfect or had been disturbed by external shocks or thermal convection, but because these particles have received extremely strong, uneven impacts.

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Diffusion is characterized by the *diffusion coefficient* or diffusion constant which is the amount of solute passing in one second across a plane of one cm^2 when the concentration gradient is unity. If the cross section of a diffusion cell is 1 cm^2, and it contains a one molar solution in the bottom, the diffusion constant is the quantity of solute which has spread in 1 second 1 cm deep into the pure solvent layered on top of the solution. The dimensions of the diffusion constant are cm^2/sec, and the values for various organic colloids range from 10^{-6} to 10^{-8}, whereas micromolecular substances, e.g. urea, have diffusion coefficients of 10^{-5} and higher.

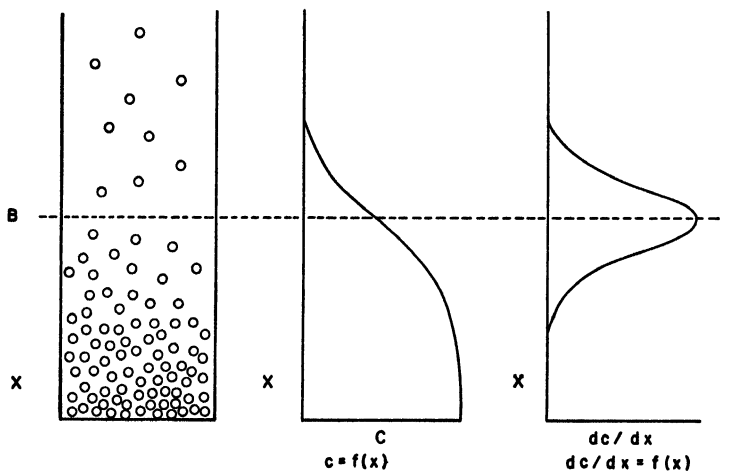

Fig. 25. Diffusion of a monodisperse colloid across the boundary B into pure solvent. The concentration (c) of the particles decreases with increasing distance (x). The change of the concentration (its first derivative dc/dx) has its maximum at the boundary B. (After H. P. Lundgren.)

There are two basic approaches in determining diffusion coefficients: (1) free boundary spreading, and (2) diffusion through porous plates. The first method uses rectangular cells, like those presented in Fig. 26. Such cells were originally designed by Tiselius and were first used for quantitative studies in electrophoresis. They are also useful for free boundary diffusion studies, since a sharp boundary can be easily formed by using a sliding joint to superimpose the solvent on the solution. The boundary spreading is observed by optical means, e.g. refraction changes at certain levels (x) at definite time intervals (t)[12]. A simpler means is the porous disc method of

Northrop and Anson[13]. The boundary is formed in a porous disc of sintered glass which separates the solution from the solvent. The amount diffused is determined by taking specimens from the solvent compartment and testing them for the amount of solute.

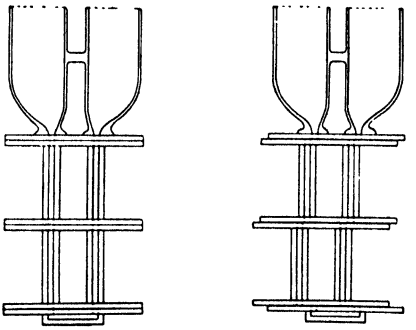

Fig. 26. Rectangular cells used in diffusion and electrophoresis experiments.

The diffusion coefficients are evaluated from the experimental data according to the law of Fick (1855), which states that the diffusing amount dm which travels in direction x (see Fig. 25) in a time dt across an area q is proportional to the concentration gradient dc/dx:

$$dm = -Dq \cdot dc/dx \cdot dt.$$

D in this equation is the diffusion coefficient, which is simply a proportionality constant. This differential equation which expresses Fick's law may be solved for certain limited cases. Introducing the experimentally found values of x, c, and t in the integrated equation the constant D can be evaluated.

The larger a particle the more slowly it will diffuse. Hence the diffusion coefficient can be used as a measure of particle size. The theoretical relations appear rather simple in cases where the particles are spherical, non-solvated, and relatively much larger than the molecules of the solvent. According to Stokes and Einstein, the following relation then holds between the diffusion constant D and particle radius r:

$$D = RT/6\eta Nr,$$

where R is the gas constant, T absolute temperature, η absolute viscosity, and N the Avogadro number. For molecular colloids the molecular weight M can then be evaluated from r, as

$$M = 4/3\pi Nr^3 d,$$

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where d is the density of the spherical molecules. The diffusion constants are also of considerable importance for estimating the molecular weights by sedimentation velocity measurements.[1]

The determination of *particle shape* by diffusion or sedimentation measurement is more difficult than the evaluation of particle size. The theory predicts that the molar frictional force, f, will increase with increased molecular asymmetry. Asymmetric, fibrous molecules diffuse more slowly, and also sediment more slowly than spherical particles. According to Stokes, the frictional force f_0 for one mole of spherical particles will be:

$$f_0 = 6\pi\eta N(3MV/4\pi N)^{1/3}.$$

TABLE 12

DIFFUSION AND SEDIMENTATION CONSTANTS AND FRICTIONAL RATIOS OF VARIOUS PROTEINS*

Protein	*Diffusion constant* D *(at 20°C)*	*Sedimentation const.* s_{20}	*Frictional ratio* f/f_0
Lysozyme (egg white)	$8.6\cdot10^{-7}$	$1.8\cdot10^{-13}$	1.58
Myoglobin (muscle)	11.3	2.0	1.11
Lactalbumin (milk)	10.6	1.9	1.16
Crotoxin (snake venom)	8.6	3.1	1.22
Oxytocin (hormone)	8.5	2.6	1.15
Pepsin (stomach juice)	9.0	3.3	1.08
β-Lactoglobulin (milk)	7.3	3.1	1.26
Insulin (pancreas horm.)	8.2	3.5	1.13
Ovalbumin (egg white)	7.7	3.5	1.16
Hemoglobin (blood)	6.3	4.4	1.24
Albumin (human blood)	6.1	4.6	1.28
Globulin fract. (serum)	3.5	7.6	1.56
Catalase (enzyme)	4.1	11.3	1.25
Amandin (almonds)	3.4	12.5	1.28
Urease (enzyme)	3.4	18.6	1.19
Thyroglobulin (hog)	2.6	19.2	1.43
Hemocyanin (crabs)	2.7	22.6	1.27
Virus (tobacco mosaic)	0.3	174	3.12

* From Lundgren-Ward, *l.c.* ref. 6.

This would be the molar fractional force for non-solvated spheres. The actual frictional force f is always greater, since the molecules are solvated and more or less asymmetric. The quantity f can be determined either from

$$f = RT/D,$$

or from the sedimentation equations (1) and (2) (p. 92). The ratio f/f_0 is called the molar frictional ratio. In Table 12 are presented some representative data for the frictional ratios, diffusion constants and sedimentation constants for some proteins.

The first proteins in the Table have molecular weights near 15,000, and those at the bottom have very high molecular weights. The frictional ratios in most cases are close to unity, which means that the macromolecules are not much hydrated, and that their shape is nearly spherical. The frictional ratio does not represent the molecular axial ratio which is much greater than f/f_0. This has been confirmed in several instances by other methods, such as electron microscopy. The particles of tobacco mosaic virus appear in the electron microscope as long rods, although the frictional ratio is only 3.12.

Efforts have been made to calculate the influence of molecular asymmetry upon diffusion. Equations for prolate and oblate ellipsoids of revolution have been proposed[14].

4. Determination of Molecular Weight by Osmotic Pressure Measurement

The sedimentation and diffusion methods have been successfully applied chiefly to proteins and similar organic spherocolloids. The osmotic pressure method is applicable to both sphero- and linear colloids having molecular weights between 10,000 and about 500,000.

The method is based on Van 't Hoff's law, according to which the osmotic pressure p depends on molar concentration of the solute c/M, and on temperature:

$$p = RTc/M.$$

This equation is valid for very dilute solutions in which the molecules of the solute do not interact mutually. For the same amount of dissolved material c (at a constant temperature) the osmotic pressure is inversely proportional to the molecular weight M.

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Osmotic pressure is a colligative property which depends upon the *number* of acting entities.

According to Van 't Hoff's law, the *reduced osmotic pressure* p/c should not change with concentration (if T is constant) since R and M are constant values. In fact, however, the reduced osmotic pressure in many cases increases with increasing the amount of the substance dissolved. This indicates that other factors are also in-

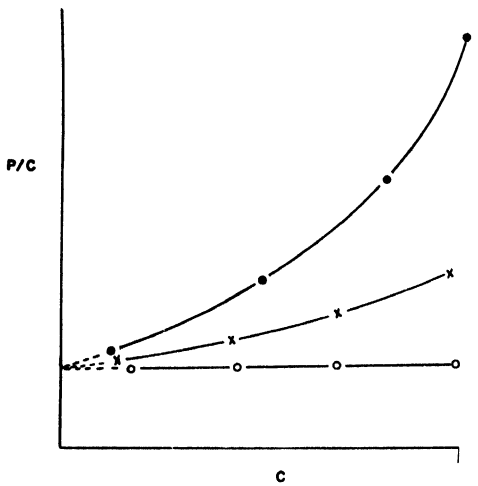

Fig. 27. The dependence of the reduced osmotic pressure p/c on concentration (c) for three different colloids.

volved. For moderately concentrated solutions of polymers the reduced osmotic pressure p/c depends on concentration as follows[15]:

$$p/c = RT/M + Bc + Cc^2 + \ldots\ldots$$

The third virial coefficient C can usually be neglected, if the concentrations are not too high. However, the coefficient B must be taken into account in osmotic pressure for linear colloids. The above equation then is reduced to the following:

$$p/c = RT/M + Bc,$$

which is an equation for a straight line. Figure 27 shows plots of p/c against concentration c. The RT/M value is the intercept on the ordinate, and B is the slope of the line. From the intercept, obtained by extrapolating the p/c to zero concentration, the mo-

lecular weight M is calculated, since R (gas constant) is known, and the temperature can easily be measured. Such evaluation of molecular weight, of course, implies the necessity of determining the osmotic pressure for a series of concentrations at a constant temperature.

The determination of osmotic pressure is based on the phenomenon of osmosis. Suppose a solution is separated from a pure solvent by a semipermeable membrane (Fig. 28), the latter having the property that only the solvent, and not the solute, can pass through it. The solvent will diffuse into the solution through the membrane, as there is a concentration difference on either side of the membrane, and there is a tendency to equalize the difference. Since the solute is unable to pass the membrane, the equalizing can occur only in diluting the solution, i.e. increasing its volume. Consequently, the liquid rises in the tube exerting a pressure. The greater the number of particles of the solute the higher the diluted solution rises in the tube. The rise continues slowly till the osmotic pressure is balanced by the hydrostatic pressure of the column of liquid. Hence the osmotic pressure may be determined by an accurate measurement of this rise.

Considerable difficulties had to be overcome in developing this method. The greatest difficulty in the application of this method to low molecular substances and semicolloids is the finding of suitable semipermeable membranes. Cellulose, nitrocellulose, or animal membranes are not suitable, because small and medium size (M = 1000–10,000) molecules of the solute will penetrate them. Hence the observed pressures will be too low. For macromolecular substances the finding of a suitable membrane is not too difficult, but other problems arise. The osmotic pressure, as mentioned before, is inversely proportional to the molecular weight, i.e. a 1% solution of a high polymer of M = 500,000 will produce a lower pressure than a 1% solution of a polymer of M = 20,000. For the same weight of solute, the number of particles will be inversely proportional to the molecular weight. Hence the pressures observed for solutions of very large molecules are quite low. The errors involved in any measurement, however, increase as the quantity to be measured decreases.

Various devices have been proposed for the measurement of osmotic pressure. Some investigators have used bag-shaped membranes, and a simple device would then be similar to that presented in Fig. 28. More common nowadays are flat membranes which are

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fastened between perforated plates. The osmometer of Schulz (Fig. 29) has such a design. A flat membrane rests on a perforated disc, and is fastened tightly to the housing by screws. The solution is added and a glass capillary with ground joint is inserted into the neck of the housing. The cell is then placed in a glass cylinder containing the solvent, and the whole device is put into a constant temperature bath. Usually a whole series of such osmometers is used, the osmosis of the same colloid being observed at various degrees of dilution. Equilibrium is reached after several days. When

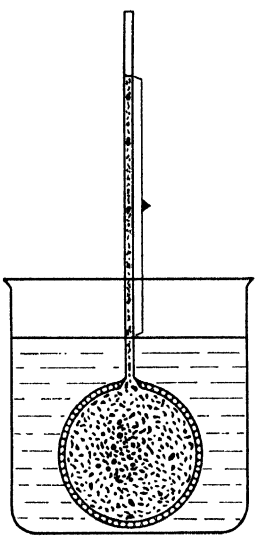

Fig. 28. Schematic sketch of an osmotic cell.

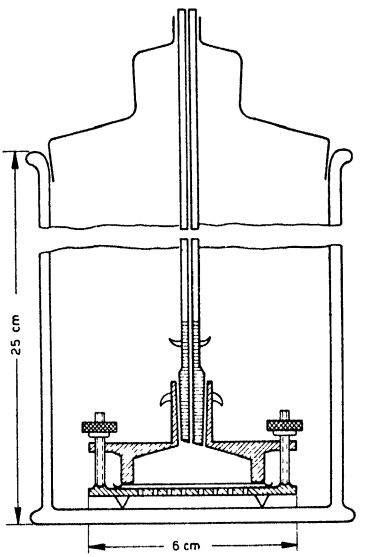

Fig. 29. Osmometer according to G. V. Schulz.

no further rise is noticeable, the height of the capillary columns is measured, and correction is made for the capillary rise of the liquid. Detailed descriptions of other osmometers can be found in handbooks[16].

Osmotic pressure can be measured also using different principles. According to the dynamic equilibrium method, the osmotic pressure is compensated by external pressure, and the rate of flow of the liquid thereby is altered[17]. Osmometers of this type usually are equipped with sensitive devices for measuring the flow rate of the

liquid. A cell is divided in half through the membrane, and either pressure or suction can be applied to both sides. The flow rate (from the solvent compartment into the solution compartment) depends on the pressure applied. The flow rate is plotted against pressure, and the osmotic pressure is equal to that counterpressure at which the rate of flow is zero. This method is suitable in cases where the osmotic pressure changes with time, i.e. the particles either disaggregate or associate themselves into larger units. In the first instance the osmotic pressure will increase with time, if the process is slow enough to be observable. Aggregation is more common, in which case the osmotic pressure will decrease. However, it must be pointed out that it is impossible in this manner to follow the aggregation processes in coarse dispersions. In this instance the osmotic effect produced by the particles is too small, and is distorted by the osmotic activity of electrolytes and other small molecules which are always present in solutions. The osmotic effect produced by the droplets of an emulsion (diameter of droplets about 0.001–0.0001 mm), even if highly concentrated, is too small to be measured conveniently. The upper limit for high polymers is M, approximately 500,000. Calculation shows that for a one per cent solution of a polymer of $M = 350{,}000$ the osmotic pressure should be only 0.7 cm of water. With even larger molecules the errors of measurement become too great.

Several improvements in the methods have been achieved recently. Noteworthy is the method of Jullander and Enoksson in which the osmotic pressure is measured by weighing[18]. The osmotic cell, resembling that of the Schulz type, is hung on one beam of a sensitive balance, and the lower part of the cell is immersed in the solvent. The movement of the solvent is registered by the increase in weight of the cell (Fig. 30). Very convenient osmometers have been designed recently by Hellfritz[19], employing a combination of the Schulz type and Fuoss-Mead osmometers. This instrument is commercially available, is simple in construction, and has the advantage over the Schulz osmometer in that the equilibrium is reached in 3–5 hours.

The results obtained by the osmotic pressure method will be discussed in detail later but, generally speaking, there is a difference in osmotic behavior between the spherocolloids and linear colloids, as pointed out by Staudinger[20]. The reduced osmotic pressure for the spherocolloids is independent of concentration, i.e. the interaction constant B is zero, even in relatively concentrated solutions.

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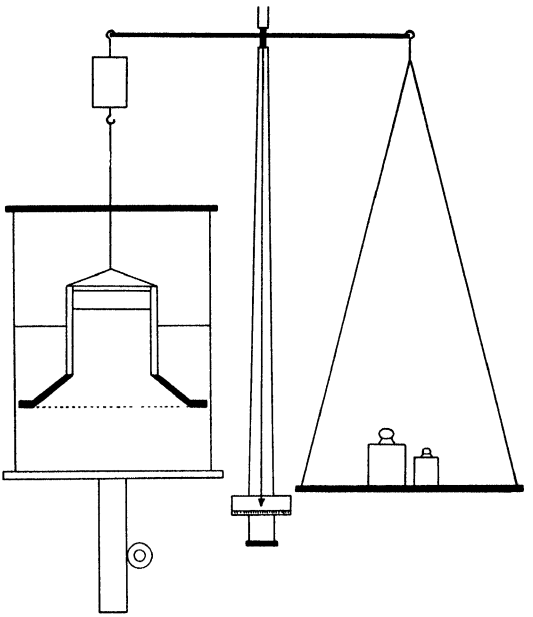

Fig. 30. Diagrammatic sketch of the osmotic balance after Svedberg, Jullander and Enoksson.

TABLE 13

THE OSMOTIC PRESSURE FOR GLYCOGEN AND FOR OVALBUMIN

Colloid	*Concentration* c *(g in liter)*	*Pressure* $p \cdot 10^3$	*Reduced osmotic pressure* $\frac{p \cdot 10^3}{c}$
Glycogen	5	0.43	0.086
(in water)	10	0.88	0.088
$M = 283{,}000$	20	1.74	0.087
	30	2.58	0.086
	50	4.15	0.083
Egg albumin			
(according to	111.7	39.0	0.349
Sørensen),	173.8	59.8	0.342
$M = 70{,}000$	238.5	82.2	0.345

This is illustrated for glycogen and ovalbumin in Table 13. The reduced osmotic pressure of linear colloids, however, rises with rising concentration in most cases, as illustrated earlier in Fig. 27. This is due to increasing interaction between the fibrous particles themselves and between the particles and solvent, as the concentration increases.

5. Molecular Weight Determination by Light Scattering

The basic principle of this method is simple: the light scattered by colloidal particles is determined (among other things) by the size of the particles. A light wave is associated with an electromagnetic field. When such energy of radiation strikes a particle, the electrons of the particle are made to vibrate, and will oscillate in unison with the incident wave, thus becoming sources of the scattered light. It was shown by Lord Rayleigh in 1870 that the intensity of scattered light depends upon the following factors: (1) intensity of the incident beam, (2) the wave length of the incident beam, (3) the difference between the refractive indexes of the particle and the medium, (4) the number of the particles which scatter, and, (5) the volume of the particles. Moreover, the intensity in certain cases may depend on the angle under which the scattered light is measured.

So far we have mentioned the importance of the wave length in scattering only when describing the phenomenon of opalescence. The short waves (blue and violet) are scattered much more than the long waves (yellow, orange, red). According to Lord Rayleigh, *the scattering is inversely proportional to the fourth power of the wave length.*

This theory of scattering was developed by Rayleigh for specific cases only, namely, for spherical molecules which are much smaller than the wave length of light, and which are in a highly diluted state (gas, a very diluted solution). Mie later (1908) elaborated a theory for the scattering by larger particles. In spite of these theoretical developments, little progress occurred in the application of the theory to experimental determination of particle size. The classical work of Putzeys and Brosteaux[21] is the most important one in organic colloid chemistry.

The work of Debye, Doty, Zimm, and others (1944–1949) gave new impetus to the development of this method, especially after

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the simple light scattering instrument of Brice[22] became available. Debye extended the theory to non-ideal solutions of polymers, and arrived at the following equation which correlates the molecular weight M to the intensity of scattered light[23]:

$$H \cdot c/t = 1/M + 2Bc. \tag{5}$$

In this expression, c is the concentration (grams per ml), t the turbidity (representing the intensity of scattered light), and H and B are constants. Here B is the interaction constant which, as in the osmotic pressure measurement, depends upon the degree of non-ideality of the solution. H is a complicated function, which is constant for any given system (solute-solvent) investigated with light of the same wave length:

$$H = \frac{32\pi^3 n_0^2 [(n - n_0)/c]^2}{3N\lambda^4}$$

In this expression n_0 is the refractive index of the solvent, n the refractive index of the solution, N the Avogadro number, and λ the wave length. The value $(n - n_0)/c$ is the specific refractive increment which must be accurately determined for various dilutions by means of a high precision differential refractometer.

It is important that the equation (5) is that of a straight line. If Hc/t is plotted against concentration c, the intercept is $1/M$, and the molecular weight is the reciprocal of this. The constant B can be evaluated from the slope. Fig. 31 illustrates such a plot. In the case of ovalbumin the line is parallel the abscissa, i.e. the constant B is zero. (It is noteworthy that the same was found by means of osmotic pressure measurement.)

The measurement of the scattered light can be made by a variety of instruments; the Brice photoelectric light scattering photometer is the most popular one (*l.c.* ref. 22). An improved recent model is presented in Figs. 32–34. Green or blue light, produced by a mercury lamp, enters the chamber through a series of filters by means of which the intensity of the beam can be conveniently varied. The light strikes the cell containing the solution, and the scattered light is detected by a photoelectric cell. The current produced is measured with a sensitive galvanometer. By rotating the stand holding the cell, the turbidity may be observed at various angles. Devices for checking the polarization of the scattered light can also be included in the path. It is interesting that the light scattered

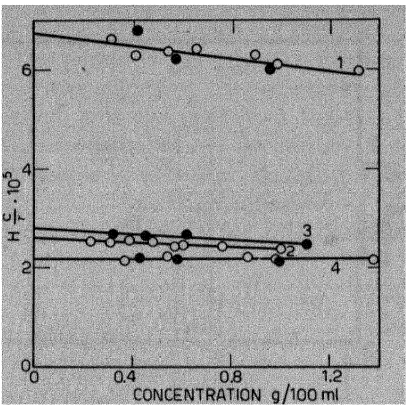

Fig. 31. The dependence of the intensity of scattered light on the concentration for several proteins (according to Halwer, Nutting and Brice, *J. Amer. Chem. Soc.*, 73 (1951) 2786).

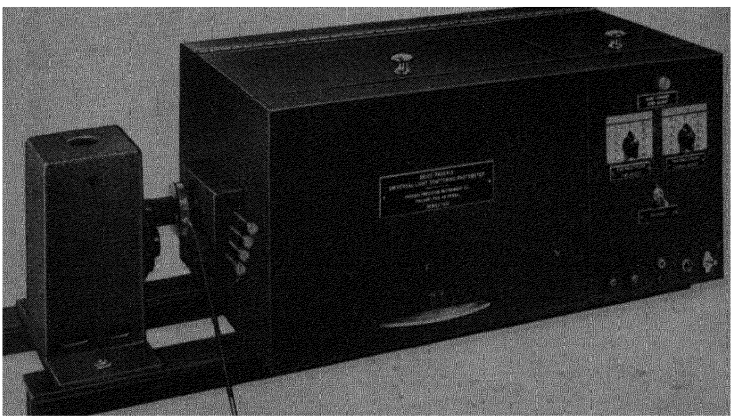

Fig. 32. The Brice-Phoenix light scattering photometer. (Courtesy Phoenix Precision Instrument Company, Philadelphia.)

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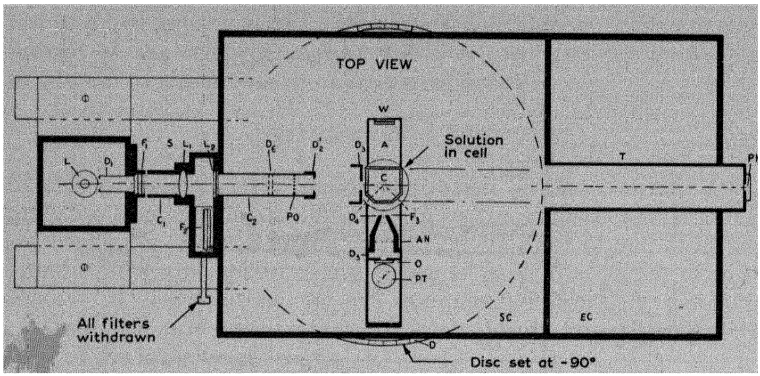

Fig. 33. Cross section of the scattering chamber of the Brice-Phoenix light scattering photometer. L – the light source (mercury lamp); PT – photomultiplier tube.

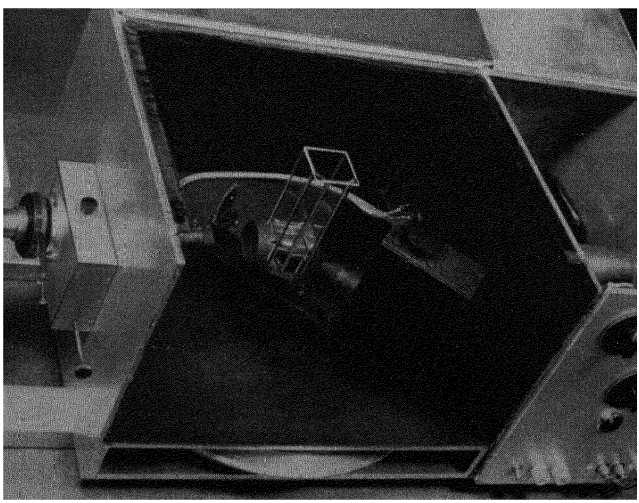

Fig. 34. Photo of the light scattering chamber of the Brice-Phoenix instrument, top and one side removed. In the center is seen the hexagonal cell. (Courtesy Phoenix Precision Instrument Company, Philadelphia.)

by very small nearly spherical particles (mol. weight about 10,000–200,000) is polarized, and its intensity at different angles is nearly the same. When the particles are large or fibrous, the scattering phenomena become very complex. The scattering changes with the angle of observation, and the scattered light is less completely polarized. The intensity distribution, depending on the angle (formed with the incident beam), is called *scattering dissymmetry*. Commonly this is the ratio of intensity found at 45° to that at 135°. This is found using hexagonal cells for the determinations.

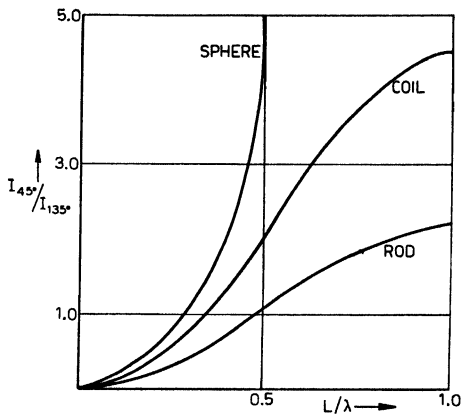

Fig. 35. The theoretical dependence of the light scattering dissymmetry on the particle shape for spherical, rod-shaped, and coiled units. On the abscissa is plotted the ratio of the length (L) or diameter and the wave length (λ), L/λ, on the ordinate the ratio of the scattering intensities at two different angles (45° and 135°).

One important advantage of the method is that it makes possible a study of the *particle shape*. Theoretical relations between the dissymmetry and particle shape have been developed and tested. Especially important are the most common cases for spheres, coils, and rods. In Fig. 35 are shown the theoretically obtained dependencies for these three most common shapes; the scattering dissymmetry is plotted against the ratio of the molecular length to the wave length employed. (The molecular "length" in the instance of spheres being the diameter of the sphere, and in cases of coiled fibrous molecules it is the mean diameter of the coil.)

One of the most convincing tests for the accuracy of the light scattering method was the study of tobacco mosaic virus, since the

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size and shape were known from direct observations in the electron microscope. Oster, Doty and Zimm measured the light scattered by the solutions of this colloid at the angles of 42.5° and 137.5° as a function of the virus concentration. The scattering dissymmetry values then were plotted against concentration of the virus, and the *intrinsic dissymmetry*, i.e. dissymmetry at zero concentration was obtained. From this figure, which was 1.94, and the theoretical curve (Fig. 35 for rods), the length of the particles was calculated. In this way, the length of the virus particles was found to be 2700 A (or 0.00027 mm), in excellent agreement with the electron microscopy and other data[24].

Stein and Doty[25] in 1945 had already made thorough studies on the molecular size and shape of cellulose acetate fractions, and found that the light scattering method gives reliable results for the molecular weights. Dissymmetry studies on the same specimens showed that the molecules in high molecular fractions of $M =$ 160,000 exhibit loop or loosely coiled shape, whereas in the lower molecular fractions ($M =$ 50,000) they are like extended rods. Later studies on high molecular weight samples of polystyrene[26] and polymethylmethacrylate[27] have shown that these very long molecules (M about 1,000,000) exist as kinked coils. Reasonably good agreement was reported between the light scattering and other methods.

The light scattering method is useful also for investigating changes in colloidal solutions. If aggregation occurs, the intensity of the scattered light increases considerably (*l.c.* ref. 28). Reversible association between two colloidal components can also be studied conveniently with this method[28]. One of the greatest experimental difficulties of the method is that the solutions employed must be carefully cleared of dust, bubbles, and other coarse material, since a few large particles may greatly affect the scattering. For this reason, the solution must be clarified either by means of centrifugation at 10,000–25,000 r.p.m. or by filtration through ultrafine filters. This imposes a limit to the study of relatively coarse systems by this method, since some of the material might be removed by the procedures of clarification.

Quite interesting results have been obtained recently by studying the scattering of X rays in colloidal solutions. The wave length of the X rays is very small; and contrary to the short waves of the visible light, they are little influenced by the molecules of liquids and colloidal particles of organic substances. The scattering effects of X rays in organic colloids are observed at very small angles. The

analysis of these *low angle X ray scattering* curves has rendered useful information regarding the size and shape of colloidal particles and macromolecules in solutions[29].

6. The Streaming Birefringence and Particle Shape

Streaming birefringence or double refraction of flow is a phenomenon exhibited by linear colloids. In a lesser amount it can be observed also with moderately asymmetric particles. Assume that a colloid possessing rodlike particles is observed in a rectangular tube between two crossed Nicol prisms. The field of vision will be dark initially, but will lighten when the colloid is forced to flow in the tube. This is explained as a result of *orientation of the particles along the stream lines*. The colloid thus oriented becomes like an uniaxial crystal, i.e. its refraction index is different depending upon the direction. The brightness can be measured by a photoelectric cell, and will give values for the double refraction of flow. Lauffer and Stanley studied the tobacco mosaic virus[30], in this manner.

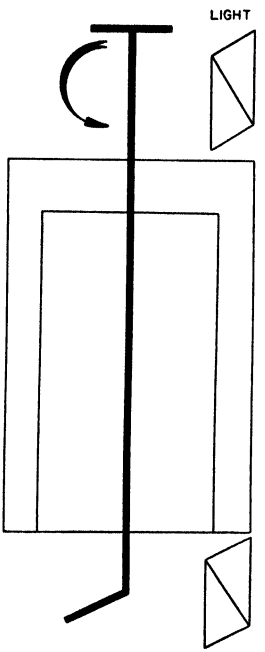

Fig. 36. Observation of the double refraction of flow in the gap between two concentric cylinders.

The effects produced in this way depend chiefly on two factors (1) the asymmetry of the particles, and (2) the orienting force. Ordinarily even the most assymmetric particles will be oscillating and rotating chaotically because of the Brownian movement. However, they become oriented along the stream lines, when the liquid flows. It is obvious that the degree of orientation must depend on the orienting force. Coiled linear molecules may be stretched out considerably under the influence of this force.

Streaming birefringence is usually studied with an instrument composed of two coaxial cylinders (Figs. 36–38). The colloid is in the larger cylinder, and the orientation is achieved by rotating one of the cylinders. Asymmetric particles then become oriented along the

stream lines, and this orientation is observed by means of crossed Nicol prisms. The field of vision lightens, leaving only a cross-shaped shadow (Fig. 39). The position of the arms of the cross change, as the speed of rotation of the cylinder is increased. It appears that the position of these arms is related quantitatively to the speed of rotation (orienting force) and to the asymmetry of the oriented particles. In Fig. 39, the crossed shadow (the so-called

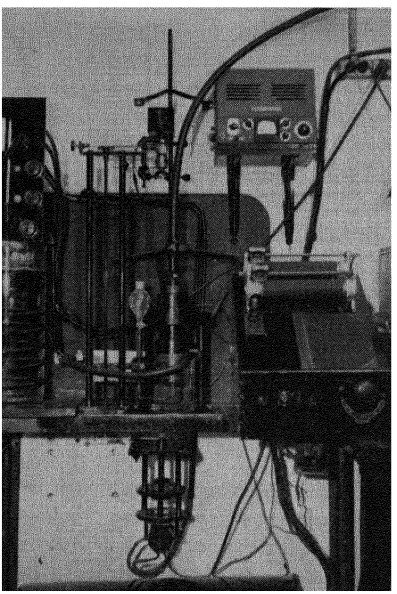

Fig. 37. General view of flow birefringence apparatus of Dr. J. F. Foster (Purdue University, Lafayette, Indiana).

cross of isocline) is shown at angle X with respect to the plane of polarization, and this angle is equal to the angle formed between the oriented rod-like particle (a) and the stream lines. The angle varies between 0 and 45°. In the case of complete orientation it is zero, and it is close to 45° when there is little orientation.

Extensive studies on streaming birefringence have been conducted by Edsall, Signer, Peterlin, Sadron, Tsvetkov, and many others[31]. Double refraction of flow has been studied on many fibrous proteins,

such as myosin of muscle, fibrinogen of blood plasma, and gelatin. Reasonable agreement was found between the estimates made from streaming birefringence data and molecular length obtained by

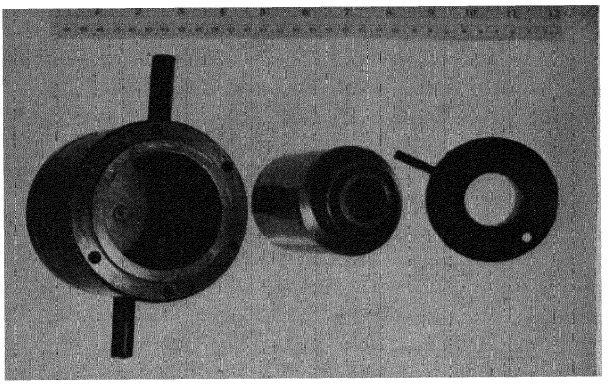

Fig. 38. Stator (left), rotor (center) and top cover (right) of the flow birefringence apparatus of Dr. J. F. Foster.

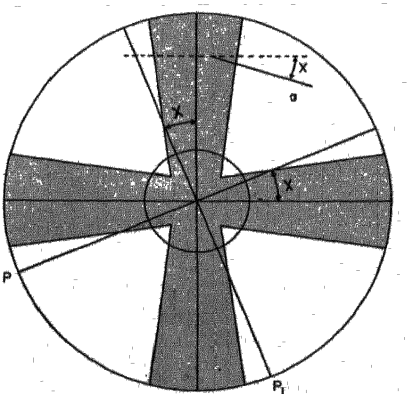

Fig. 39. Schematic illustration of the cross-shaped shadow indicating orientation of rod-shaped particles along the stream lines.

means of other methods. Many linear polymers also have been investigated by this method. Fig. 40 shows the results of Signer and Gross with nitrocellulose. The graph shows the dependence of

the extinction angle X, on the velocity gradient (G) for the various fractions. For the relatively low molecular fractions the angle X is close to 45°, since it is difficult to orientate short chains, even at high velocity gradient (orienting force); the longer the chains the more easily they become oriented.

It is interesting that the long molecules of cellulose and its derivatives are comparatively stiff, as indicated before in the case of cellulose acetate (see p. 110). This was confirmed for nitrocellulose by streaming birefringence studies. Mosimann[32] compared the calculated length for straight rods (knowing the length of a single radical and the number of radicals, from molecular weight deter-

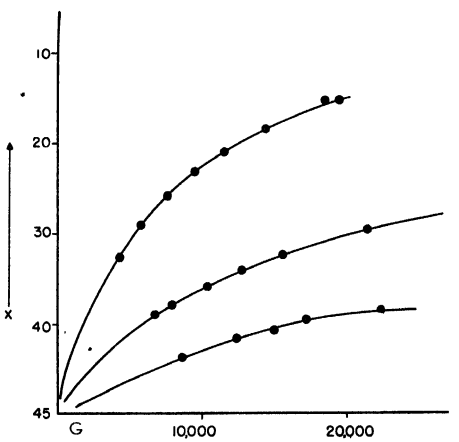

Fig. 40. The dependence of the extinction angle X on the velocity gradient for several specimens of fractionated nitrocellulose.

minations) with the length obtained by streaming birefringence and other methods. According to these data the molecules are very *long* and *thin*. The molecules of the lower molecular fractions (M = 6000–30,000) are nearly *extended rods*, whereas the molecules of the very high polymeric fractions (M = 80,000–600,000) are gently coiled into *loops* and *spirals* (see sect. 15, Chapter 9).

There are more flexible linear molecules which show a somewhat different behavior at high streaming velocity. The molecules of polystyrene, polyisobutylene, and many other vinyl polymers appear to be *kinked strings* in solution. All of these exhibit streaming birefringence, which can be explained in these cases by assuming

that the "strings" are stretched out under the influence of the shearing force[33].

Accurate estimation of molecular shape and size from streaming birefringence studies is, however, possible only in some limited cases. The theory is very complicated, and it is far from being settled on all points. Very disturbing in accurate evaluations is the polydispersity (polymolecularity) of linear colloids.

7. Colloidal Particles in Electron Microscope

The simplest and most straightforward way to study the size and shape of colloidal particles would be to make them visible in a suitable microscope. This is impossible by means of the ordinary light microscope, since the colloidal particles are smaller than the wave length of visible light. A considerable achievement in this direction was the *ultramicroscope* of Siedentopf and Zsigmondy (1903). In this instrument particles are strongly illuminated and observed on a dark background. Colloidal particles act as scattering centers, which are detectable and can be counted. However, not the particles themselves but the diffraction patterns around them are visible. Hence, the size can be determined only indirectly, and the shape cannot be studied at all. Moreover, only a substance with refractive index differing greatly from that of the dispersion medium is well suited for such studies. The ultramicroscope served well for the study of inorganic colloids (gold and silver sols), but it was of much less use in the study of organic colloids.

Electron microscopy is based on the fact that electron beams exhibit the properties of short waves. The wave length of the electron rays can be conveniently altered by changing the voltage. According to de Broglie,

$$\lambda = 12.247 \cdot V^{-\frac{1}{2}} \cdot 10^{-8} \text{ cm.}$$

In this equation λ is the wave length, and V the voltage. For example, if $V = 15{,}000$ volts, the wave length of the electron rays will be about 0.1 A. Such waves penetrate thin layers of matter, and would be sufficiently short for resolving the colloidal patterns in great detail. Since even higher voltages are obtainable, the method has great potentialities in resolving molecular structure and even showing single atoms.

The glass or quartz lenses of ordinary light microscopy are replaced in the electron microscope by electrostatic or electro-

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magnetic coils which act as lenses. The electron source is an incandescent tungsten filament, and the electrons travel through the coils (acting as lenses) in vacuum. The colloidal preparation to be observed is in a very thin layer, and in a solid state, since liquids would evaporate under vacuum. Many difficulties have had to be overcome in the construction of the instrument, as well as in elaborating the methods for the preparation of specimens. The first electron

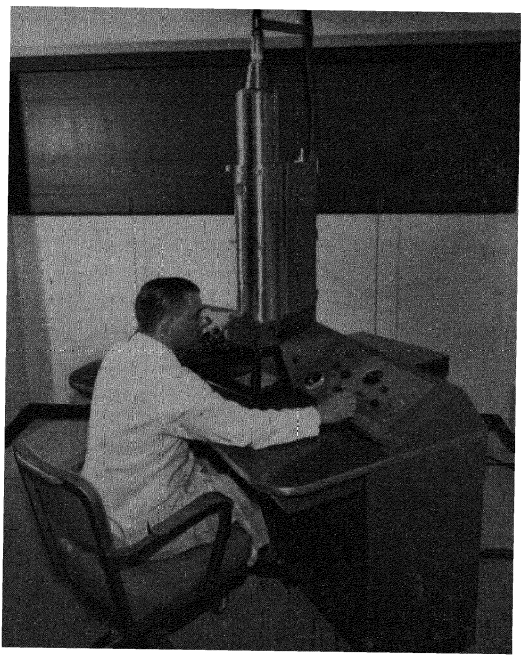

Fig. 41. The electron microscope of the Radio Corporation of America. The instrument is in use in the Virus Laboratory of the University of Texas M. D. Anderson Hospital and Tumor Institute, and is under the supervision of Dr. L. Pirochovski (at the instrument).

microscopes were built in Germany in the early thirties by Ruska, von Borries, von Ardenne and others[34], and independently they were developed in the U.S.A., England and other countries. Conveniently usable instruments are now offered by several companies.

Although the potentialities in magnification power are very great, the best pictures are obtained in the magnification ranges of about 5000 to 20,000 times. Photographic enlargements up to 200,000 are still good. Hence, a particle with a diameter of 10 A or 0.000,001 mm will appear as 0.2 mm large spot. An instrument produced by the Radio Corporation of America (RCA) is shown in Fig. 41.

The contrasts of the electron microscopic images depend on the mass and density differences of the specimen through which the electrons have to pass. The particles to be investigated usually are placed in a very thin layer on a supporting substrate, e.g. a col-

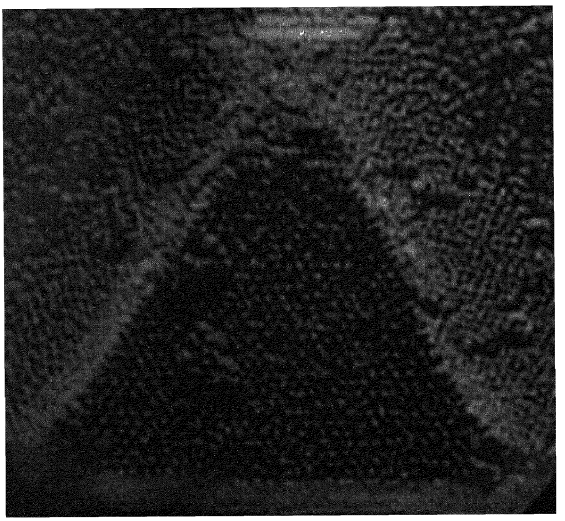

Fig. 42. Electron micrograph of the surface of a crystal of the protein edestin, showing spherical molecules having a molecular weight of 300,000. Magnification 200,000 times. (Courtesy Dr. C. E. Hall, Massachusetts Institute of Technology.)

lodion film. (The supporting substrate does not interfere with the picture, as the nitrocellulose molecules do not affect much the electron beam.) If a drop of a colloidal solution is placed on the supporting film, and the specimen placed in the instrument, the solvent will evaporate, and the particles remain on the film. Very high dilutions, of course, must be used, so that individual particles will be distinguishable. Ingenious methods have been developed in order

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to enhance the sharpness of the images, e.g. collodion replicas from the surfaces are obtained, and the replica pictures can be sharpened by shadowing them with metal vapor.

A variety of organic material has been studied by means of electron microscopy. Important information regarding the structural details of microbes, viruses, and bacteriophages have been obtained. Single large molecules of proteins have been photographed (Fig. 42). Hall has obtained very revealing pictures of edestin (molecular weight 300,000) and catalase molecules (molecular weight 250,000) by means of this method[35]. In both cases the molecules appear as small beads. The tobacco mosaic virus particles appear like rods of various lengths. The particles of fibrinogen appear fibrous, and it seems that they are built of smaller spherical subunits. One of the first carbohydrate molecules made visible was that of iodobenzoyl-glycogen[36] which also appeared to be spherical, as predicted earlier by Staudinger and his associates. The long and thin molecules of cellulose derivatives or rubber are still too thin for the electron microscope. The images obtained with various kinds of fibrous materials show bundles of many linear molecules.

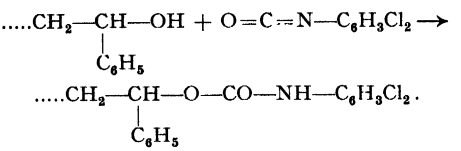
8. Determination of Molecular Weight by Chemical Methods

Chemical methods are important for the molecular weight determination of macromolecular compounds. There are two possible ways to do this. The first possibility is to determine chemically the *end groups* of linear macromolecules. A classical example is the work of Carothers and associates[37] on polyesters. The polymers have the following constitution:

$$HO—CH_2CH_2...CH_2—CO—(O—CH_2CH_2...CH_2CO)_n—O—CH_2CH_2...CH_2\cdot COOH.$$

The macromolecules of such polyesters have the OH group at the one end and the carboxyl group COOH at the other end. The latter can be determined simply by acidimetric titration. It is obvious that the number of equivalents used for titration is inversely proportional to the molecular weight of the polymeric acid. The OH groups also can be determined, e.g. by means of acetylation. Reasonable agreement between the chemical method and other methods of molecular weight determination was achieved with relatively low molecular material. The upper limit is about $M = 25{,}000$. For very large molecules the end group content becomes vanishingly small, and the accuracy of the method becomes too low, to be of great value.

In some cases it was possible to establish the branching of chains by means of the end group determination. Kern and associates polymerized styrene in the presence of bromobenzoyl-peroxide and found, according to the amounts of bromine detected in the polymer, that each molecule contains three to four atoms of bromine[38]. With another catalyst, polystyrene samples containing hydroxyls as end groups were prepared; the high-molecular alcohol was then treated with 3,4-dichlorophenyl-isocyanate, and the end groups of the latter were determined by analyzing for chlorine. The amount of chlorine found indicates that 2.2–2.4 end groups are present in these chains, hence they are only slightly branched[39]. The molecular weight of this specimen was estimated to be 17,300. The following reaction with the isocyanate occurs at the chain ends:



The presence of the respective end groups in the polystyrene samples was ascertained also by means of infrared spectroscopy.

Another possibility of applying the chemical method is independent of structural assumptions, the only prerequisite being the presence of a unique structural unit in small amount. For example, a protein molecule may contain just one atom of iron. If A is the atomic weight of this trace element, and a its analytically determined percentage, the molecular weight

$$M = 100A/a .$$

The smaller the percentage, a, the higher the molecular weight. For example, it was found that the hemoglobins from mammalian blood contain 0.335 % of iron, hence the molecular weight of hemoglobin would be $100 \cdot 55.85/0.335 = 16{,}700$. This is the *minimum* molecular weight calculated with the assumption that only *one* atom of iron is present in the molecules. The molecular weight cannot be smaller than this but it certainly may be some multiple of this minimum, since more than one atom of iron can be present in the molecule. Other metals present in certain enzymes have also been analyzed, and minimum molecular weights have been determined for various proteins. Furthermore, the amino acids present in small amounts, the sulfur content, and the amount of other specific constituents

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can be determined. For example, careful analytical comparisons for β-lactoglobulin suggested a minimum molecular weight of 42,000[40]. In this case the chemical molecular weight agrees reasonably with that obtained by physical chemical methods, and this is the best evidence that it is the correct molecular weight. In the case of hemoglobin the chemical molecular weight is smaller than the physico-chemical (see Chapter 26).

9. The Terms 'Molecule' and 'Molecular Weight'

The terms 'molecule' and 'molecular weight' are used differently by various authors, thus causing some confusion. The term 'molecular weight' for colloidal particles should be used only for macromolecules, not for micelles or macromolecular aggregates. For example, the particle weight found for colloidal solutions of soaps is micellar weight, as the soap particles are aggregates of many small molecules of soap. Soap micelles cannot be called molecules. This is not because they are unstable, or because the soap colloids are polydisperse, but because a micelle is held together by Van der Waals forces (cohesion). It must be pointed out that *polydispersity or polymolecularity is not a criterion of the micellar structure.* All polymers are polymolecular, but the particles in these colloids usually are macromolecules of various sizes, not micelles. The only criterion in distinguishing true macromolecules from micelles is the *structure.* In a macromolecule the atoms are linked by true primary bonds *throughout the whole molecule. The molecular weight is the sum of the weights of the atoms which are linked in the particle by primary valency bonds*[41]. There is no limit to the size of such particles. Moreover, it does not matter that very long molecules, such as isolated molecules of cellulose, are relatively unstable, and can be cleaved easily under ordinary chemical handling at room temperatures. And, finally, it does not matter that all macromolecules of a polymer in a solution are not of the same size.

It was repeatedly admitted by Staudinger and others that such criteria do not solve all the difficulties in classification. Most difficult are the proteins. In the papers of Svedberg and many other authors all particle weights of proteins determined by means of the ultracentrifuge were called molecular weights. In many cases, however, these 'molecular weights' changed depending upon the solvent. For example, the particle weights of hemocyanin and other respiratory blood proteins of lower animals became smaller in weakly

alkaline or weakly acid solutions. The 'molecules' of the tobacco mosaic virus also dissociate reversibly into smaller units. The molecular weight of insulin determined by ultracentrifugation or by osmometry was found to be 12,000, 36,000 or 48,000, whereas by the chemical method 12,000 or even 6,000 was found (Chap. 26). The problem of the chemical constitution of insulin is close to its final solution, and it seems that 6,000 will be the true molecular weight for this protein. The subunits of 6,000 apparently are joined by secondary bonds into the larger particles of the relative weight 12,000, 36,000 or 48,000. If the definition given for the molecular weight of macromolecules should be applied *strictly*, the protein 'molecules' which reversibly dissociate into small subunits ought to be called micelles. However, a distinction between the various types of micelles also could be made. It seems to be useful to distinguish the *soap type micelle* from the *protein subunit associate*. Only the former is a true micelle, whereas the latter resembles a chemical complex. A *true micelle* is an aggregate of *many small molecules*, whereas the reversibly dissociable protein particle is composed of a *small number of large subunits*. In true micelles the chief binding force is cohesion, in "micellar" proteins hydrogen bonding is involved in addition to cohesion and possibly some other not yet quite clarified linking. Especially in the cases where the number of subunits is small, the entities can be called molecules rather than micelles. An insulin particle of mol. weight 48,000 could be called *associate of subunits* or *associated macromolecule*. A much larger particle, e.g. appearing in a state of flocculation, however, is an *aggregate*.

Many more problems arise in evaluating the results of molecular weight determinations for macromolecular substances, especially when the results obtained with different methods are compared. These problems will be discussed in the next chapter.

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CHAPTER 8

Fractionation and Molecular Size Distribution

1. The Various Molecular Weight Averages

In the previous chapter a comparison of the molecular weights obtained by different methods was avoided. The reason is simple: the results obtained with polydisperse (polymolecular) systems are not expected to be the same, if different methods are employed. One exception is Table 11 (p. 94) where the molecular weights of some proteins obtained by sedimentation velocity are compared with those obtained by means of the sedimentation equilibrium method. In this instance the agreement is reasonably good because the proteins mentioned are relatively monodisperse. Many of the natural and all of the synthetic polymers, however, are polymolecular mixtures of polymer homologs. The molecular weight determined by any method for a specimen of rubber, polystyrene, or nylon is an *average value.* It appears that the various molecular weight averages, e.g. those obtained *for the same specimen* of a polymer by osmotic pressure and light scattering, are *not the same.* This is not due to errors involved in measurement but *to inherent effects of molecular size distribution on the values of average molecular weights.*

The ordinary molecular weight as found by the osmotic methods or chemical analysis is the *number-average molecular weight* M_n. It is the ratio of the total mass m to the total number of moles n, i.e. m/n. This number-average molecular weight M_n is obtained by methods which measure the effects caused by the *number* of molecules. In a polymer which contains many similar molecules of

various sizes this value will depend on the molecular weights M_1, M_2, M_3.... of each species, and number of moles n_1, n_2, n_3 .... of these species as follows:

$$M_n = \frac{n_1M_1 + n_2M_2 + n_3M_3 + \ldots}{n_1 + n_2 + n_3 + \ldots} = \frac{\Sigma n_iM_i}{\Sigma n_i}.$$

When the molecular weight of each species is known, and the number of moles also is known, the number-average molecular weight can be calculated by multiplying the number of moles of each species by its molecular weight, adding the individual results, and dividing this sum by the total number of moles. Suppose one has a polymer composed of 10 moles with $M = 10{,}000$, of 80 moles with $M = 50{,}000$, and of 10 moles with $M = 100{,}000$. The *number-average* molecular weight then will be:

$$M_n = \frac{(10 \cdot 10{,}000) + (80 \cdot 50{,}000) + (10 \cdot 100{,}000)}{100} = 51{,}000.$$

(This is just an example for illustration. The polymers usually are composed of a large number of molecular species which are similar, hence a complete analysis of the number and size of each species is impossible.)

The important fact is that the molecular weight of this mixture, as determined by sedimentation or light scattering studies, would never approximate the same 51,000. In these latter methods the effects produced by the larger molecules will strongly overshadow the effects produced by the smaller molecules. Light scattering or sedimentation velocity measurement gives the *weight average molecular weight* M_w, It is defined as follows:

$$M_w = \frac{m_1M_1 + m_2M_2 + m_3M_3 + \ldots}{m_1 + m_1 + m_1 + \ldots} = \frac{\Sigma n_iM_i^2}{\Sigma n_iM_i},$$

where m_1, m_2, m_3 are the masses of each species. (Each value of m is obtained by multiplying n by M. Thus the molecular weight appears in denominator and as its square in the numerator.) For our previous example with the three kinds of molecules then

$$M_w = \frac{(10 \cdot 10{,}000^2) + (80 \cdot 50{,}000^2) + (10 \cdot 100{,}000^2)}{(10 \cdot 10{,}000) + (80 \cdot 50{,}000) + (10 \cdot 100{,}000)} = 59{,}019.$$

Hence the average molecular weight calculated as weight average is nearly 16% larger in this example than the number average weight M_n.

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There are several other molecular weight averages. One of the most important ones is the viscosity-average molecular weight, as obtained by viscosity measurements (see the next chapter). *The viscosity molecular weight average* M_v is calculated as follows:

$$M_v = \left(\frac{\Sigma n_i M_i^{a+1}}{\Sigma n_i M_i}\right)^{\frac{1}{a}}.$$

In certain cases the constant a is unity, and the viscosity average then equals the weight average. If the linear molecules are stretched out, the longer components affect the viscosity much more than the shorter ones. In polydisperse spherocolloids however the larger molecules do not greatly affect the viscosity.

The z-average molecular weight is obtained by the sedimentation equilibrium method. The value of M_z is larger than M_w, and is given by:

$$M_z = \frac{\Sigma n_i M_i^3}{\Sigma n_i M_i^2}.$$

Similar problems of polydispersity arise in cases of micellar colloids and dispersions. For coarse systems the size distribution is directly observable in the light microscope or the electron microscope.

The various molecular weight averages have been compared for the same polymer specimen in several instances. The molecular weight of a polystyrene sample was determined by osmometry and sedimentation methods. The solvent was the same in all experiments (carbon tetrachloride), and the determinations were made at several concentrations of the polymer. By osmotic pressure methods, a number average molecular weight of 50,000 was obtained. The weight average by sedimentation was found to be 104,000 and the M_z average was 148,000[1]. Hence, the ratio is approximately 1:2:3.

Smaller differences were found for three fractionated specimens of methyl esters of polymethacrylic acid[2]. The results obtained by osmometry (M_n), sedimentation and diffusion (M_w) are presented in Table 14.

The light scattering method has given weight averages which do not differ greatly from the number averages in Table 14. For example, a molecular weight of 172,000 was found with the osmotic method and 191,000 with the light scattering, or 223,000 and 256,000 respectively[3].

The molecular weight averages obtained with various methods

TABLE 14

NUMBER AND WEIGHT AVERAGE MOLECULAR WEIGHTS FOR THREE SPECIMENS OF METHYL ESTERS OF POLYMETHACRYLIC ACIDS (after Meyerhoff and Schulz)

Specimen	M_n	M_w
Fraction A	61,000	77,000
Fraction B	128,000	148,000
Fraction C	201,000	306,000

depend considerably on the *degree of homogeneity or monodispersity*. The less homogeneous a specimen the greater the differences will be in the average molecular weights obtained by various methods. The differences are small for the proteins, and for an absolutely monodisperse colloid the various molecular weight averages should be the same, i.e. $M_n = M_w = M_v = M_z$.

A recent report[4] compares data obtained in different laboratories with a high molecular specimen of polystyrene, using various methods of molecular weight determination. The weight average as obtained by light scattering varied within the limits 485,000–590,000; the number averages calculated from osmotic pressure were 270,000–640,000, the average being 455,000 ± 100,000; the molecular weight as found for this sample by sedimentation velocity and diffusion was 450,000 (or 840,000 using a diffusion constant obtained differently). The data show that the degree of accuracy in these determinations is relatively low if results of various authors are compared.

2. The Size Distribution Curves and Degree of Polydispersity

The degree of polydispersity or polymolecularity is expressed by means of the size distribution curves. On the abscissa is plotted the molecular weight, on the ordinate the amount of the particular species of molecules. For example, the amount of each fraction in grams can be plotted against the weight average molecular weight M_w or against the degree of polymerization P. In Fig. 43 the distribution of the M_w is presented for a relatively monodisperse protein and for a very polydisperse polymer.

Several experimental methods are available for the study of molecular weight distribution. The most straightforward one would be measurement of the particles under the microscope or electron

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microscope, and a count of the number of the various sizes appearing in the specimens. This method, of course, would be applicable only to relatively coarse systems, and even then there would be serious limitations; moreover, it would be very time consuming.

Another method to consider would be sedimentation in the analytical ultracentrifuge. This method is capable of revealing the presence of different components present in a mixture of a colloidal material. For instance, upon centrifuging blood plasma several sedimenting boundaries appear in the pictures. However, this method, too, has serious limitations, the greatest of which is its lack of sensitivity. Each of the protein components appearing in the

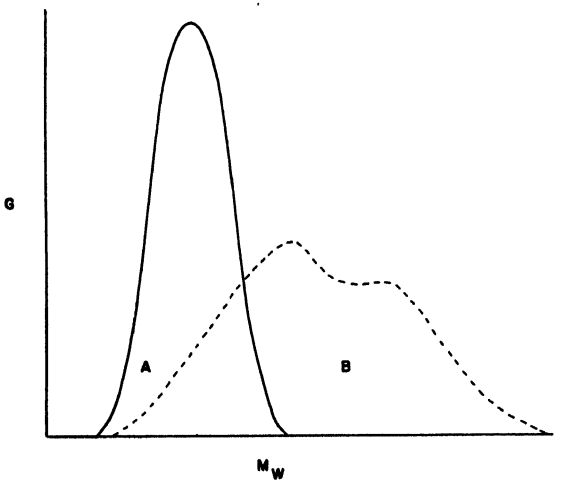

Fig. 43. The distribution curves of a relatively monodisperse protein (A), and of a polydisperse polymer (B). On the abscissa is plotted the molecular weight, on the ordinate the amount of a certain component.

sedimentation diagrams of blood plasma as homogenous is known to consist of several components. The number of the components is greater than the number of boundaries or peaks.

Fractionations by means of centrifugation or ultracentrifugation are successful only if a component which is much heavier than the other components has to be removed, e.g. cells or cell fragments from a protein solution. Hence, separation by fractional preparative ultracentrifugation is not feasible, if, for example, a mixture with molecular weights of 32,000, 34,000, 38,000, 45,000 and 50,000 must be separated quantitatively.

3. Fractionation of Polymers

The size or molecular weight distribution data can be obtained by *fractional precipitation*. The classical procedure of preparative fractionation is carried out by gradually adding a precipitant to the solution of a polymer. This precipitant usually is a liquid, miscible with the original solvent of the polymer but a non-solvent for the polymer under study. For example, a solution of nitrocellulose in acetone can be fractionated by gradually adding water (or better —a water-acetone mixture) with stirring. The higher molecular components precipitate first, i.e. when the concentration of added water is very low. This first fraction is removed by centrifugation, and more water is added to the remainder of the solution until a second fraction precipitates. This is removed like the first fraction, and the precipitation is continued by adding more of the precipitant and removing one after another the subsequent fractions. Usually about 6–12 fractions are obtained from a specimen of a polymer. The average molecular weight is the largest for the first removed fraction, and the last is usually semicolloidal, i.e. of relatively low molecular weight. None of the fractions, however, is absolutely homogeneous but they all are polymolecular. The distribution curves of the fractions are steeper than the curves of the original polymer, i.e. the fractions are more homogeneous than the original sample, but it is impossible to obtain completely homogeneous fractions. Some improvement in the homogeneity is obtained by dissolving and refractionating each of the fractions. Nevertheless, even several times refractionated specimens of polymers are mixtures of similar molecules, although the size distribution range may be rather small, e.g. between $M = 60{,}000$ and 70,000 for 98% of the whole fraction.

A sample fractionation of acetyl cellulose is presented in Table 15,[5]. The original sample of cellulose acetate was dissolved in an acetone-water mixture, and the fractions were precipitated by the addition of water.

Several other methods are available for preparative fractionation. A polymer may be fractionated by cooling the solution. The higher molecular components usually are less soluble at a lower temperature than the smaller molecules, so that these higher molecular components precipitate on cooling. Another method is extraction of the lower molecular components from the solid polymer by a suitable solvent. The older literature on this subject has been compiled by Cragg and Hammerschlag[6].

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TABLE 15

FRACTIONATION OF CELLULOSE ACETATE, M (average) = 52,000

Fraction	%	*Number average degree of polymerisation*, P	*Molecular weight*
A 1	5.31		
A 2	8.75	420	110,000
A 3	8.81	375	99,000
A 4	9.01	328	86,500
A 5	8.56	278	73,500
A 6	7.92	260	68,000
A 7	10.10	245	64,500
A 8	7.86	205	54,500
A 9	19 65	185	48,500
A 10	6.10	150	39,000
A 11	5.20	92	
A 12	2.73	50	

All of these fractionation procedures are based on the observations of Staudinger and his colleagues[7] that the higher molecular weight specimens of the same polymer are less soluble in certain solvents than lower molecular specimens. Hence, a smaller amount of a precipitant is needed for the former than for the latter. This was predicted theoretically by Bronstedt[8], and the applications were further developed by Schulz[9]. Denoting by v_0 the original volume of a solution of a polymer, and by v the volume of a precipitant, necessary to cause the appearance of turbidity, the *precipitability* γ can be expressed as follows:

$$\gamma = v/(v_0 + v)\,.$$

According to Schulz, the precipitability γ is inversely proportional to the degree of polymerization P or molecular weight:

$$\gamma = a + b/P^m, \qquad (1)$$

where a, b, and m are constants which differ from polymer to polymer and also vary with the solvent and precipitant used. Equation (1) is that of a straight line if $m = 1$, which is the case for linear polymers.

This approach can be exploited not only for *molecular weight*

determinations (if the constants a and b are known) but it proved to be even more important in the study of *size distribution* of polymers. This application was introduced into the field of polymers by Morey and Tamblyn[10]. The precipitant is added slowly, with uniform stirring, and the amount of precipitated material is measured by estimating the turbidity with a photocell. Of course, the method must be calibrated with well defined fractions of known molecular weight. This method is simpler and less time consuming than the preparative fractionation, and it is used in some industrial laboratories for the determination of the degree of homogeneity of polymers. Some improvements in this method have been suggested recently[11].

Several other methods have been proposed for the determination of molecular size distribution. A colloid can be fractionated by filtration through ultrafilters of various pore diameters. This method proved to be of some value for relatively coarse dispersions, such as bacteria and the viruses. However, it is of little use for linear polymers. An interesting idea was put forward by Odén about 40 years ago: the pan of a sensitive balance was hung near the bottom of the container in which particles precipitate, and the amount of precipitate was weighed directly without removing the fraction. This method might be feasible for polymers precipitated by non solvents, and some success has been achieved recently by Hengstenberg[12].

According to Boyer, the molecular weight distribution of heterogeneous polymers can be obtained by measuring the volume of precip.tate which corresponds to increasing the amount of non-solvent to a dilute solution[13]. The gel-like precipitates of polystyrenes have been investigated so far.

A more accurate determination of the molecular weight distribution was proposed by Schulz[14]. According to this procedure, only the very high molecular and the lowest molecular fractions must be separated, and their amount and average molecular weight must be determined. Moreover, the average molecular weight (or degree of polymerization) of the whole sample must be known.

The degree of inhomogeneity was defined by Schulz[15] as the ratio of the weight average molecular weight to the number average molecular weight, or more exactly:

$$U = M_w/M_n - 1, \text{ or}$$

$$U = P_w/P_n - 1,$$

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U being the degree of inhomogeneity, and P_w and P_n weight and number average degrees of polymerization respectively. For monodisperse systems $P_w = P_n$, and $U = 0$. The broader (more flat) the distribution curve the greater is U. Hence differences in the weight and number average molecular weight values obtained by exact methods give information about the degree of inhomogeneity of a particular specimen of a macromolecular substance.

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CHAPTER 9

The Viscosity of Organic Colloids

1. Basic Terms[1]

Viscosity characterizes resistance to flow. Glycerin is an example of a viscous liquid, whereas ether has a low viscosity. Glycerin flows slowly from a bottle, water moves faster, and ether even faster than water. The viscosity of solutions depends on the properties of both the solvent and the solute. Water is the most common solvent or dispersion medium of a moderately high viscosity. Aqueous solutions of micromolecular substances, e.g. sugar, sulfuric acid, sodium hydroxide, have a higher viscosity than pure water. The viscosity becomes greater the higher the concentration.

The viscosity is a very important property of colloids, as has been indicated in the previous chapters. First, viscosity of colloidal solutions depends on the molecular shape and size of the macromolecules or micelles. The usefulness of any polymer for certain industrial purposes is evaluated partly from the viscosity data. Viscosity is important also for the characterization of protein and polysaccharide solutions in clinical research, and in the food product industry. Second, viscosity can be easily measured with a high degree of precision.

The resistance to flow in liquids is caused by the attractive forces acting between the molecules. The molecules in highly viscous liquids are polar, e.g. water, sulfuric acid, formamide. These molecules, according to Debye, are dipoles, the positive and negative charges being distributed asymmetrically. Consequently, the molecules tend to associate.

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This mutual aggregation of water molecules can be explained also as hydrogen bonding between the H atoms of one molecule with the O atoms of another molecule. It is obvious that such H bonded liquid must have a higher resistance to flow than another one in which the attractive forces are less pronounced, e.g. ether, benzene. The Van der Waals attraction between non-polar molecules is much weaker than the hydrogen bonds.

Viscosity of liquids, however, does not depend solely on the polarity of the molecules, but also on the molecular size and shape. Mineral oil, for instance, is more viscous than water. The hydrocarbon molecules in the oil are non-polar but they are relatively large and long. The high resistance to flow in such oil is caused by the entanglement of the long, wormlike molecules.

The boiling point of viscous liquids is higher than the boiling point of non-viscous liquids. The same can be said about the heat of evaporation.

For a quantitative treatment of the viscosity of liquids it is useful to consider two layers of molecules moving one over another. Viscosity characterizes the friction between these layers. This frictional force, f, according to Newton, increases with the area A of the gliding layers and with the difference in velocities of their movement. If v_1 is the velocity of the one layer, and v that of the other (and if $v_1 > v$), the frictional force f will be proportional to the difference $v_1 - v$. Furthermore, this frictional force for different liquids will depend on the specific molecular attraction indicated above. This is usually denoted by the viscosity contant η. The greater this attraction the greater will be the frictional force f, if A and $v_1 - v$ are constant; hence:

$$f = \eta A(v_1 - v) .$$

A flowing liquid can be considered as composed of an infinite number of layers gliding one over another with various velocities. The frictional force depends on the velocity gradient $\mathrm{d}v/\mathrm{d}x$ in the direction x, perpendicular to the flow lines, therefore:

$$f = \eta A \mathrm{d}v/\mathrm{d}x .$$

The *viscosity coefficient* η characterizes the internal friction for different systems, when A and $\mathrm{d}v/\mathrm{d}x$ are unity. If $A = 1$, and $\mathrm{d}v/\mathrm{d}x = 1$, $f = \eta$. The unit of *absolute viscosity* is the force acting on 1 cm^2 area, and forcing adjacent layers to glide with a speed of 1 cm/sec. This unit is called *poise*, after Poiseuille, who made

important discoveries concerning the flow of liquids in capillaries. 0.01 of a poise is centipoise, and 0.001 poise is known as millipoise. The dimensions of η are dyne cm^{-2} sec. The absolute viscosities of various liquids are recorded in Table 16.

TABLE 16

VISCOSITIES OF PURE LIQUIDS (at 20° C) IN CENTIPOISES

Water	1.008	Benzene	0.647
Methyl alcohol	0.593	Chlorobenzene	0.800
Ethyl alcohol	1.200	Nitrobenzene	2.013
Acetic acid	1.220	Chloroform	0.563
Acetone	0.329	Glycerin, approx.	800

The reciprocal of the absolute viscosity $1/\eta$ is called fluidity. The ratio of the absolute viscosity to the density of the liquid is known as kinematic viscosity.

2. The Flow Through Capillaries

The viscosity of a liquid or solution can be measured either by moving a solid body through the liquid or by allowing the liquid to flow through a capillary. The flow through narrow capillaries was thoroughly studied by Poiseuille more than a hundred years ago. He found that the volume V of a liquid which flows through a capillary is directly proportional to the flow time t, the driving pressure p, and the fourth power of the capillary radius r^4; moreover, the volume V is inversely proportional to the length of the capillary l, and to the viscosity η. According to Poiseuille:

$$V = \pi t r^4 p / 8 l \eta . \tag{1}$$

From this equation, which is known as the law of Poiseuille, the absolute viscosity of a liquid can be calculated, if V, t, r, p, and l are known.

The absolute viscosities, however, are seldom used in the characterization of colloidal solutions. Much more useful for this purpose are the relative viscosities, the specific viscosities, and other terms which correlate the viscosity and concentration.

It is important for our purposes to know to what extent the viscosity of a solvent is changed by the presence of colloidal particles or macromolecules. For this purpose the flow times of a colloidal

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solution and solvent are determined and compared. If the same volumes of the solution and the solvent will drain through the same capillary, the values of V, l, and r will be constant. Together with $\pi/8$ they can be joined in a constant K, and equation (1) then can be simplified as follows:

$$\eta = Kpt. \tag{2}$$

The pressure p depends on the level of the flowing liquid in the tube. If the starting level is kept the same for the solution and solvent, p will depend only on the difference in densities. Usually these density differences are so small that they do not influence the result significantly. Hence p can be included in the constant K. Consequently, the only value to be determined accurately is the flow time. If a fixed volume (e.g. 2.0 ml) of a solution flows t_1 seconds, and the same volume of the solvent t_0 seconds, the ratio t_1/t_0 expresses the *relative viscosity* of the colloid. Since the solution is more viscous than the solvent, the relative viscosity $\eta_{rel} > 1$. When the density of the solution d and solvent d_0 is also taken into account, the relative viscosity

$$\eta_{rel} = \frac{t_1 d}{t_0 d_0}.$$

The term *specific viscosity*, which is even more useful than the relative viscosity in colloid science, is defined as the rise in viscosity of the solvent produced by the dissolved substance:

$$\eta_{sp} = (t_1 - t_0)/t_0 = \eta_{rel} - 1.$$

The specific viscosities of moderately concentrated spherocolloids are less than unity, usually about 0.03 to 0.1. The specific viscosities of linear colloids are much higher.

3. Reduced Viscosity. Intrinsic Viscosity

Since relative and specific viscosities both depend on the concentration of the solute, the particular values must be supplemented with data on concentration. For this reason the term *reduced specific viscosity* or simply *reduced viscosity* η_{sp}/c was introduced. When the specific viscosity is divided by the concentration c of the colloid, the frictional properties of the system are better characterized.

The most common way of expressing the concentration of colloids is in grams per 100 ml of solution. Unfortunately different authors

express c differently. Professor Staudinger, and most of his associates, calculate c in grams per liter[2], the resulting values of reduced viscosities thus becoming 10 times less. For polymers of known constitution Staudinger used also "base-molar" or "radical-molar" concentrations, for which the concentration was expressed in terms of the radical weight of the repeating unit per liter of solution. For instance, one "base-mole" per liter of a polymethylene....$(CH_2)_n$.... would be 14 grams per liter. Other authors have suggested that concentration be expressed in grams per milliliter. We shall use, in further discussions about viscosity, the most common convention, i.e. c in grams per 100 ml.

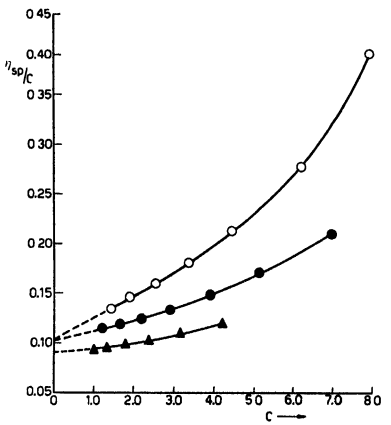

Fig. 44. Extrapolation of the reduced specific viscosity η_{sp}/c to infinite dilution. The graph represents three examples of abnormal globulins isolated from human blood serum. The intrinsic viscosity (intercept on the ordinate) for two of the proteins is the same—approximately 0.11.

○ "Fraction G" No. 135
● "Fraction G" No. 205
▲ "Fraction G" No. 208.

The reduced viscosity of linear colloids increases considerably with concentration. Hence, for the characterization of a linear colloid the function reduced viscosity versus concentration must be studied. It is customary to present such functions graphically. The *intrinsic viscosity* then is obtained by graphical *extrapolation of the reduced viscosity to zero concentration* (see Fig. 44). This is denoted most frequently by $|\eta|$. Many other proposals have been made for expressing the internal friction of linear colloids[3].

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Methods of Measurement
4. The Capillary Viscometer of Wilhelm Ostwald

The flow time through a capillary can be measured most conveniently using the viscometer of Ostwald (Fig. 45). It is a U-shaped tube, one arm of which contains the capillary. The liquid is introduced into the wider arm and is pulled by means of suction through the capillary into the upper bulb of the other arm, just a little over the upper mark. The liquid then is allowed to flow, and the flow time is determined by means of a stopwatch. The knob of the stopwatch is pressed at the moment the level of the liquid passes the upper mark, and the watch is stopped when the level passes the lower mark. The time measured is the flow time t. It is determined with an accuracy of at least 0.1 second.

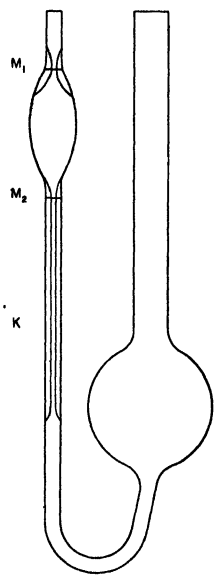

Fig. 45. The capillary viscometer of Wilhelm Ostwald.

All viscosity measurements should be made at constant temperature. Viscosity decreases considerably with increasing temperature, and vice versa. This is quite comprehensible, if one considers that the motion of any kinetic units increases with temperature. It is obvious that the molecular attraction and internal friction must decrease when the intensity of the motion increases. Therefore the viscometer must be kept in a constant temperature bath, and sufficient time should be allowed for the solution to attain the temperature of the bath which should be kept constant within the limits of at least $\pm$ 0.03° C.

The choice of the proper capillary is the most important consideration in the construction and application of the Ostwald viscometer. The evaluation of the results is based on the law of Poiseuille. This law was derived on the assumption that the flow in the capillary is "laminar" or "streamlined". The layer of liquid at the wall of the capillary can be considered as stationary, and the next adjacent one presumably remains parallel on gliding. In order to achieve such laminar flow, the rate of flow must be very low. If the flow rate is high, turbulence will result, and the Poiseuille law becomes invalid. The radius of the capillary is especially important

since the flow rate increases with the fourth power of the radius. According to G. V. Schulz[4], the most suitable capillaries for high precision measurements are those with r of 0.14 to 0.24 mm. The length of the capillary l is not very important, because with increasing length of the capillary l the pressure p also increases. (See equation 2.) For a flow time of 100 seconds the volume of the upper bulb must be about 0.5–1.0 ml. According to Schulz, the flow is essentially laminar, and no hydrodynamic corrections are needed, if the ratio of the volume V to flow time t, i.e. V/t is about 0.01–0.005. One must, however, realize that the volume of the liquid introduced into the tube is greater than that of the upper bulb. It is impossible to use only 1.0 ml of a liquid for a viscometer with an upper bulb of 1.0 ml volume, due to air bubbles formed in the capillary limb upon suction. Five ml of liquid are often pipetted into the tube, whereby V, i.e. the volume between the marks of the upper bulb, is only about 1 to 2 ml. If V is 0.5–1.0 ml, a smaller volume than 5.0 ml is sufficient. Flow times of 100 to 300 seconds are the most common in precise viscosity measurements.

Moreover, drainage errors and surface tension effects must be taken into account in certain cases[5]. It is also obvious that the capillary must be perfectly clean. It should be clamped vertically in the constant temperature bath. The solutions to be used must be free of any solid particles, such as filter paper fibers. Gas bubbles and frothing should also be avoided[6].

Many changes and improvements in the design of the Ostwald capillary viscometer have been proposed during the last 40 years. The viscometers of Bingham, of Ubbelohde, and of Hess are the most important modifications of the original Ostwald viscometer[7].

5. Rotating Cylinder Viscometers

In 1890, M. Couette proposed measuring internal friction by rotating a cylindrical container filled with liquid. A smaller coaxial cylinder is then rotated (Fig. 46). The liquid will also move, and this movement will be transmitted to the smaller cylinder. The higher the viscosity of the liquid the greater will be the torque produced on the hanging cylinder. This torque effect can be measured in various ways, for instance by a mirror attached to the wire, reflecting a light beam onto a scale. Another factor which determines the amount of torque is the angular velocity w of the outer cylinder. If a is the

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angle of rotation of the suspended cylinder, and η the viscosity of the liquid,

$$a = Kw\eta.$$

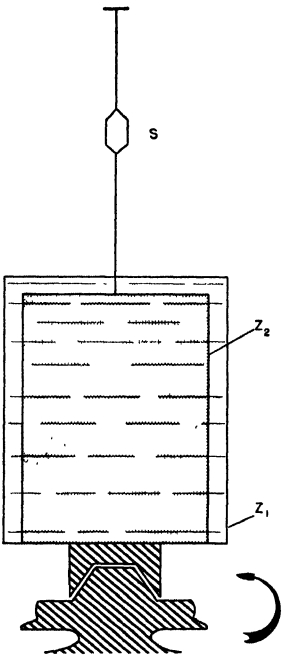

Fig. 46. The rotating cylinder viscometer of Couette.

In this equation K is a constant determined by the dimensions of the instrument*; i.e. the clearance between the cylinders, the height of the cylinders, and the properties of the wire. The angular velocity w of the container produces the shear stress in the liquid, which corresponds to the pressure p in the capillary viscometer.

The chief advantage of the rotating cylinder viscometer is the ease with which the shear stress can be varied. Therefore these instruments are particularly suitable for highly viscous colloids. It will be shown later that the viscosity of many highly viscous systems, especially of the linear colloids, is not constant but varies with the shear stress, or with the velocity gradient dv/dx produced by the shear stress[8].

The disadvantages of this type of instruments are (1) the temperature control is not satisfactory, and (2) more of the test substance is required than for the capillary viscometer.

6. Other Types of Viscometers

Viscosity can be studied also by determining the sinking rate of solid bodies, or by watching gas bubbles ascending to the top. The rate w of a falling ball with the radius r and density d is inversely proportional to the viscosity of the medium η. According to Stokes:

$$w = 2/9 \cdot 981 r^2(d - d_0)/\eta,$$

where d_0 denotes the density of the liquid. The sinking rate w is determined by watching the ball passing between two marks in a cylinder filled with the colloid to be studied. The time required to

* This K has nothing in common with the K of equation (2).

cover this distance between the marks is determined by means of a stopwatch as in the case of flow rate measurements.

The falling ball viscometer of Hoeppler was designed for such purposes. The cylinder is fastened in a slanting position to retard the sinking. The temperature control is maintained by placing the cylinder inside another one in which water is circulating from a constant temperature bath (see Fig. 47). The shear stress and velocity gradient in this instrument can be changed by using balls which have different densities.

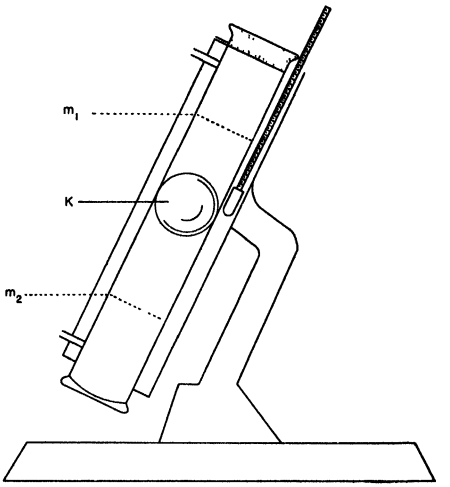

Fig. 47. The falling ball viscometer of Hoeppler.

The Viscosity of Spherocolloids

7. Viscosity and Concentration

The viscosity of any colloidal solution increases with increasing concentration of the dispersed phase. A solution which contains 10% albumin is more viscous than a 2% solution. The quantitative relationship existing between viscosity and concentration was derived theoretically by Einstein in 1906. He came to the conclusion that viscosity should increase linearly with the volume fraction φ occupied by the particles:

$$\eta_{rel} - 1 = K\varphi = 2.5\varphi .$$

Einstein's equation was derived with the assumption that the dispersed particles are rigid spheres, and that they are much larger than the molecules of the liquid in which they are dispersed.

Experiments with suspensions of various kinds have proven the validity of Einstein's equation. For example, the viscosity of yeast cell suspensions increases linearly with the concentration of the cells[9]. The same was found by Staudinger and associates for rubber

milk (latex)[10]. Staudinger also proposed changing Einstein's equation to a more convenient form. If c is the concentration of the dispersed substance (grams per 100 ml), and d its density:

$$\eta_{sp} \cdot d/c = K_e = 0.025.$$

The variations of viscosity with concentration are presented in Table 17.

TABLE 17

THE INCREASE OF VISCOSITY WITH CONCENTRATION OF RUBBER MILK

c *grams per 100 ml*	η_{sp}	η_{sp}/c	K_e
0.96	0.023	0.024	0.021
1.93	0.052	0.027	0.024
2.82	0.072	0.026	0.023
3.93	0.109	0.028	0.025
4.82	0.141	0.029	0.026
6.80	0.24	0.035	0.032
13.6	0.58	0.043	0.039

From Table 17 it may be seen that specific viscosity increases linearly up to a concentration of about 5%. In this low concentration region the values of the reduced viscosities are constant, and the constant K_e is 0.025, or very nearly so. When the concentration is increased above 5%, K_e is no longer constant, but is increasing. This means that Einstein's equation becomes invalid when the concentration of a suspension is very high.

Extensive experimental studies by several authors have shown that the magnitude of K_e may differ from 0.025 (or 2.5 in the original equation of Einstein) even in diluted spherocolloids. The magnitude of K_e for hemoglobin solutions in water is 0.033, and for glycogen in water it is 0.13. There are several reasons for these differences. First, K_e depends on the shape and compactness of the particles. Glycogen molecules are not very compact but have a rather loose, meshy structure. Second, K_e depends upon the degree of solvation, i.e. attachment of the solvent to the particles. Strongly hydrophilic substances, such as proteins or polysaccharides, are more solvated than rubber particles in latex.

The increase of *reduced viscosity*, in highly concentrated dispersions is caused by mutual attraction of the particles when they

are packed very closely. It is obvious that the internal friction of such systems must thereby increase.

Certain spherocolloids, such as serum albumin, show a slight increase of the reduced specific viscosity η_{sp}/c with concentration c. This relation is linear (up to c approximately 8%), and it can be expressed by the equation:

$$\eta_{sp}/c = [\eta] + kc.$$

The constant k represents the slope of the straight line, if η_{sp}/c is plotted against c. The greater the slope constant k the more flexible or asymmetric are the particles or macromolecules.

8. Viscosity and Particle Size

The viscosity of spherocolloids is independent of particle size as confirmed by numerous experimental measurements. For example, Table 18 contains data concerning the viscosity of glycogen solutions.[11]

TABLE 18

THE VISCOSITY OF GLYCOGEN FRACTIONS OF DIFFERENT MEAN MOLECULAR WEIGHTS IN 0.1 *N* CALCIUM CHLORIDE

Molecular weight	*Reduced viscosity*	K_e
20,300	0.081	0.12
52,000	0.086	0.13
135,000	0.088	0.13
450,000	0.085	0.13
930,000	0.081	0.12
1,530,000	0.078	0.12

The same was found for solutions of glycogen acetate in chloroform. The reduced viscosity of solutions with smaller glycogen acetate molecules was practically the same as that which contained very large molecules. The constant K_e in this case had the value of 0.15. The viscosities of each fraction were also measured at different concentrations. It was found that K_e remains constant if the concentration does not exceed 3%. In a 10% solution of a high molecular glycogen acetate the reduced viscosity was found to be 0.278, and K_e had the value of 0.42.

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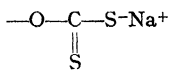
The independence of the viscosity from particle size indicates that the smaller particles of a particular substance have the same spherical shape as the larger ones. The larger glycogen molecules have been seen and photographed under the electron microscope in which they appeared as spherical objects. It may be concluded from the viscosity data that the smaller molecules (e.g. of molecular weight 20,000), which are invisible in the electron microscope, also have a spherical form.

9. The Influence of Electrolytes on the Viscosity

A great deal of thought has been devoted to the problem of how electrical charges on the particles might influence the viscosity[12]. The equations derived, however, could not be verified satisfactorily by the experiments.

If the particles of a dispersion are charged, the viscosity of the dispersion decreases upon addition of a neutral salt. For example, the reduced viscosity of serum albumin (dissolved in water, pH 5.1) is 0.039, and it decreases to 0.031 upon addition of sodium bromide (0.5 *M* per liter). This decrease of viscosity is even more pronounced when weakly alkaline solutions of the spheroproteins are investigated. The reduced viscosity of glycogen xanthate, which carries the ionogenic groups



also is decreased by added electrolytes. According to Staudinger, the reduced viscosity of this colloidal electrolyte in water is 0.660; this value is reduced to 0.048 upon addition of sodium chloride (0.1 *M* per liter of solution).

The failure of the theoretical explanations which treated these "electro-viscous effects" seems to be due to the fact that the particle shape was completely disregarded. The studies which have been carried out recently on linear polyelectrolytes, however, indicate that variation of the charge produces considerable change in the shape and volume of the molecules (see sect. 12 of this chapter).

Variations in net charge produce changes in the shape and configuration even of globular macromolecules. Serum albumin, for instance, is a spheroprotein; the viscosity of its solutions increases when they are acidified or when alkali is added to the aqueous

solution. It is known that the net charge of the albumin molecules in acidic solution (and also in alkaline solution) is greater than at the isoelectric point. It was believed that the surplus net charge on the surface of the rigid molecules was causing the viscosity rise. Recent experiments on the viscosity, optical rotation, and other properties have shown that the molecules of this spheroprotein are not rigid, but expand in acidic solutions[13]. It is obvious that the swollen molecules occupy a larger volume than unswollen units. Water is immobilized in the swollen particles. The more the solvent becomes immobilized the greater will be the viscosity of the solution.

The molecules of spheroproteins, glycogen xanthates, and similar spherocolloids possess a certain degree of flexibility. The viscosity changes of their solutions upon addition of electrolytes are due chiefly to the changes in molecular volume and shape. Repulsion of equally charged parts of the same molecule may cause expansion. Upon addition of neutral salts this net charge is diminished, the molecules contract, and the viscosity decreases[14].

In concentrated colloids, of course, the conditions are much more complex, especially if the concentration of the micromolecular electrolyte is also high.

Viscosity of Linear Colloids

The viscosity of linear colloids is much *higher* than the viscosity of spherocolloids. While the reduced viscosity of the latter is about 0.03–0.08 in most cases, the linear colloids have values approximately of 0.5–2 and higher, even in diluted solutions. Einstein's equation is not valid for linear colloids. Furthermore, the viscosity of linear colloids depends on the molecular weight of the polymer, when the concentrations are the same. For spherocolloids, the viscosity is practically independent of molecular weight, as was shown in section 8 of this chapter.

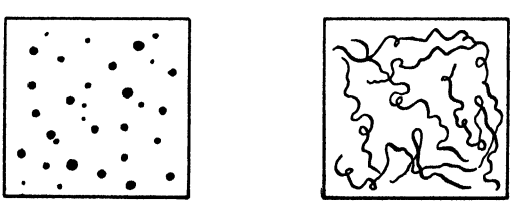


Fig. 48. Sphero- and linear colloids. The linear (fibrous) colloids are very viscous.

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According to the vast experimental and theoretical evidence which has been accumulated in the last three decades, the chief factor determining the viscosity is *particle shape*. When the particles in the solution are corpuscular, the viscosity is low; when the particles are fibrous, the viscosity is high.

10. Viscosity and Concentration of Linear Colloids

The *reduced* viscosity of linear colloids increases with concentration. Data concerning fractionated nitrocellulose in butyl acetate solutions are presented in Table 19. The increase of the *specific* viscosity

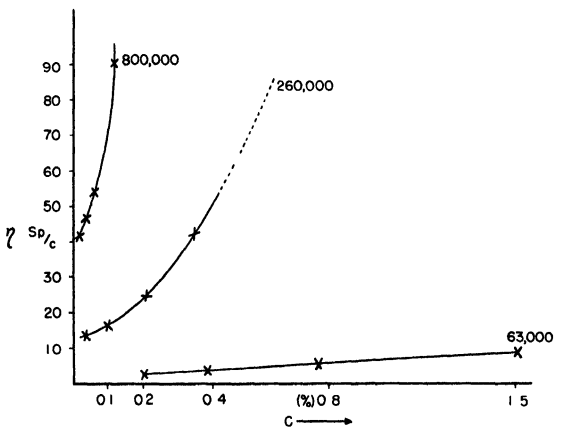

Fig. 49. The dependence of the reduced specific viscosity on concentration (c in grams per 100 ml) for three specimens of nitrocellulose. The viscosity of the high molecular nitrocellulose (mol. weight 260,000 and 800,000) increases sharply with concentration (c), whereas the reduced viscosity of a lower molecular nitrocellulose (mol. weight 63,000) does not change much with c. (Data of H. Staudinger.)

is more striking, even in diluted solutions (third column of the Table). It is noteworthy that for the relatively low molecular fractions (mean molecular weight 63,000) the increase of viscosity with concentration is less pronounced than for the higher molecular fractions. This is illustrated in Fig. 49. For the lower molecular fraction of nitrocellulose, the reduced viscosity increases linearly with c. In the case of a high molecular fraction (mean mol. weight 260,000) the curve turns upwards sharply.

TABLE 19

THE VISCOSITY OF NITROCELLULOSE SOLUTIONS IN BUTYL ACETATE

T = 20° C

(According to Staudinger and Sorkin)

Mol. weight of the fraction	c *grams in 100 ml*	*Specific viscosity* η_{sp}	*Reduced viscosity* η_{sp}/c
63,000	0.188	0.670	3.56
	0.375	1.593	4.25
	0.750	4.575	6.09
	1.50	17.85	11.9
	3.0	104.2	34.7
260,000	0.0315	0.444	14.1
	0.0630	1.060	16.8
	0.125	2.858	22.8
	0.250	10.80	43.4
	0.500	68.0	136 0
800,000	0.0063	0.265	42.0
	0.0126	0.585	46.4
	0.10	26.9	266.3

Great variation has been observed in the viscosity behavior of linear colloids. Endeavors at theoretical correlation of the viscosity and concentration have been only moderately successful. Huggins derived the following equation:

$$\eta_{sp}/c = |\eta| + k|\eta|^2 \cdot c\,.$$

This relation proved to be valid for linear colloids in dilute solutions[15]. In this equation c is the concentration of the colloid, $|\eta|$ is the intrinsic viscosity, and k is a constant. The constant k is different for various colloids. For instance, it is 0.38 for polystyrene dissolved in benzene or toluene. Cellulose acetate, according to Bartovics and Mark, has a k value of 0.70[16]. The magnitude of k increases with increasing heterogeneity (polymolecularity) of the solute[17]. It depends also on temperature, but appears to be independent of the molecular weight of polymer-homologous fractions of the same substance when the solvent is the same. The Huggins' constant is high (2.5–5, or greater) if the fibrous particles are electrically charged[18].

Other equations have been proposed, and the following seem to be reasonably valid for linear colloids even at relatively high con-

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centrations. According to Bungenberg de Jong, Kruyt and Lens[19]:

$$\ln\eta_{rel} = ac + bc^2 \text{ } (a \text{ and } b \text{ being constants}).$$

Bredee and De Booys[20] introduced a third term, and a third constant C:

$$\eta_{sp} = ac + Bc^2 + Cc^3.$$

More popular, however, is the equation of Fikentscher:

$$\log \eta_{rel}/c = 0.75\, K^2/(1 + 1.5\, Kc) + K.$$

The Fikentscher constant K is different for each colloid, and is often used for the characterization of colloidal solutions.

The linear equation

$$\eta_{sp}/c = |\eta| + kc$$

is valid for some linear colloids at low concentrations c. This is the same equation which expresses the dependence of the viscosity on concentration for spherocolloids with flexible particles (p. 143).

11. Dependence of the Viscosity on Molecular Weight

The pioneering work in this important branch was done by H. Staudinger in 1925–1935, as mentioned in Chap. 2. It was shown that the intrinsic viscosity of linear molecular colloids increases linearly with the degree of polymerization P (or the molecular weight). A very simple and convenient method for the estimation of the degree of polymerization or length of linear macromolecules thereby was available. The original equation of Staudinger,

$$\lim_{c \to 0} \eta_{sp}/c = K_m \cdot P,$$

rendered reliable results especially under the following conditions. First, the polymer should be dissolved in a good solvent in which the macromolecules do not associate. Second, the viscosity must be determined at very high dilutions. (Even at moderate concentrations of 0.5–1% the fibrous molecules may become entangled and abnormally high viscosities will be obtained.) Third, the molecules must be truly linear, not branched or tightly coiled. And finally, the molecules must not be extremely long. Good agreement between the molecular weights obtained viscosimetrically and by means of sedimentation in the ultracentrifuge was found in cases where the above mentioned conditions have been realized. The weight-average

molecular weights are obtained with these methods. Some examples are presented in Table 20.

TABLE 20

MOLECULAR WEIGHT OF METHYL CELLULOSE AND NITROCELLULOSE DETERMINED BY MEANS OF VISCOMETRY

	Molecular weight by	
	Ultracentrifugation	*Viscosity*
Methyl cellulose I[21]	14,100	12,300
Methyl cellulose II	24,300	25,000
Methyl cellulose III	38,100	38,000
Nitrocellulose I[22]	10,000	11,600
Nitrocellulose II	18,700	21,300
Nitrocellulose III	23,300	22,500

Mark, Kuhn, Houwink, Flory and other authors have shown that more accurate values for the viscosimetrically determined molecular weights can be obtained, if Staudinger's equation is modified as follows:

$$|\eta| = K_m \cdot P^n, \qquad \text{or} \qquad |\eta| = K \cdot M^a. \tag{3}$$

In this equation, a is another constant which has a definite value for each polymer-solvent combination as has the other constant K*. Equation (3) holds for a wider range of molecular weights than the original Staudinger's rule. By means of this equation (3), good agreement has been obtained between molecular weights determined by viscometry and other methods.

The viscosimetric method of molecular weight determination is very convenient, and gives sufficiently accurate results if the constants K and a are known. In order to determine these constants, samples of known molecular weight M should be used. (M should be determined by several other precise methods.) Unfortunately, the properties of a single polymer (e.g. rubber, nylon) may vary with the method of preparation, and the constants K and a are hardly reproducible. Some of them are compiled in Table 21.

The constants K and a depend also on the method by means of which the molecular weights were determined. For example, the

* This constant K, of course, is different from the constants denoted by the same symbol in the previous sections.

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TABLE 21

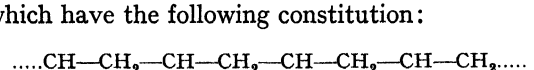
THE CONSTANTS K AND a FOR VARIOUS LINEAR COLLOIDS (from P. J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, 1953, p. 312).

Polymer	*Solvent*	*T°C*	*Mol. weight range* · 10^{-3}	K · 10^4	*a*
1. Polystyrene	Benzene	25	32 - 1,300	1.03	0.74
2. Polystyrene	Methyl ethyl ketone	25	2.5–1,700	3.9	0.58
3. Polyisobutylene	Diisobutylene	20	6 – 1,300	3.6	0.64
4. Polyisobutylene	Cyclohexane	30	6 – 3,150	2.6	0.70
5. Polyisobutylene	Benzene	24	1 – 3,150	8.3	0.50
6. Polyisobutylene	Benzene	60	6 – 1,300	2.6	0.66
7. Natural rubber	Toluene	25	40 – 1,500	5.0	0.67
8. Poly-(methyl methacrylate)	Benzene	20	77 – 7,440	0.84	0.73
9. Poly-(vinyl acetate)	Acetone	25	65 – 1,500	1.76	0.68

measurements on the polystyrene # 1 were made from osmotic pressure data which render the number average molecular weights, while in the polystyrene # 2 the molecular weights were determined by light scattering. This is the chief reason that the constants are so different in these two cases. For the polyisobutylene (# 3–6) and rubber the reference method was the osmotic method. For the case # 8 the reference method was sedimentation, and for case # 9 it was again the osmotic method.

12. The Viscosity of Ionized Linear Colloids

Ionized linear colloids show several anomalies of viscosity. One of the simplest and best investigated examples is polyacrylic acid and its salts which have the following constitution:



The viscosity of aqueous solutions of polyacrylic acid varies with concentration in very intricate fashion, depending upon the degree of ionization. The reduced viscosity is high at low concentrations of the colloid. It decreases with rising concentration, becomes constant, and then rises again. The anomalies disappear if neutral salts are

added to the solutions. According to recent extensive studies on polyacrylic acid[23] and other ionized linear colloids[24], the viscosity of these colloids decreases upon addition of neutral salts. The reason for this decrease is a change of the molecular shape[23, 24, 25]. At high degrees of ionization the molecules are stretched out, as the equally charged segments repel each other. At low degrees of ionization the fibrous molecules coil up. Added electrolytes decrease the electrostatic repulsion between segments, and the molecule becomes less asymmetric.

The molecular weight of ionized linear colloids can be determined from viscosity measurement if the following precautions are taken into account. The constants K and a of equation (3) depend on the degree of ionization, and on the presence of neutral salts. At very low degrees of ionization of the macromolecules, a is close to 0.5, and the molecules are coiled up. At maximum ionization the molecular fibres stretch out, and a increases. The influence of added salts on the intrinsic viscosity of polyelectrolytes was thoroughly investigated by Fuoss[26]. He proposed the following equation:

$$|\eta| = D + A/(1 + BC^{\frac{1}{2}}).$$

A, B, and D are constants, and C is the salt concentration.

The dependence of the viscosity on concentration of a linear polyelectrolyte has been studied by several authors. Eirich *et al.*[27] studied solutions of polyvinyl-pyridinium bromide, a linear vinyl polymer with pyridinium rings at the sides. The nitrogen of these pyridinium radicals carries positive charges. Viscosity, streaming birefringence, sedimentation, diffusion and electrophoretic mobility measurements were made. The concentration of the colloid was varied, and the Huggins constant k was found to be very high (2.7–5.2)[28].

13. Viscosity and Axial Ratio of Asymmetric, Rigid Particles

Theoretical considerations, based on the laws of hydrodynamics, have shown that the greater the asymmetry the higher must be the viscosity. This was proved to be true in experiments with macroscopic models of glass rods[29].

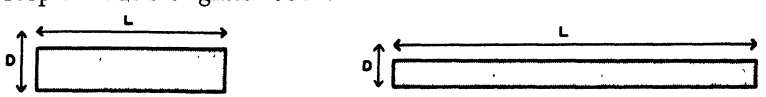


Fig. 50. The axial ratio L/D for rod-shaped particles.

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Further experimental evidence was obtained by the study of tobacco mosaic virus. Electron microscopic studies have shown that this virus possesses long, rodlike particles[30]. The viscosity of its solutions is high and when the long rods are broken down into shorter ones, the viscosity decreases.

Several authors have tried to correlate viscosity with the axial ratio of the rodlike particles. If L is the length of the rod, and D its diameter (short axis), the axial ratio will be L/D. Simha[31] derived the following equation:

$$\eta_{sp}/\Phi = \frac{(L/D)^2}{15(\log 2L/D - 3/2)} + \frac{(L/D)^2}{5(\log 2L/D - \frac{1}{2})} + \frac{14}{15},$$

where Φ is the volume fraction occupied by the particles.

Reasonably good agreement between the axial ratio L/D calculated from this equation and the axial ratio obtained by means of other methods has been found[32].

14. Flexible Linear Macromolecules in Different Solvents

Molecular colloids can be dissolved directly only in solvents which are chemically similar to the solute. The solvent molecules penetrate into the bulk of the solid polymer because they have a certain affinity for the macromolecules. The latter become solvated, and by virtue of thermal motion they are separated from the bulk and go into solution. It is obvious that the adhering layer of solvent molecules protects the macromolecules from mutual association. Moreover, this layer must also influence the flexibility of the fibrous particles. When this layer is thin and loose, and when it has holes, the flexibility will not be much impaired. In this case the molecules will coil and aggregate easily. If, however, the protective envelope is thick and compact, the macromolecules will be well protected from mutual association, but their flexibility will be much impaired. Consequently, the particle shape may vary when a certain molecular colloid is dissolved in various solvents. Since viscosity depends on this shape, it is obvious, that the viscosity must be different in various solvents.

All the possible solvents which are available for a linear polymer can be classified into two groups: *good and poor* solvents. The intrinsic viscosities in the former are higher than in the latter. This is quite understandable on the basis of the above outlined solvation phenomena. In a good solvent the macromolecules are stretched

out. In a poor solvent they are coiled up in more or less compact, globular units. Fig. 51 illustrates this.

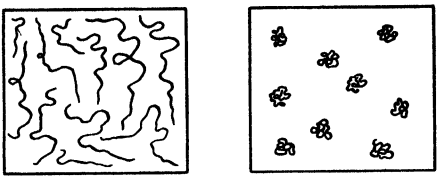

Fig. 51. Linear colloids in a good (left) and in a poor (right) solvent.

For example, a polystyrene in aromatic solvents has the intrinsic viscosity of 1.20–1.30 (at 25° C), whereas in aliphatic solvents it is only 0.65–0.75[33]. The former are much better solvents for polystyrene, because of the similarity in constitution.

The degree of coiling can be estimated by determining the constant a (equat. 3, also Table 21). The smaller the value of a the more coiled are the linear macromolecules.

Flory and Fox[34] have presented a thorough theoretical treatment of this subject. The interaction of the solute with the solvent (solvation) is characterized by a constant α. The following equation was developed for highly flexible, uncharged linear macromolecules

$$|\eta| = KM^{\frac{1}{2}}\alpha^3, \tag{4}$$

in which M is the molecular weight. It was shown that the interaction constant α varies with the solvent and the polymer, and methods were developed to determine this constant. Conditions can be found when α is unity, in which case equation (4) becomes

$$|\eta| = KM^{\frac{1}{2}}.$$

Important in this is the temperature at which the viscosity is measured. This must be done at the extrapolated critical miscibility temperature Θ for the polymer with a poor solvent, and it is determined in the following way. First, the precipitation temperatures of solutions of polymers must be determined. These temperatures T_c are determined for a series of fractions of the same polymer, and the T_c values are plotted against the reciprocal of the square root of the molecular weight $M^{-\frac{1}{2}}$. These plots give straight lines, and extrapolation to the ordinate renders the desired temperature Θ. In order to simplify the nomenclature and avoid con-

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fusion, Fuoss has suggested naming this Θ value the *Flory point*, and the constant K the *Flory constant.*

Cragg *et al.*[35] suggested that the molecular weights of flexible linear polymers be determined in poor solvents instead of good ones. When the viscosities are measured at the Flory point of a particular solute-solvent system, the relation $|\eta| = KM^{\frac{1}{2}}$ holds. Cyclohexane is a poor solvent for polystyrene, and the Flory point for this system is 34.3° C. The flexible macromolecules in such a poor solvent are strongly coiled. The interactions between the solute and solvent are minimal. It was shown by Cragg and associates that the system then is something like a spherocolloid. The reduced viscosity depends very little on shear, and also very little on concentration. Generally, such a system is more "ideal" than if a good solvent were used. The only hazard would be the possibility of association in such poor solvents. Slight association among spherical molecules, however, does not affect the viscosity appreciably.

Flory and Fox showed that the constant K in equation (4) depends on the molecular weight of the linear polymer, and on the mean distance L between the ends of the kinked chain molecule:

$$K = F(L^2/M)^{3/2}. \tag{5}$$

In this equation (5) F represents a constant which is the same for all linear polymers in any solvent.

$$F = 2.1 \cdot 10^{21}.$$

When the molecular weight M and the intrinsic viscosity are known, and the interaction constant α is also determined, the values of L can be calculated from (4) and (5). Flory and Fox [34] have presented such calculations for polystyrene and polyisobutylene (see Chap. 17).

Polyvinyl pyrrolidone is another linear polymer which has been studied by means of viscosity, light scattering and other methods. It was concluded[36] that the molecules of this polymer in solution are randomly kinked chains encompassing immobilized liquid. For a sample of $M = 249{,}000$ the mean diameter of the molecules was found to be 360 A; for a higher molecular fraction of M about a million, the diameter was 930 A.

15. Flexibility and Length of Linear Macromolecules

It was found that the flexibility of dissolved linear macromolecules depends also on their original length, i.e. on the molecular weight.

Solutions of nitrocellulose and of methylcellulose have been thoroughly studied in this respect. Mosimann investigated various fractions of nitrocellulose dissolved in acetone[37]. Viscosity, streaming birefringence, and sedimentation in the ultracentrifuge were studied for very high molecular ($M = 613{,}000$) and low molecular (down to $M = 6{,}200$) fractions. The results are presented in Table 22.

TABLE 22

FLEXIBILITY OF NITROCELLULOSE MOLECULES IN ACETONE. LENGTH IN ANGSTROMS

Sample	M	*Length, calculated for completely stretched molecule*	*Length from sedimentation in ultra-centrifuge*	*Length from viscosity*	*Length from streaming bire-fringence*
I	613,000	1190	435	560	365
II	199,000	386	224	306	198
III	80,000	153	138	148	105
IV	30,000	58	54	75	
V	6,200	13	14	15	

The length for the completely stretched chains was calculated from X ray data. The actual length in acetone was calculated from viscosity data according to a theoretical relation developed by Burgers[38]. It is noteworthy that the viscosimetric method gave nearly the same values for length as the ultracentrifuge and streaming birefringence. Moreover, it is remarkable that for the very large molecules ($M = 613{,}000$) the experimentally obtained length is smaller than that which was calculated for the completely stretched chain. This indicates that the long molecules are not like straight rods but that they may resemble loops or spirals in solution. In the case of the relatively shorter molecules, however, the stretching seems to be complete. These shorter molecules can be visualized as having the shape of a bristle hair.

Hydrodynamic theories correlating the viscosity, diffusion and streaming birefringence with the flexibility and coiling of linear macromolecules have been developed by Huggins, Brinkman[39], Debye and Bueche[40], Kirkwood and Riseman[41], Kuhn[42], and Peterlin[43].

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Anomalous Flow Properties of Colloidal Solutions

16. Non-Newtonian Systems. The Influence of the Temperature

It was observed long ago that many colloidal solutions do not follow the law of Poiseuille (see equations 1 and 2). According to this law the flow time should decrease proportionally if the pressure is raised. In many instances this is not the case. The viscosities of moderately concentrated solutions of linear colloids will appear different when measured at different pressures in a capillary viscometer. Similar differences will show up also if the viscosity of such systems is measured in a rotating cylinder viscometer at different speeds of rotation. Such systems are called non-Newtonian because they do not behave in accord with the Newtonian concept that viscosity is independent of shear stress. For the characterization of such anomalous systems it is expedient to determine the viscosities at various stresses.

The flow properties of linear colloids depend on temperature, concentration, and on other variables. The solutions of linear colloids become Newtonian if the temperature is sufficiently high. The anomalies in flow properties disappear when the temperature is raised to about 50–60° C. This indicates that the anomalies are caused by orientation (and even association) of the asymmetric particles, since thermal motion counteracts against such orientation (and association).

The anomalies also depend on the concentration of the colloid. They appear even in spherocolloids if the concentration is raised over 3–5%, and if the temperature is moderate (such as room temperature). Linear colloids show these anomalies in much lower concentrations of about 0.5 or even 0.1%, depending upon temperature. Moreover, the appearance of flow anomalies depends upon the length of the macromolecules. The higher the molecular weight of linear particles the more likely that anomalies will show up, even at very low concentrations of the colloid.

Fig. 52 shows how the specific viscosity changes with the shear stress (velocity gradient). A polystyrene of molecular weight 600,000 was used. It was dissolved in tetralin, and the viscosity was measured at 20° C. It is obvious that there was little change of viscosity with stress only when the concentration of the colloid was very low (0.01–0.05%). At a concentration of 0.2% the curve turned up, when the stress was decreased. At a high shear stress (high pressure

in a capillary viscometer) the viscosity appeared lower than at a low stress.

The dependence of the deformation on stress applied has been studied not only with diluted linear colloids but also with more concentrated systems. Even gels, oils, greases, and pastes are studied by this approach, as the data appear to be important for the characterization of such systems. The branch of colloid science including such studies is called *rheology*.

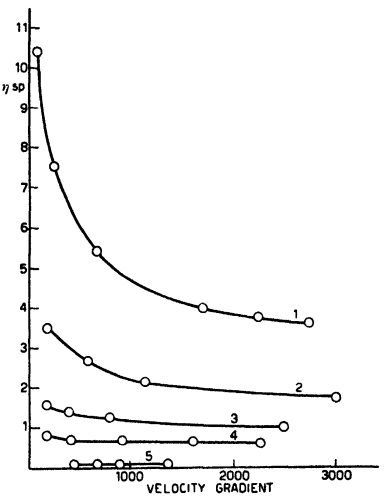

Fig. 52. The dependence of the specific viscosity on the velocity gradient at 20° C for polystyrene in tetralin (after H. Staudinger). The various curves represent the polymer of various concentrations; curves 1, 0.40%; 2, 0.20%; 3, 0.10%; 4, 0.05%; 5, 0.01%.

The decrease of viscosity exhibited by increased shear stress is caused by orientation along stream lines only, if the particles are rodlike. Matted coils can be oriented only after they are first stretched out. This seems to happen to a certain degree at very high shear stresses[44]. For example, polyisobutylene (dissolved in petroleum ether) was subjected to very high shear stresses, and it was shown by streaming birefringence studies that a certain stretching does indeed take place. The molecules then are oriented along the stream lines to a certain degree.

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17. Gelation Phenomena. Thixotropy

The viscosity anomalies of relatively concentrated colloids are caused not only by orientation phenomena, but by the association of particles in clusters. The more asymmetric the particles the more likely that they will meet each other and eventually become linked. The solution thereby may even set. The conditions for such association and formation of structures throughout the solution are favorable if the temperature is sufficiently low, and if the system is not mechanically disturbed. The viscosity increases enormously upon such internal structuration of a solution. Such aggregates, however, are not stable, and the structures may be destroyed by a slight

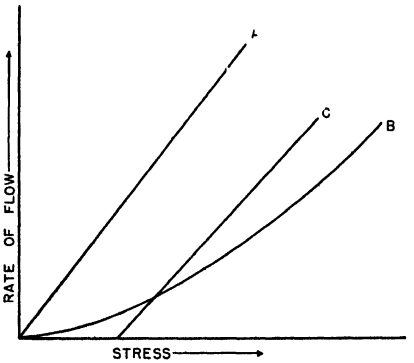

Fig. 53. The dependence of the flow rate on the driving pressure (stress) for three different systems.

increase in temperature, or by shaking. H. Freundlich was one of the first who investigated these phenomena. It was observed that many colloidal solutions set when left undisturbed, and that the jelly can be liquified again upon vigorous shaking. Such reversible sol-gel transformations were called thixotropic, and the phenomenon itself thixotropy.

Since the viscosity in these instances is not constant, instead of viscosity the terms *consistency* or *apparent viscosity* were proposed. The consistency usually decreases with increasing shear stress. Non-Newtonian systems are characterized by measuring this consistency at various stresses, e.g. measuring the flow rate at various pressures. If flow rate is plotted against stress, the following dependencies may result in various cases (Fig. 53). For Newtonian liquids the

flow rate will increase linearly with the stress (case A), and the line will run through the origin of the coordinates. In the cases of non-Newtonian systems (curve B), however, the rate will be very low at low stresses, and will increase strongly when the stress is raised. Thixotropic systems show such behavior, since high stress causes disruption of the weak structures, the consistency decreases, and the flow rate increases. Finally, case C illustrates the so called *plastic flow*. It may happen that there will be no flow at all if the stress is low, and the consistency will then decrease only upon applying a certain minimum stress. The minimum stress required to produce such plastic flow is called the *yield value*[45].

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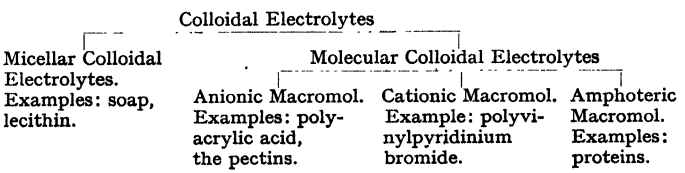
CHAPTER 10

Organic Colloids in Electrical Field

With respect to electrical properties, all organic colloids can be classified into two main groups: non-conducting colloids, and colloidal electrolytes. In non-conducting colloids the solvent (dispersion medium) is a nonpolar lipophilic liquid, such as hexane, benzene, or chloroform. Solutions of rubber or polystyrene in benzene are typical examples of such colloids. Well purified nonpolar colloidal solutions do not conduct electricity, and they will not be treated in this chapter.

1. Classification of Colloidal Electrolytes

The colloidal electrolytes can be classified, first, into micellar and molecular colloidal electrolytes. The molecular colloidal electrolytes themselves can be divided further into anionic macromolecules, cationic macromolecules, and amphoteric colloids, as illustrated in the following chart.



As any classification, this is not complete. Low conductivity values have all dispersions in aqueous, alcoholic or similar ionizing media. Moreover, electrical properties, though not very pronounced, can

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be observed with many molecular colloids which are not genuine colloidal electrolytes, such as glycogen, nitrocellulose, or polyvinyl-pyrrolidone. The weak conductivity observed in these cases may be due either to some impurities or to a few dissociable (ionogenic) groups of these macromolecules. In the case of synthetic linear polymers, such as polyvinyl-pyrrolidone or polyvinyl alcohol these may be the end groups. This is certainly true for the polyesters which have an ionogenic carboxyl group at one end of each molecule. At moderate or high degrees of polymerization, however, the influence exhibited by these end groups is very weak, therefore, these macromolecules are not included in the class of colloidal electrolytes. The conductivity and other electrical properties in these cases, as well as in the true colloidal electrolytes, depend very much on the properties of the solvent. The higher the *dielectric constant of a liquid* the greater its ionizing power for any ionogenic molecules introduced. Hence in aqueous colloids the electrical properties will be more pronounced than in such solvents as alcohol, acetone or ether.

All the colloidal electrolytes mentioned in this chapter will be *colloidal polyelectrolytes*, i.e. each micelle or macromolecule carries *many* ionogenic groups.

2. Conductivity

Conductivity (or conductance) of solutions is measured by using low intensity alternating current of high frequency, and comparing the unknown resistance of the solution with a known resistance. *Specific conductivity, k,* is the reciprocal of the specific resistance and is expressed in reciprocal ohms or mhos. (The specific resistance is the resistance in ohms of a column of substance 1 centimeter deep and 1 cm^2 in cross section[1].) *Equivalent conductivity*

$$\lambda = 1000k/c,$$

where c denotes the concentration of an electrolyte in gram equivalents per liter.

It was found experimentally that the conductivity of various colloidal solutions varies widely, depending on the degree of purity as well as on the kind of dispersed phase. For example, well dialyzed solutions of starch or proteins had a lower conductivity than undialyzed solutions. It was realized long ago that low-molecular electrolytes, which are present as impurities in practically all

hydrosols, contribute greatly to the conductivity. When they are removed by dialysis, the conductivity decreases considerably. For this reason, the conductivity measurement is a very important means of *testing the purity* of colloidal solutions. Of course, only the presence of extraneous electrolytes can be determined by this method, since the presence of non-electrolytes, such as alcohol, will not affect the conductivity.

Very important was the observation that in some hydrosols the conductivity could be brought to low values upon continued dialysis, while in some other cases the most highly purified colloids still conducted well. Examples of the first type are solutions of glycogen, starch, and polyvinyl alcohol. The conductivity of these colloids, if they are well dialyzed, is close to that of distilled water. The soap colloids, on the other hand exhibit considerable conductivity, even if they are well dialyzed. This proved that the *colloidal particles themselves are charged and contribute significantly to the conductivity.*

Soaps, synthetic detergents, and other micellar colloids were studied extensively by McBain and his associates, Lottermoser, and others[2]. The equivalent conductivity was measured at various concentrations, and the following interesting observations were made. When the equivalent conductance is plotted against concentration, it is almost constant at low concentrations. At these low concentrations the soap behaves as a strong electrolyte which, according to the modern concept of Debye, Hückel and Onsager, is fully dissociated[3]. In this very low concentration region, the conductivity decreases linearly with concentration, this decrease being expressed by the linear equation of Debye, Hückel and Onsager:

$$\lambda_0 - \lambda = (A + B\lambda_0)c^{\frac{1}{2}},$$

where λ_0 is the equivalent conductivity at infinite dilution, and A and B are constants. There are no particular anomalies at these concentrations, and it can be assumed that any of the compounds investigated, e.g. dodecyl sulfonic acid, is present under these circumstances in the form of simple small ions. Upon further increase in concentration, however, the conductivity drops abruptly (Fig. 54). This is the critical concentration (see p. 60) of micelle formation. This critical concentration decreases, as the hydrophobic chain is lengthened. At still higher concentrations the equivalent conductivity passes through a minimum and then remains approximately constant. The *specific* conductivity is rising with increasing

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concentration, and at very low concentrations the conductivity of all the electrolytes, regardless of chain length, approaches a common value (Fig. 55).

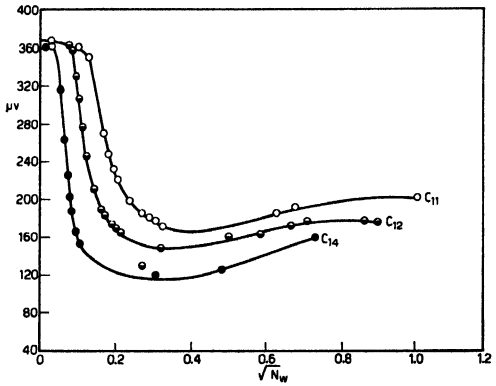

Fig. 54. The change of the equivalent conductivity with concentration, according to McBain. On the abscissa is plotted the square root of concentration (M/l). (From McBain, *Colloid Science*, D. C. Heath Publ., Boston, 1950, p. 243.)

Conductivity measurements have been very important in the study of micellar colloids. Important also was the observation that the *osmotic activity of the solute decreased at the critical point of micelle formation to a much greater extent than the conductivity*. This is illustrated in Fig. 56 for the solutions of sodium desoxycholate, a sodium salt of one of the bile acids. This is the best kind of evidence that at a certain concentration the molecules of the desoxycholate are associated into micelles. The anions actually are the associating entities, as the sodium cations remain free regardless of whether the anions are associated or not. Hence the decrease of osmotic activity far below the decrease of equivalent conductivity is explained simply by the association of the anions. The same is true for all other colloidal electrolytes of this type. In the case of cationic detergents, such as tetradecyl-trimethyl-ammonium bromide, the tetradecyl-trimethyl-ammonium cations are "pushed out" of the solution at a certain critical concentration, e.g. they cling together forming micelles; the bromine anions remain free. The osmotic activity again decreases more than the conductivity. This indicates that the *number of particles* (on which the osmotic activity depends) has decreased more than the *number of charges*. Hence, the charged sites of the associating ions must remain on the outside of the

micelle. This is in agreement with the concept of the structure of micelles as outlined in Chapter 5 (see also Chapter 34).

It might be worthwhile to consider the situation in a dilute solution of soap before and after micelle formation. Before the micelle formation the anions $R{\cdot}COO^-$ and cations Na^+ are distributed at random, each anion tending to hold several cations in its vicinity, and vice versa. After the micelles are formed, the anions of the soap lie in ordered structures, the lipophilic 'tails' turned inside the micelle, and the polar 'heads' being turned outward toward the water. The sodium 'counter ions' (gegenions) will now stick in the vicinity of these polar 'heads' which are the carboxyl ions. Hence the whole system becomes more organized, i.e. its entropy decreases. The charge density is very high on the surface of the micelle, is low in the spaces between the micelles, and is zero inside them.

The *macromolecular polyelectrolytes* resemble micellar colloids to a certain degree. The conductivity of macromolecular polyelectrolytes was studied by Fuoss and colleagues[4], and some of their results are presented in Fig. 57. On the abscissa is plotted the square root of the concentration, on the ordinate the equivalent conductivity of sodium polyacrylate and polyvinyl-N-butylpyridinium bromide. There is no abrupt decrease in equivalent conduc-

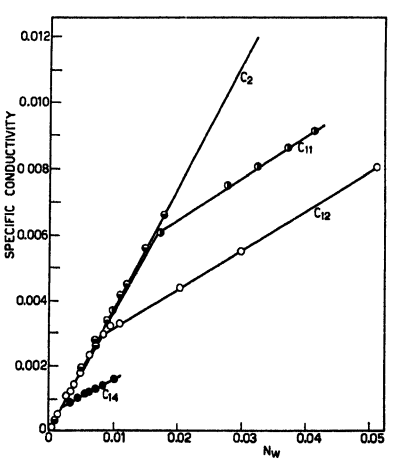

Fig. 55. The dependence of the specific conductivity on concentration, according to McBain (ref. see Fig. 54).

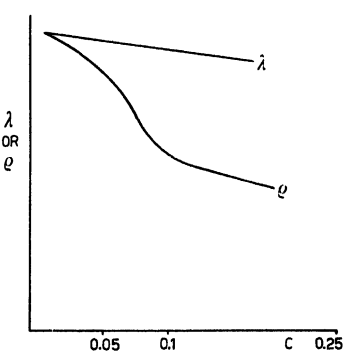

Fig. 56. Comparison of osmotic pressure (ϱ) and equivalent conductivity (λ) of sodium desoxycholate according to McBain and Johnston.

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tivity at any given concentration, hence *no micelles are formed* in the solutions of these polymers. Generally the conductance curves of such polyelectrolytes resemble those of strong electrolytes in solvents of low dielectric constant. These polyelectrolytes also differ considerably from micellar electrolytes in the distribution of the

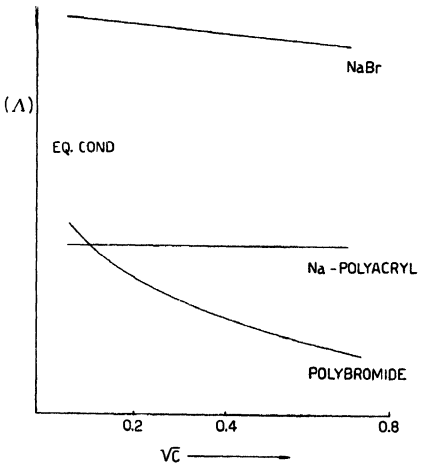
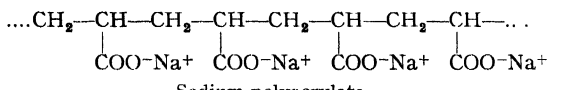
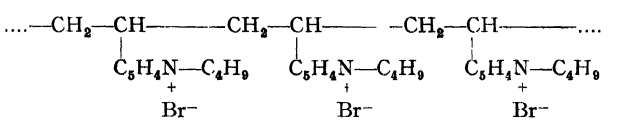


Fig. 57. The dependence of the equivalent conductivity on the square root of concentration of sodium bromide, sodium polyacrylate, and polyvinyl-N-butylpyridinium bromide (according to Fuoss and associates).

charges. The molecules or polyions of these substances have the following structure:



Sodium polyacrylate



Poly-(4-vinyl-N-*n*-butylpyridinium) bromide

Relatively short molecules of such polymers (mol. weight 5000–20,000) will be stretched out, but very long chains will coil up to a certain degree, especially when the ionization is decreased. The charges in these cases are distributed evenly over the whole mole-

cule, but when coiling occurs a certain fraction of the counter ions will be trapped inside the coil.

This, according to Fuoss, explains the conductivity resemblance of these polyelectrolytes to ordinary weak electrolytes. The gegenions are not as free as in micromolecular electrolytes. Because of the relative immobility of the macro-ions, the small gegenions will not be able to move quite as freely about in the solution, but will be held close (or even inside) the large macro-ions by electrostatic attraction. This was confirmed by Wall and associates[5] who measured the rate at which counter ions in the bulk of a solution exchange with added radioactive ions. It was found that the counter ions can be divided into two categories: those belonging to the ionic atmosphere around the polyacrylate ion which exchange very rapidly, and those trapped inside the coiled polyion which exchange slowly.

It is also worthy of mention that the osmotic activity of sodium polyacrylate in salt-free solution is determined to a close approximation by the sodium ions only. The macromolecular anions contribute practically nothing to the osmotic pressure. This explains the fact that the osmotic pressure of sodium polyacrylate is independent of the molecular weight of the polymer[6]. Moreover, the osmotic activity of the sodium ions is lower than expected, because the freedom of the sodium ions is restricted by the large polyvalent anions.

3. Electrophoretic Phenomena

The movement of small particles under the influence of direct current was observed more than hundred years ago by Reuss. This phenomenon, *electrophoresis*, is important in the study of electrically charged organic colloids, especially the proteins. A colloidal solution as a whole is electrically neutral, like any solution of an electrolyte. The number of charge units carried by the particles is equal to the number of opposite sign charges carried by the counter ions.

The simplest method of studying electrophoresis is to introduce the colloid into a U-shaped tube, immerse electrodes into the solution, and connect the device into a direct current circuit (Fig. 58). If the particles are colored, their movement can be observed easily. The concentration of the particles increases at the one electrode and decreases at the other electrode. Many qualitative and semiquantitative observations with various colloids were made

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in the 19th century and in the beginning of this century by Quincke, Picton, Linder, Burton, Svedberg, and others. It was found that most natural colloids move to the anode, hence the particles must be negatively charged. A very important discovery was made by Hardy in 1899. He noticed that the particles of proteins may be either negatively or positively charged depending on the acidity of the solution. One of the first improvements in the techniques was to connect the colloid with the electrodes by means of a salt solution. Attempts were made to measure the migration at different voltages.

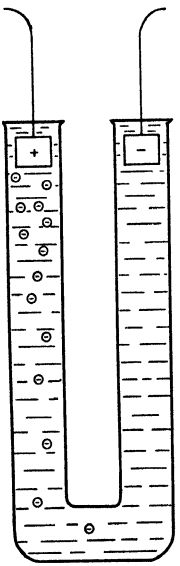

Fig. 58. Electrophoresis of negatively charged particles.

Substantial experimental improvements in electrophoretic technics were introduced by Svedberg and Tiselius in 1926. In order to detect better the migration of the colorless proteins, they introduced fluorescence photography. The fluorescence was caused by irradiation with ultraviolet light, and the intensity of fluorescence was measured at different points of the electrophoresis cell. The position of the moving boundary thus could be established with greater precision than before. Improvements in the construction of the cell were also made. One of the most difficult problems was to find a suitable way of producing a sharp initial boundary between the protein solution and the conducting buffer solution. Moreover, great difficulty was experienced in avoiding disturbance of the boundary by convection during electrophoresis. The development of the method was continued and in 1937, Tiselius[7] described a new electrophoresis apparatus in which the electrophoretic mobility of components in mixtures could be measured. The individual boundaries were followed by Toepler schlieren method, which had been used earlier by Lamm for diffusion measurements. Also, the U tube was divided into compartments to allow the isolation of the components separated by the electric field. Although more improvements, especially in the optical method of precise measurement of the boundary shift, were introduced later by Svensson, Longsworth, and others, it is essentially in the form that the apparatus was described in 1937 by Tiselius that it is used today.

The electrophoresis tube in the Tiselius apparatus is rectangular in

cross section and is composed of several sections which can be made to slide one over the other (Fig. 26), thus separating the contents of the tube. The protein solution to be tested is first dialyzed against a buffer of definite pH, in order to maintain the ionization of the protein, and a certain ionic strength. The protein then is poured into the lower compartment, and the buffer into the upper. The cells are connected with the electrode compartments which are large tubes containing a concentrated chloride solution in which are immersed reversible silver–silver chloride electrodes. The cell and the electrodes are immersed into a constant temperature bath which is operated at the temperature of maximum density for the buffer solution in order to minimize the disturbing effects of convection. (The current causes heating in the solution, and the heat exchange with the surrounding of the cell causes a certain temperature gradient in the cell.)

The migration of the charged components is observed by means of optical methods based on the measurement of refractive index gradients. The schlieren scanning method according to Longsworth, and the cylindrical lens schlieren system according to Philpot and Svensson are the most common methods. A variety of instruments have been designed by various companies; for example, the Klett-Tiselius instrument has all the essential elements mounted on an optical bench resting on concrete.

The basic features of the optical schlieren method, as applied to the study of electrophoretic phenomena, may be illustrated with the aid of the diagram shown in Fig. 59 taken with the following explanations from the paper by Longsworth and MacInnes[8].

"The image of the slit, S, illuminated by the lamp L and condenser C, is brought to focus in the plane P by the lens D. The

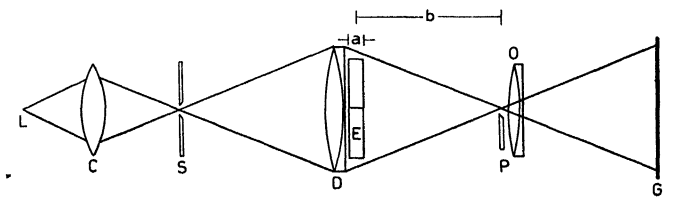

Fig. 59. Observation of boundaries with the schlieren method. L, 5-volt, 18 ampere ribbon filament lamp; C, projection condenser; S, 0.3 × 15 mm horizontal slit; D, Dallmeyer portrait lens 6 *D*, 4 in. diameter, 24 in. focal length; E. electrophoresis cell; P, schlieren diaphragm; G, groundglass or photographic plate; O, Dallmeyer R. R. lens, 3 in. diameter, 24 in. focal length.

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schlieren diaphragm, a screen with a sharp, horizontal upper edge, is placed in the plane P and may be moved vertically, a micrometer adjustment being used. The cell C, in which the electrophoresis is carried out, is placed as near the lens D as the thermostat construction permits. The camera objective O, placed immediately behind the schlieren diaphragm, is focussed on the cell and forms a fullsize image on a ground-glass or photographic plate at G."

"In the absence of refraction gradients in the electrophoresis cell all of the light is brought to focus in the image of the illuminated slit at P and enters the camera objective. If, however, a boundary is present in the cell the refraction decreases with increasing height through the boundary, and the pencils of light through this region are deflected downward. These deflected pencils are intercepted by the schlieren diaphragm and fail to reach the screen. Thus the region at G conjugate to the boundary appears as a dark band on a light background." A more compact and refined instrument is offered by the Beckman Corporation, Specialized Instruments Division (Belmont, Calif.) (Figs. 60 and 61).

The Tiselius method proved to be one of the most valuable tools

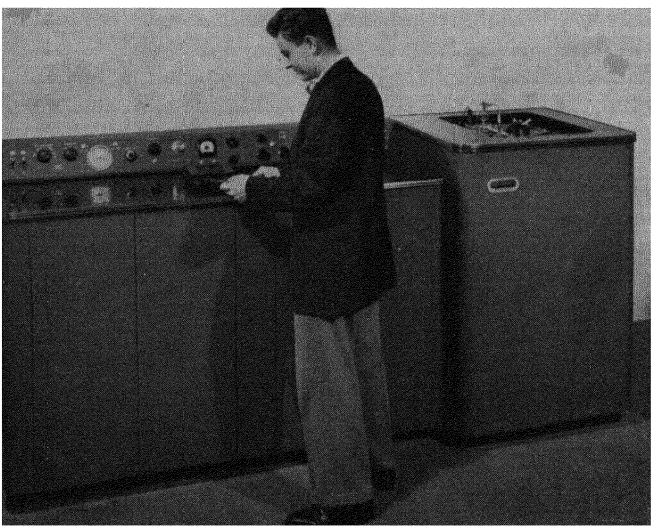

Fig. 60. Spinco Model H electrophoresis instrument.

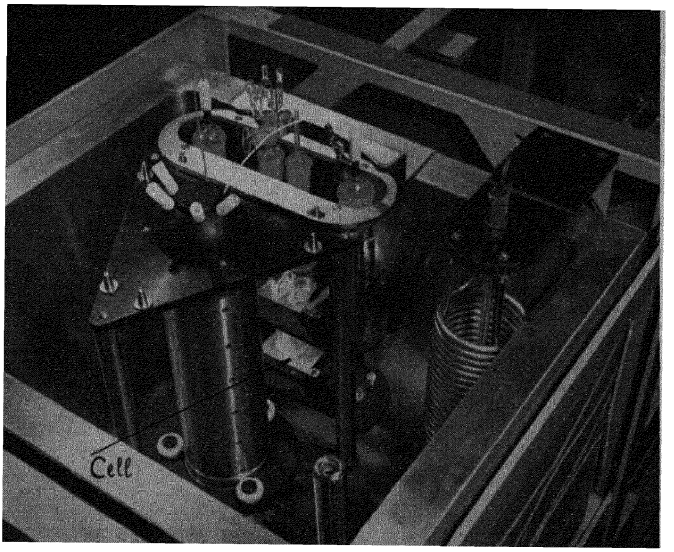

Fig. 61. The interior of the electrophoresis compartment of the Spinco Model H instrument.

in the study of proteins and other colloidal electrolytes. Moreover, the same instrument, without the application of current, has proven quite suitable for the measurement of diffusion. By changing the size and design of the cell, the instrument can also be adapted easily for preparative purposes. Proteins usually are mixtures of many components which have different mobilities, hence after a sufficiently long period of migration the components can be separated more or less completely. A great variety of electrophoresis instruments have been designed for preparative purposes by other authors. They differ from the Tiselius device chiefly in that the cell is composed of a greater number of compartments. For example, the apparatus of H. Theorell (1934) has eight compartments on each side of the U-tube; the compartments are short cylinders with ground glass joints, and the contents of each compartment can be separated by properly adjusted thin discs[9].

The method of Tiselius is most important as an analytical tool, especially for the *characterization of closely related proteins*. In many cases proteins appearing homogeneous in the ultracentrifuge were shown by electrophoresis to be composed of several components.

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The presence of electrophoretically different components is detected by the cylindrical lens system in the form of several peaks representing the refractive index gradients of each component. There are, for instance, more globulin peaks in the electrophoresis pictures of blood plasma than on the sedimentation pictures obtained by the same optical method. This indicates that the globulins which appear essentially homogeneous in sedimentation are not homogeneous in electrophoresis. The molecules may be of the same size but some of them carry higher net charge at a certain pH than others.

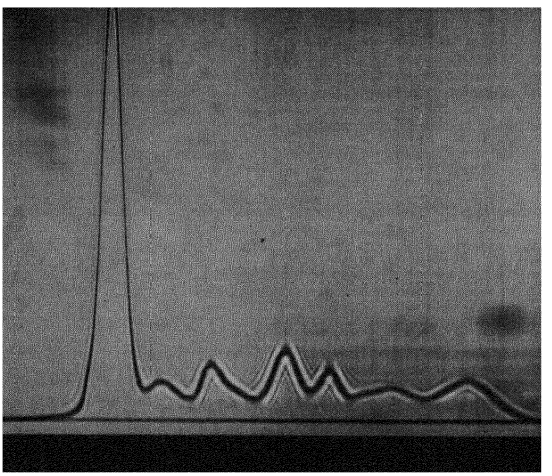

Fig. 62. Electrophoresis diagram obtained by means of the Tiselius method with the Spinco Model H instrument. Human plasma (approx. 1.15% protein), the high peak is that of albumin.
(Courtesy Spinco Division of Beckman Corporation, Belmont, Calif.)

Moreover, not only can the presence of different components be demonstrated, but the *amounts* of each component can also be estimated by measuring the areas under the peaks obtained by photography. An example of an electrophoresis diagram of human blood plasma is presented in Fig. 62.

The mobility of a protein molecule differs greatly depending on the acidity of the solution, as noted by Hardy. This is due to the

amphoteric nature of the proteins. The macromolecules of proteins contain the acidic carboxyl groups and the alkaline amino groups (together with other groups of lesser acidic or alkaline nature), and the net charge of the molecule will depend, primarily, on the *chemical composition* of the protein. Proteins composed predominantly of the dicarboxylic acids will be more acidic, as they will contain more carboxyl groups than amino groups. Likewise, those having an excess of diamino acids will be basic (Chap. 25 and 26). Those having an excess of free carboxyl groups, when dissolved in water, will have a negative charge, whereas those with excessive amino groups will be positive, as shown in Fig. 63. Most proteins contain a slight excess of carboxyl groups, and hence carry a weak negative net charge in neutral solutions. If a little alkali is added

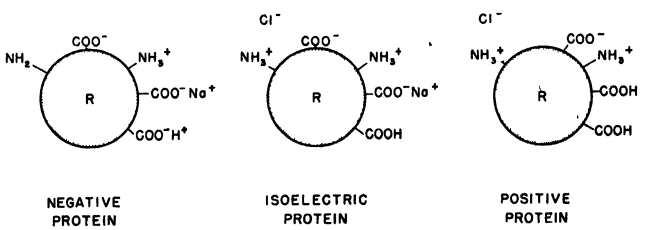

Fig. 63. Schematic presentation of negative, isoelectric, and positive protein particles.

to such a protein solution, the net charge increases, since the dissociation of the carboxyls is increased (and the dissociation of the amino groups is diminished):

$$R\begin{smallmatrix}\diagup\mathrm{COOH}\\ \diagdown\mathrm{NH_2}\end{smallmatrix} + \mathrm{NaOH} = R\begin{smallmatrix}\diagup\mathrm{COO^-Na^+}\\ \diagdown\mathrm{NH_2}\end{smallmatrix} + \mathrm{H_2O}.$$

Upon the addition of acid, however, the dissociation of the carboxyls will be suppressed and the dissociation of the amino groups will be promoted:

$$\mathrm{HOOC{-}R{-}NH_2 + HCl = HOOC{-}R{-}NH_3^+ + Cl^-}.$$

Consequently, the protein molecules in acidic solution will acquire a positive net charge. As counter ions in this case are the anions of the added acid, whereas in alkaline solution the counter ions are the cations of the added alkali. It is conceivable that at a certain definite pH or acidity a protein may have an *equal number of positive and negative charges on its molecules.* This is the so-called

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isoelectric condition. As the number of plus charges equals the number of minus charges on each molecule, the *net charge* at the isoelectric pount is *zero.* Hence, at a certain pH corresponding to the isoelectric point the protein molecules will not move under the influence of a direct current.

The number of free carboxyl groups and free amino groups in the proteins is estimated indirectly by the amino acid analysis (Chap. 25 and 26), and directly by potentiometric titration of a well purified preparation which is dissolved in water. From the number of equivalents of acid or alkali used the number of the free alkaline and acidic groups can be estimated. If now the electrophoretic mobility of proteins is compared at a certain pH, those possessing a higher net charge will move faster, provided the molecular size and shape are similar. An alkaline sodium barbital (veronal) buffer of pH 8.6 is the most commonly used solvent in testing the electrophoretic mobility of proteins, and at this pH the albumin of blood plasma has a considerably higher mobility than the other proteins present (Fig. 62).

The electrophoretic mobility is defined as the velocity of the particle in a potential gradient of one volt per cm. For most proteins the mobilities are between $10 \cdot 10^{-5}$ and $-10 \cdot 10^{-5}$ $cm^2 \cdot volt^{-1} \cdot sec^{-1}$ depending on the nature of the protein, the temperature, and on the pH of the solution. For example, the mobility coefficients of all plasma proteins at pH 8.6 are negative (the proteins move to the positive pole), and their values are as follows: albumin 5.9. α_1-globulin 5.1, α_2-globulin 4.1, β-globulin fraction 2.8, fibrinogen 2.1, and the γ-globulin fraction approximately 1.5, all these coefficients being multiplied by 10^{-5}. Attempts to correlate the mobility quantitatively with the net charge and molecular size and shape have been moderately successful. The theory would be very simple, if the protein molecules were rigid unsolvated spheres without the atmosphere of counter ions around them. In such case the mobility u would be proportional to the charge q and inversely proportional to the frictional force f. The frictional force f can be expressed either as $f = RT/D$, where D is the diffusion constant, or as $f = 6\pi r\eta$, where r is the radius of the sphere and η the viscosity of the medium. The charge q may be expressed in terms of the number of elementary charges ε (the charge carried by one electron), the number of chemical equivalents e (e.g. equivalents of acid bound per gram of protein) and molecular weight M: $q = eM\varepsilon$. Introducing these expressions[10] for f and for q, the following equation is obtained for

the dependence of the mobility u (in a unit field) on the number of net charge and molecular weight:

$$u = eM\varepsilon/6\pi r\eta . \tag{1}$$

This would be applicable to an idealized case where there is no ionic atmosphere around the moving particles. As the latter really exists, the equation (1) must be supplemented by further terms. The ionic layer screens the particles from the external electric field, and it also produces the movement of the liquid medium in the direction opposite to that of the particles. Theoretical studies of Debye, Hückel, Henry and others showed that these effects can be accounted for by introducing a factor k, which is the reciprocal of the "thickness" of the ionic layer, and a factor a (closest approach of the counter ions to the particle). Moreover, Henry introduced a function $F(ka)$ which approximates unity when the product $k \cdot a$ is small, but which approaches 3/2 for large values of $k \cdot a$. For spherical molecules generally then:

$$u = eM\varepsilon \cdot F(ka)/6\pi r\eta\,(1 + ka) . \tag{2}$$

Since the value of k and a cannot be measured by a reliable method, equation (2) can be applied only by making simplifying assumptions[11].

It is interesting that the particle size parameters M and r appear in equations (1) and (2) in both numerator and denominator. This indicates that for spherical particles the mobility should not depend on particle size, if the *charge density* on the surface of the particles remains the same. This was verified experimentally by observing the mobility of large particles of glass, carbon, or silica coated with a layer of protein[11], directly in a small cell under the microscope. The mobilities calculated for these large particles were essentially the same as those for the protein molecules at the same pH. The molecular shape, however, has a definite influence on the electrophoretic mobility, as asymmetric particles have greater resistance to flow than spherical particles.

4. Electrophoresis on Paper

The method of free boundary electrophoresis is of great value for the study of colloidal electrolytes, though it is a complicated and expensive method. Moreover, using even the smallest cells, relatively large amounts of material are needed. In the search for a suitable

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micromethod, it was found that ordinary filter paper is a suitable supporting material for electrophoresis on a micro scale. Only one drop of a test solution of suitable concentration is needed for this method. Since the migration occurs in a framework of fibers, no precautions about the blurring of the boundary are required. Moreover, the method is rapid and inexpensive. It is now becoming a standard procedure in all laboratories dealing with analytical testing of proteins and similar substances[12]. A strip of filter paper is impregnated with a buffer solution and placed on a support of glass or plastic. The ends of the paper are immersed into the buffer which is connected with the electrode compartment. Between 0.01 and 0.03 ml of a protein solution are placed on the paper at a certain position marked in advance with pencil. Direct current is applied across the length of the strip, and the electrophoresis is continued 6–20 hours. The paper is then removed and dried, and the positions of the proteins are revealed by developing the strip, i.e. staining with a suitable dye. (This staining substance must of course selectively stain the protein, not the whole strip.) After the excessive dye has been washed out of the strip, the positions of the proteins appear as colored spots. When an alkaline buffer is used, all components of a certain protein mixture will either move to the positive electrode or stay near the point of origin. Those with the highest mobility will appear farthest from the origin, and the others will show up in the order of their mobilities. The particular components can then be recognized best by comparison with a standard material run at the same time and under the same conditions.

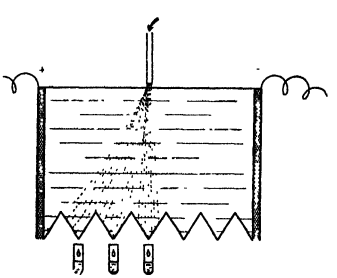

Fig. 64. Electrophoretic separation according to Grassmann.

Although this micromethod is less accurate than the free boundary method for the determination of mobilities, it serves well for many purposes as a semiquantitative method. The amounts of the separated components can be estimated either by cutting the paper, extracting the dye, and determining it colorimetrically, or by direct measurement of the color on the strip by means of a densitometer.

According to Grassmann[13], the paper electrophoresis method can be used also for preparative purposes. A sheet of paper is stretched in a vertical position between two vertical electrodes. The buffer

and the sample are applied continously at the top and travel down the sheet at the same time that the electrically charged components are being pulled to one side by the current (Fig. 64). The lower side of the sheet is serrated in a pattern which enables one to collect the dripping fractions in tubes. The paths of electrically charged dyes are directly visible on the sheet. The tracks of colorless proteins can be made visible by staining, if so desired. Electrically neutral components, of course, will descend vertically, and can be collected in the middle tubes.

Very sharp separation of protein components can be accomplished using *starch gels* as supporting media[14].

5. Electro-osmosis and Related Phenomena

The atmosphere of counter ions around the particles confers charge to the dispersion medium or solvent. The sign of this charge is opposite that of the particles. Hence, as the particles move toward the one pole the liquid tends to move toward the opposite pole. This can be visualized best by means of an apparatus in which the solid dispersed phase is held immobile, and the movement of the liquid is carefully observed. This can be done by a device as illustrated in Fig. 65. A tube is plugged in the middle with a porous material (wool, ion exchange resin), and filled with a dilute buffer solution. The positive and negative electrodes are on either side of the plug. As the current passes, the liquid will move toward one of the electrodes. This can be observed best if the ends of the tubes are drawn in the form of capillaries which can be calibrated in order to measure the volume of the liquid displaced. This phenomenon of electro-osmosis was observed long ago, and was studied quantitatively. It was found that the volume of the displaced liquid is directly proportional to the area of the diaphragm, to the applied potential gradient, and to the dielectric constant of the liquid; it was inversely proportional to the viscosity of the liquid, and to the distance between the electrodes. Moreover, the volume was directly proportional to the potential at the interface, diaphragm-liquid.

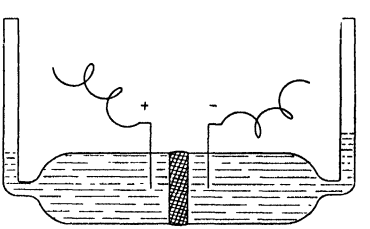

Fig. 65.
The phenomenon of electro-osmosis.

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This, so-called, *zeta* (ζ) *potential* depends on the *charge at the surface.* It is conspicuous that the effect of electro-osmosis is due to the same causes as electrophoresis: in a unit electrical field the electro-osmotic effect is determined by the charge at the interface and the resistance to flow.

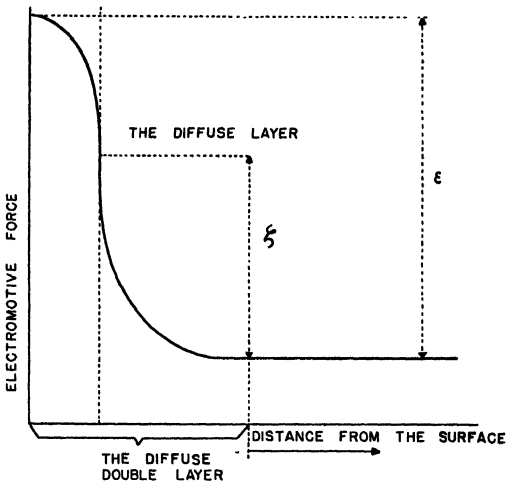

Fig. 66. The electrokinetic or zeta potential, and the electrothermodynamic potential.

Since the applied electrical force, dielectric constant, viscosity, and volume of the displaced liquid can be measured easily, the zeta potential can be estimated. It can be estimated likewise in electrophoresis, if the migration of the particles is measured. The zeta potentials of many solid-liquid systems have thus been calculated.

It must be pointed out that the electrokinetic or zeta potential must be distinguished from the electrothermodynamic potential. The latter is developed between the solid surface of any particle and the liquid, whereas the zeta potential is developed between the liquid and the fixed layer of the surface, as illustrated in Fig. 66[15].

The fixed layer is formed of the solvating liquid and ions which either genuinely belong to the surface or are adsorbed from the surroundings. This fixed layer moves with the particles in electrophoresis, and it stays with the solid plug in electro-osmosis. The thickness of this tightly bound layer is considered monomolecular, and it is considered part of the 'double layer'. The diffuse part of it extends into the liquid and contains the counter ions.

Electro-osmosis is frequently applied in industry, for example in facilitating the filtration of difficultly filterable slurries, pastes, and similar products. The separation of liquid from the mass is facilitated by applying direct current to a filter press.

There are several other quite interesting phenomena closely related to electrophoresis and electro-osmosis. Suppose that the direct current source in the electro-osmosis apparatus (Fig. 65) is disconnected and replaced in the circuit by a galvanometer. It would not show any current, if the liquid remains stationary in the tube. However, if the liquid is forced to flow through the porous plug, the galvanometer will indicate a current. This *streaming potential* is due to the displacement of the charges equilibrated in the double layer around the solid. The displacement of the liquid causes also some displacement of the counter ions. In electro-osmosis outside potential causes a movement of liquid; in forcing the liquid to flow, the movement causes streaming potential. Similarly, one may expect an opposite effect to electrophoresis: The movement of particles should cause a potential. This might be expected e.g. in sedimentation in the ultracentrifuge, where charged particles are displaced by an external force. This *sedimentation potential* is extremely difficult to measure, and so far very little investigation has been carried out in regard to this phenomenon.

The phenomenon of *electrodialysis* is of much greater practical importance than those just mentioned. It was found by Pauli, Morse, Pierce and Dhéré (1903–1915) that the dialysis of electrolytes can be greatly facilitated by the application of a direct current. For this purpose an apparatus as illustrated in Fig. 67 is used. The

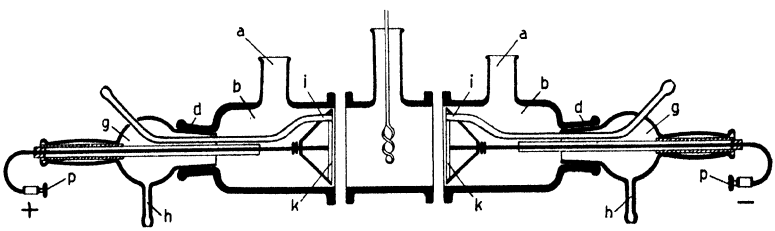

Fig. 67. Electrodialysis according to Pauli.

colloid to be purified is introduced into the middle section which is separated from the right and left sections by semipermeable membranes. Distilled water is circulated on both sides of the colloid,

and the removal of electrolytes is facilitated by direct current applied by means of platinum electrodes. The latter are placed in the water compartments near the membranes. Electrodialysis is also applied for some industrial purposes. For instance, whey can be liberated by this method from undesired salts. Such a method is used in the Netherlands. Whey is a waste product of cheese and casein factories, and its salt content would be too high for ordinary uses. By liberating whey from salts a valuable food material containing proteins and milk sugar is obtained[16].

An interesting modification of the electrodialysis technique is the *electrodecantation* process developed by Pauli[17]. When the colloid in the middle chamber of an electrodialysis apparatus is not stirred, *stratification* in layers can be observed. A layer rich in colloid will be found on the bottom, and a highly diluted layer on the top. This stratification is due to the density gradient formed at one of

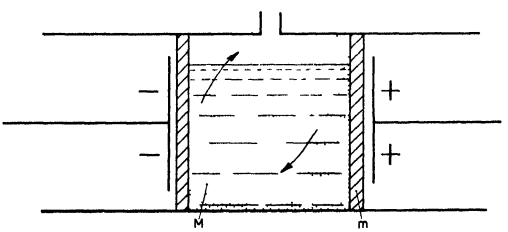

Fig. 68. Stratification of a colloid on electrodialysis without stirring.

the membranes. The colloid, because of its charge, will be pulled toward one of the membranes, hence the density near the membrane increases, and the dense solution sinks to the bottom (Fig. 68). According to Pauli, very pure and concentrated colloids can be prepared in this way, and the apparatus can be made to work continuously, by removing the dense lower layer and supplying more solution. It is obvious that only colloids with electrically charged particles will show this layer formation, as the particles must be caused to move in the electrical field. It is also conceivable that preparative separation of multi-component mixtures might be feasible. This idea was developed by Kirkwood, Cann, and colleagues[18] and is now known as the method of *electrophoresis convection*. A potential gradient is applied across a narrow vertical channel (Fig. 69) containing the mixture to be fractionated. The components with the highest mobility will reach the wall first, sink, and

become concentrated in the bottom compartment. The less mobile components will be enriched in the top container. Repeated runs give increasingly pure preparations. It was possible by this method to achieve the resolution of the gamma-globulins into many components.

Further development of this method is due to Polson[19] of Cape Town, South Africa. He found that separation by electrophoresis convection is accelerated in oblique channels. A multimembrane electrodecantation apparatus was developed which can be used for continuous separation of protein fractions. The supernatant from

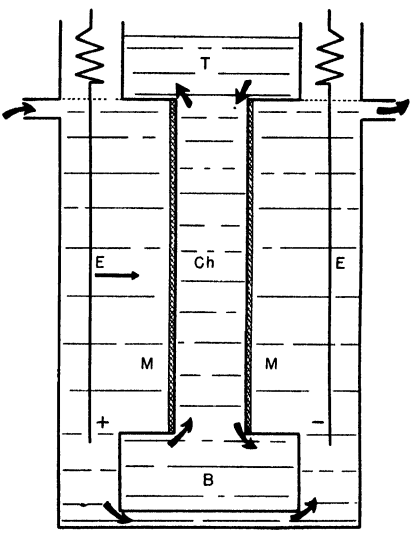

Fig. 69. Electrophoresis-convection cell according to Kirkwood and Cann. T – top reservoir; Ch – channel; B – bottom reservoir; E – electrodes; M – membranes.

the first separation section is passed into the bottom of the second, and the supernatant of this section is passed into the bottom of the third channel. Various fractions of the plasma proteins have been obtained in this way. Moreover, the method was successfully applied for the separation of trypsin from pancreas, and for the concentration of poliomyelitis virus from mouse brain.

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6. Orientation in an Electrical Field

The polar character of large molecules and colloidal particles can also be studied by measuring the dielectric constant of the solutions. Water possesses a high dielectric constant due to the polar nature and mutual orientation of the water molecules. The dielectric constant of solutions containing large polar molecules, such as proteins, is even higher than that of pure water. The determination is carried out by measuring the capacitance of a condenser containing the solution in question between the plates. The electrical field applied leads to orientation of polar molecules, whereby the capacitance is increased. Hence, the measurement gives information about the molecular orientation of molecules.

The studies of this subject indicated that the response of the molecules depends very much on the frequency of the alternating field. Orientation is hindered by the frictional force which tends to prevent the molecules from changing their positions, and by the Brownian movement which tends to disorient them. In a low frequency field both the large molecules and the water molecules will become oriented, and a high dielectric constant will be measured. If the frequency is increased over a certain limit, the large protein molecules will be too sluggish to follow the orienting torque, the measured dielectric constant will be smaller than that measured with a lower frequency, and it will increase in time as the orientation completes. Finally, at extremely high frequencies the large polar molecules will not be oriented at all. The dependence of the dielectric constant on the frequency is called *dielectric dispersion.* It was shown by Oncley[20] that it is possible to correlate the dielectric dispersion data with the molecular size, shape and hydration. The quantitative theory is somewhat complex, but it should be clear from the previous statements that the molecular orientation of the large polar molecules will depend on the size, shape, and hydration. Moreover, it must depend on the polarity which usually is expressed by the *dipole moment* (see Table 23).

It is interesting to note that the axial ratios of several proteins calculated from such data agree reasonably with the data obtained by other methods. For example, for egg albumin, assuming that the molecules are prolate ellipsoids of revolution the axial ratio 5:1 was obtained, for serum albumin 6:1, and for beta-lactoglobulin 4:1. These should be the axial ratios when hydration of the protein is neglected. Assuming a certain amount of hydration the axial

ratios will be even closer to those obtained with other methods. The dipole moments of proteins are very high, as they depend, among other things, on the molecular weight. For example, the dipole moment of myoglobin is 170 Debye units, and for serum albumin it is 380.

TABLE 23

DIELECTRIC CONSTANTS AND DIPOLE MOMENTS OF SOME COMPOUNDS

Compound	*Dipole moment, debyes*	*Dielectric constant*
Water	1.85	80
Methyl alcohol	1.68	34
Ethyl alcohol	1.70	26
Methyl amine	1.3	10
Ethyl amine	1.3	6 3
Diethyl ether	1.1	4.3
Diethyl glycol	2.3	41
Acetone	2.8	21
Acetic acid	0.8	6.4
Methyl acetate	1.7	7.3
Formamide	3.2	84
HCN	2.6	95
Carbon tetrachloride	0.0	2.2
Chloroform	1.1	5.0
Benzene	0.0	2.3
n-Hexane	0.0	1.8

Dielectric constant measurements at very high frequencies have provided important information about the hydration of proteins. At very high frequencies, the dielectric constant values of the protein solutions depend solely on the freedom of the solvent molecules (water). It was shown by Buchanan *et al.*[21] that a certain amount of the water in such solutions behaves as if it were restricted in its motion. About 0.4 gram of water per gram of protein appears to be bound. Two thirds of this water has a partial freedom to orientate, and one third seems to be tightly bound at the protein surface.

The evaluation of the dielectric measurements for ionized linear polymers is much more complex, because of the polydispersity of the polymers, and the flexibility of the chains[22].

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CHAPTER 11

The Structure of Colloidal Particles

1. Macromolecules and Micelles

A variety of structural aspects must be considered in discussing the structure of colloidal particles. In order to simplify the picture, it is useful to distinguish between two large groups: *molecular* and *micellar colloids*. The particles in molecular colloids are *large molecules* which are built according to the same structural principles as the usual small molecules of organic compounds. All atoms in the large molecules or macromolecules are linked by *primary valency bonds*. In *micellar colloids*, on the contrary, the particles are *aggregates of many small molecules*. In the micelles, the small molecules are not linked by primary bonds but by Van der Waals forces (cohesion). A more complex structure is observed in particles such as the proteins; both primary and secondary bonds and Van der Waals forces are all involved in the linking of the subunits. It was suggested that such particles be called *associated macromolecules*. Different structural aspects appear in the dispersions. In the emulsions of oils the molecules in the droplets stick together because of cohesion. However, in a dispersion of a space polymer the particles can be considered as giant molecules.

Micelles are ordinarily less stable than macromolecules. This is especially true with respect to stability against the dissolving influence of solvents. Micelles can be dissolved completely, i.e. disintegrated to the small constituting molecules by a suitable solvent, e.g. when alcohol is added to a soap sol. Macromolecules are much more stable to solvents. However, the chemical stability of macro-

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molecules is very different. It depends on both the *chemical properties* of the macromolecule and the reagent as well. Moreover, it depends on the *constitution* of the macromolecules. For example, the long, thin molecules of cellulose are much more sensitive to oxidative degradation than the corpuscular molecules of glycogen. This difference in stability may be likened to the ease with which a long thread could be cleaved, as contrasted to a compact bead.

2. Physical Evidence about the Presence of Macromolecules

According to Staudinger, the following facts prove the existence of macromolecules: (1) the identity of molecular weight values in various solvents, (2) the identity of the physical and chemical molecular weights, and (3) the identity in the degree of polymeriza-

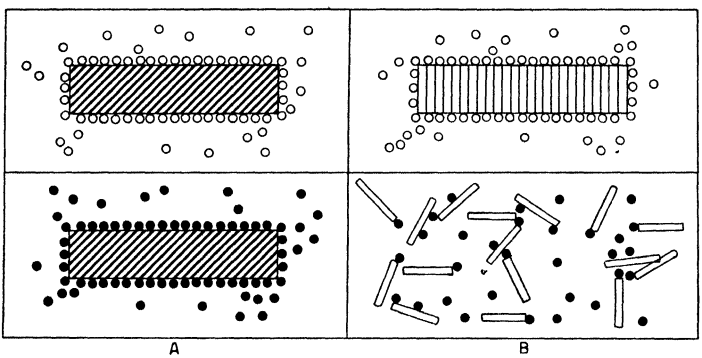

Fig. 70. Molecular (A) and micellar (B) colloids in two different solvents. The macromolecules remain of the same size, the micelles may dissociate into separate small molecules when the solvent is changed.

tion after chemical changes of polymers [1]. The fact that the same molecular weight is obtained for a specimen in various solvents is well illustrated in Fig. 70. If the particles were micelles, it would be most probable that different size micelles would be formed in different solvents. The micelle formation, as outlined in Chapter 5, is caused by solubility differences of the various parts of the solute molecule. Micelles of soap in water are formed because of the insolubility of the hydrocarbon part of the soap molecule, and because of the solubility of the polar carboxyl ion. There is no reason whatsoever for the formation of any polystyrene micelles in benzene.

The identity of the chemical and physical molecular weights is another important evidence for the existence of macromolecules. The chemical end group determination was applied successfully for several relatively low molecular polymers. The chemical method has also given important results for the chemical molecular weights of the proteins (Chap. 7). In none of the cases is the chemical molecular weight of a protein below 1000 but it is usually in the tens of thousands, e.g. about 16,000 for hemoglobin. In several cases the chemical molecular weight of proteins is identical with the physical molecular weight (see Chap. 26).

There is one more important physical evidence for the existence of macromolecules—the relatively high heats of formation of large molecules in polymerization reactions (see Table 4, p. 37). True chemical bonds are involved in these reactions, and the mechanism of activation and chain propagation is well investigated in many cases (see Chap. 4). It is impossible to investigate in a similar way the formation of the natural polymers, such as cellulose or rubber; but there is no reason to assume that, because of some differences in the conditions of synthesis, natural rubber might have a micellar structure, contrary to the molecular structure of synthetic rubber.

3. Chemical Reactions with Macromolecules

The most convincing proof of the existence of macromolecules of natural polymers is brought by Staudinger's *polymer-analogous interconversions*. The meaning of this monstrous term is quite simple: it is possible to perform chemical reactions with large molecules without destruction of the carbon backbone. Staudinger and his associates have proved that various derivatives can be prepared from starch, cellulose, rubber, and other natural polymers, and that the degree of polymerization remains the same throughout the conversion. In addition, the original polymer could be obtained again on proper treatment of the derivative. It must be pointed out that earlier work by other authors has yielded different results, probably because not enough precaution was observed in avoiding oxidative cleavage of the chains. If the cleavage is avoided, the reactions with large molecules can generally be performed in the same way as with simple micromolecular compounds. A *macroradical* remains unchanged in the chemical conversions; it is the carbon backbone in the polyvinyl derivatives and rubber, and the glucose-ring chain in the cellulose. Various atomic groups can be hooked

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on and removed from the chain without splitting it. The molecular weight, of course, is changed on these conversions but the degree of polymerization remains the same. The following examples of polymer-analogous reactions of Staudinger and associates illustrate this important concept of organic colloid chemistry.

(1) *Polyethylene to chlorinated polyethylene.* Polyethylene can be chlorinated without the cleavage of the carbon chain, if contact with oxygen is carefully avoided. The results of osmotic determination of degree of polymerization are presented in Table 24. (The degrees of polymerization or molecular weights are average values, as the samples always are polymolecular.)

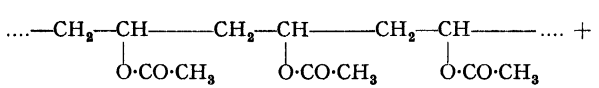
TABLE 24

THE CONSTANCY OF THE AVERAGE DEGREE OF POLYMERIZATION FOR POLYMER-ANALOGOUS CHLORIDES OF POLYETHYLENE (after Staudinger)

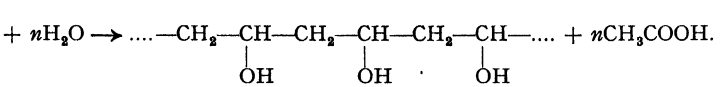
	Chlorine, %	M	P
Polyethylene (I)	0	23,000	1650
Chlorinated I, a	47.2	44,000	1720
Chlorinated I, b	66.9	67,000	1660
Chlorinated I, c	67.1	67,000	1660
Chlorinated I, d	71.6	80,000	1700

Upon the substitution of chlorine the macromolecules become more bulky but the degree of polymerization remains the same.

(2) *Polyvinyl acetate to polyvinyl alcohol.* Polyvinyl acetate is a vinyl polymer having many exposed acetate groups. Staudinger and Warth[2] have shown that the macromolecules can be saponified without affecting the carbon backbone, if oxygen is excluded from the system. The reaction occurs according to the following equation:



Polyvinyl acetate chain



Polyvinyl alcohol chain

The results of molecular weight determinations of three specimens of the original polyvinyl acetate and the resulting polyvinyl alcohols are presented in Table 25.

TABLE 25

Polyvinyl acetates in acetone		*Polyvinyl alcohols in water*	
M	P	M	P
75,000	870	40,000	910
126,000	1450	64,000	1450
152,000	1770	77,000	1750

It must be pointed out that in this case the molecular weights of the polymer derivative have been determined in another solvent than in the case of the original polymer.

(3) *Acetylation of starch and recovery of the original starch upon saponification of the triacetate.* This important polymer-analogous conversion was accomplished by Staudinger and Husemann[3]. The molecular weight of several well fractionated preparations of starch was determined osmotically using formamide as solvent. The specimens then were acetylated in the absence of oxygen by means of acetic anhydride and pyridine. The acetates were isolated, and their molecular weights were determined in acetone and in chloroform solutions. The same degrees of polymerization were found as in the starches before acetylation. The acetates finally were saponified and reconverted into the original starches. The molecular weight in formamide solutions again was determined, and molecular weights very close to the original ones were obtained. The results of these molecular weight determinations are presented in Table 26.

TABLE 26

POLYMER-ANALOGOUS INTERCONVERSIONS OF STARCH
(according to Staudinger and Husemann)

Starch fractions formamide solutions		*Triacetates in acetone*		*Triacetates in chloroform*		*Starch recovered from triacetates (in formamide)*	
M	P	M	P	M	P	M	P
30,000	185	54,000	190	53,000	190	30,000	185
62,000	380	122,000	390	110,000	390		
91,000	560	155,000	540	155,000	540	93,000	570
153,000	940	275,000	960	275,000	960	140,000	870

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Similar polymer-analogous acetylations and saponifications have been done with glycogens of even higher degrees of polymerization than the mentioned starches. The same values for the molecular weights of the recovered glycogens were found as for the original glycogens.

(4) *Polymer-analogous conversions with cellulose.* Various polymer-analogous conversions have been made with cellulose and its derivatives by Staudinger and his associates. It was found that even the nitration of cellulose can be accomplished without appreciable hydrolysis. If the nitration is performed with a mixture of nitric and phosphoric acid, the nitration is very fast, and the nitrates formed are relatively stable toward hydrolytic decomposition. The degrees of polymerization in these cases were determined by the viscosimetric method. Samples of cellulose were dissolved in copper tetrammine hydroxide (Schweizer's reagent), and the respective fractions of nitrocellulose were dissolved in acetone. A cellulose specimen which had an average degree of polymerization of 716 gave a nitrate with the average degree of polymerization of 670; another cellulose with the P of 1540 gave a nitrocellulose with the P of 1350; figures of 2000 for the cellulose and 1800 for the corresponding nitrocellulose, and 3400 and 3540 respectively for the very large molecules were obtained[4].

Specimens of cellulose were acetylated, the acetylated celluloses were investigated, and the acetates were reconverted into cellulose. It was found that the degree of polymerization remains the same after these changes. For example, a sample of cellulose acetate in *m*-cresol had a degree of polymerization of 1180, and the cellulose recovered after saponification of the acetate had a degree of polymerization of 1165. Another fraction of acetate had a P of 1660, and the corresponding cellulose had a P of 1680[5].

The saponification of the triacetate can be formulated as follows:

$$\underset{\text{cellulose triacetate}}{(C_6H_7O_2)_{1000}(O\cdot CO\cdot CH_3)_{3002}} + n OH^- \longrightarrow \underset{\text{recovered cellulose}}{(C_6H_7O_2)_{1000}(OH)_{3002}}$$

In this example an arbitrary degree of polymerization of 1000 was chosen. Each radical in the cellulose has three OH groups; with two additional end groups the number of hydroxyls and the corresponding acetyls (in the case of complete acetylation) then amounts to $3P + 2 = 3002$.

Further data on cellulose and its derivatives will be presented in Chapter 21.

(5) *Polymer-analogous reactions with unsaturated polyesters.* Interesting polymer-homologous series of unsaturated polyesters have been studied recently by Batzer *et al.*[6] in Staudinger's laboratory. Polyesters of maleic, fumaric, and acetylenedicarboxylic acid and hexanediol have been prepared, and the molecular weight of each has been determined by various methods. By polymer-analogous hydrogenation, the unsaturated polyesters then were transformed into saturated polyesters, and the molecular weights of the latter were determined. Some of the results are presented in Table 27.

TABLE 27

POLYMER-ANALOGOUS HYDROGENATION OF UNSATURATED POLYESTERS
(according to Batzer)

Material		*Before hydrogenation*		*After hydrogenation*	
		M_v *(viscosimetry)*	M_n *(osmot.)*	M_v	M_n
Polyesters from maleic acid + hexanediol,	MH6	28,600	29,900	28,100	29,800
ditto	MH8	30,300	32,000	28,900	30,000
ditto	MH8III	45,700	43,500	43,000	43,000
Polyester from fumaric acid + hexanediol,	FH15	26,800	25,200	27,000	25,600
ditto	FH16	33,000	33,600	33,100	33,000
ditto	FH18	41,800	42,600	50,000	44,000
Polyester from acetylenedicarboxylic acid and hexanediol	28AH8	35,500	38,200	38,200	38,300
	31AH10	55,200	55,000	54,600	55,400
	31AH11	63,900	64,500	64,200	65,200

It is obvious that the molecular weight does not change after hydrogenation. Furthermore, it is remarkable that there is a close agreement between the viscosimetric and osmotic molecular weights, an indication that the particular fractions were relatively homogeneous. The linearity of the molecules in this case was confirmed by chemical end group determinations. The chemically determined molecular weights agreed closely with the osmotically and viscosimetrically determined values.

Chemical methods of step-wise degradation applied to various macromolecular compounds, and their chemical derivatives, have been of great importance in the clarification of the structure of

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starch, glycogen, the proteins, and other naturally occurring macromolecular substances. These methods will be mentioned in Chap. 21–28.

4. Structural Studies by Means of X-ray Analysis

The chemical constitution of molecules is studied chiefly by chemical analysis and synthesis. The complete structure of thousands of organic compounds had been established long before any of the accurate physical methods were applied for the study of molecular structure. However, the solution of structural problems of complicated organic molecules was greatly facilitated by the modern physical chemical methods. Much detailed information has also been obtained by these methods regarding intra-atomic distances in molecules, bond angles, and molecular size and shape.

Structural analysis by means of X-rays is the most important single physical method in the study of organic macromolecules and micelles. During the last three decades, the method has been applied successfully in various ways, and it has helped greatly in establishing the structure of cellulose, rubber, the synthetic polymers, proteins, and many other colloids. There are three principal ways of applying these techniques to the study of organic colloids:

(1) Structural analysis by the study of single crystals,
(2) Structural analysis by the study of fibrous material as a function of the degree of deformation, and
(3) The study of solutions by means of low angle X-ray scattering.

The information provided by the first method, i.e. the study of single crystals, is the most important one. By the study of simple organic compounds, often in connection with other accurate methods, it was possible to establish the actual configuration of the carbon chains in various compounds, to determine the atomic distances and bond angles, as well as the positions of the constituent atoms in space.

The experiments are accomplished as follows[7]. The X-rays are produced by bombarding a metal target (anti-cathode) with high speed electrons in vacuum. Copper is one of the most common anti-cathode materials. The X-rays produced by a copper target have the wave lengths between 1.3 and 1.54 A, and they can be made practically monochromatic by crystal monochromators or by filtering through thin foil of nickel. A beam of the monochromatic X-radiation then is directed toward the object to be investigated.

When it strikes the object, the ray will be scattered. If the object is quite amorphous, no definite diffraction pattern will be detectible. When the object is made up of repeating units whose distances are nearly the same as the wave length of the ray, a diffraction pattern of the scattered X-rays will be observed (Fig. 71). This may be detected by means of a photosensitive film placed behind or around the object to be studied. The diffraction pictures revealed after developing the film may be very different depending on the material. Diffuse concentric rings are obtained from liquids, gels or unoriented fibers. Such rings indicate the presence of some repeating units which are not oriented in any way. Sharp concentric rings indicate the presence of well defined repeating units which are not oriented

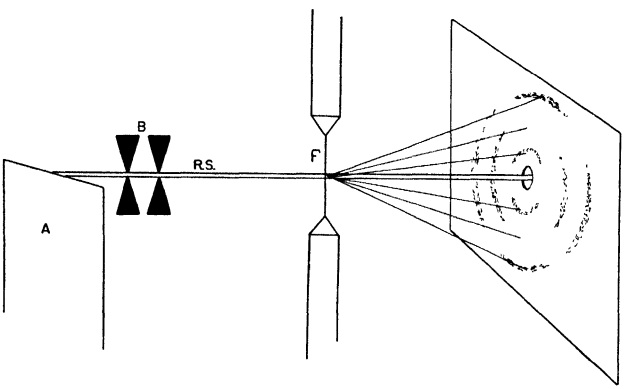

Fig. 71. Diffraction of X-rays. A – anticathode; B – collimating pinholes; R. S. the X-ray beam; F – a fiber hit by the X-ray beam.

in crystalline arrays. Arcs and spots with rings indicate more or less complete orientation of the repeating units. Finally, distinct symmetrically arranged spots indicate crystalline structure.

These diffraction patterns are due to the atomic planes in the crystal which act as a diffraction grating (Fig. 72) to reinforce the waves which are in phase, and cancel others which are out of phase. According to Bragg (1913), a simple relation exists between the angle Θ of reflection of the reinforced beam, the distance between the reflecting planes (atomic layers) d, and the wave length λ of the X-ray:

$$n\lambda = 2d \sin \Theta,$$

n being an integer. Hence, a series of reflections will be produced depending on $n(n = 1, 2, 3, 4 \ldots)$. For a constant value of d, and using the same wave length, a regular series of spots will be formed at certain angles. Measuring these angles, and knowing the wave length, the distance d between the atomic planes can be calculated.

Even the simplest organic substances have very complex structures, and many reflecting planes usually are present in the crystals. Hence, several values for the atomic distances d will be revealed by the X-ray diffraction patterns. The more complex the crystal the more spots will appear in the X-ray spectrum. For example, fatty acid crystals show three major spacings (d). The one distance is greater than the others, and changes with the molecular weight of

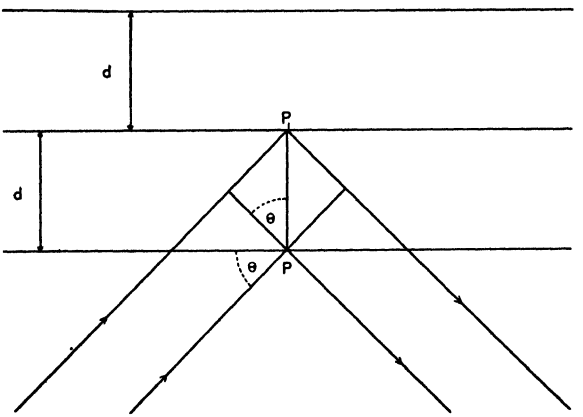

Fig. 72. Atomic planes in a crystal. d – distance between planes; θ – reflection angle.

the acid. This is the spacing exhibited by the long axes of the molecules. The two smaller d-values are practically independent of the molecular weight, as they depend on the cross-section of the hydrocarbon chains and the carboxyl groups which are in all fatty acids the same.

In this connection, the results obtained with concentrated soap solutions are interesting[8]. Three diffraction rings are usually observed in addition to the diffuse water halo. The interpretation of the spectrum shows that the soap molecules are arranged in rows, the short spacing being 4.5 A. This is the same value as obtained with the fatty acid crystals, and it represents the diameter (short axis) of the hydrocarbon chain. The second spacing resembles that of the

long spacing of the fatty acid crystals; it is about 40–60 A, depending on the soap, and corresponds to approximately twice the length of the soap molecule. This would indicate that the molecules are arranged in bundles composed of two parts (Fig. 7, p. 61), the heads of each bundle being turned toward the water. This spacing is only slightly dependent on the dilution. The third spacing is larger than the second, and it is increased with the degree of dilution, indicating that this spacing may represent the distance between the micelles. It was also found that the diffraction patterns change quite appreciably with variation in the experimental conditions. This is not suprising, if one considers the many possibilities of micellar arrangement. Rod-shaped micelles, long needles, leaflets and several other shapes have been considered. In concentrated solutions, the colloid becomes more organized, as the asymmetric micelles tend to arrange themselves in layers and other pseudo-crystalline arrangements.

The study of colloidal solutions by means of low angle X-ray scattering has also proved to be a fruitful approach. Schulman and Riley[9] have studied by this method the potassium oleate micelles in concentrated solutions (5 to 27%). They came to the conclusion that the diameter of the micelles varies between the limits of 100–550 A depending on the concentration of the soap. The solutions of many proteins also have been studied by this method. Beeman and colleagues in a recent study[10] have investigated bovine serum albumin, and a human serum albumin component—mercaptalbumin. They have come to the conclusion that either a right prism or a rectangular parallelepiped is the most probable molecular shape, the dimensions being 82.5, 27.5, and 63 A respectively (for the assumed prism).

Macromolecular substances in X-ray studies may appear to be either partly crystalline and partly amorphous, or completely amorphous. However, even those which appear amorphous become more or less completely oriented upon stretching the solid material. A wealth of information has been collected by this method by Astbury, Hermans, Katz, Mark, Meyer and many others on the structure of macromolecular substances[11]. It must be pointed out, however, that the X-ray analysis of polymeric fibers and plastics is incapable of establishing the molecular size.

Sponsler and Dore were the first to make a detailed study of the structure of cellulose fibers by means of X-ray analysis[12]. They advanced the modern concept that the glucose residues are linked

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in chains arranged parallel to the fiber axis. The principle spacings were also calculated. The dimensions of the unit cell, i.e. the smallest volume that contains the three-dimensional array, were determined very accurately by Meyer and Misch[13]. However, Meyer was unable to determine the length of the molecular chains. He made the erroneous conclusion that cellulose is composed of relatively short micelles. The molecular length can not be determined by the X-ray analysis. The macromolecules extend through many unit cells along the fiber. It was also discovered by X-ray analysis that cellulose and other macromolecular substances are not built entirely of crystalline material, but that some part of the substance is amorphous or incompletely oriented (Fig. 73). Hermans found that the crystalline portion of solid polyethylene comprizes about 50% of the total material[14]. The amount of the crystalline portion decreases with increasing temperature.

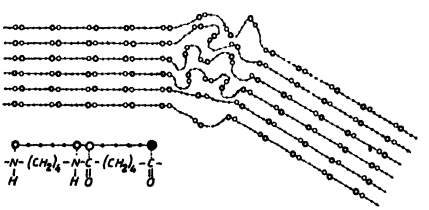

Fig. 73. Amorphous and crystalline sections in a polymer (from Hess and Kiessig, *Z. physik. Chem.*, 193 (1944) 196).

Important discoveries about the structure of gelatin and collagen were made by Katz and his associates[15]. They studied the structure as function of water content. It was found that some spacings were dependent on the water content, and some were not. The latter was identified as the peptide chain itself, whereas the water-dependent spacings showed the distance between the chains. This aspect has been developed further by subsequent investigators.

Certain data connected with the stretching of solid polymers has been most illuminating. Rubber, polyvinyl alcohol, and similar polymers appear in X-ray analysis to be amorphous, but the diffraction pattern changes on stretching the material. Instead of the diffuse concentric rings, segments and spots appear under the influence of tension (Fig. 74). This is explained by the *orientation of the fibrous molecules*. In the undisturbed material the flexible molecules are completely disoriented. A piece of such solid can be

compared with a coil of entangled steel wires. When the coil is placed under tension, the wires stretch out, and become more nearly parallel. Several spacings can be measured from the diffraction patterns of stretched linear polymers.

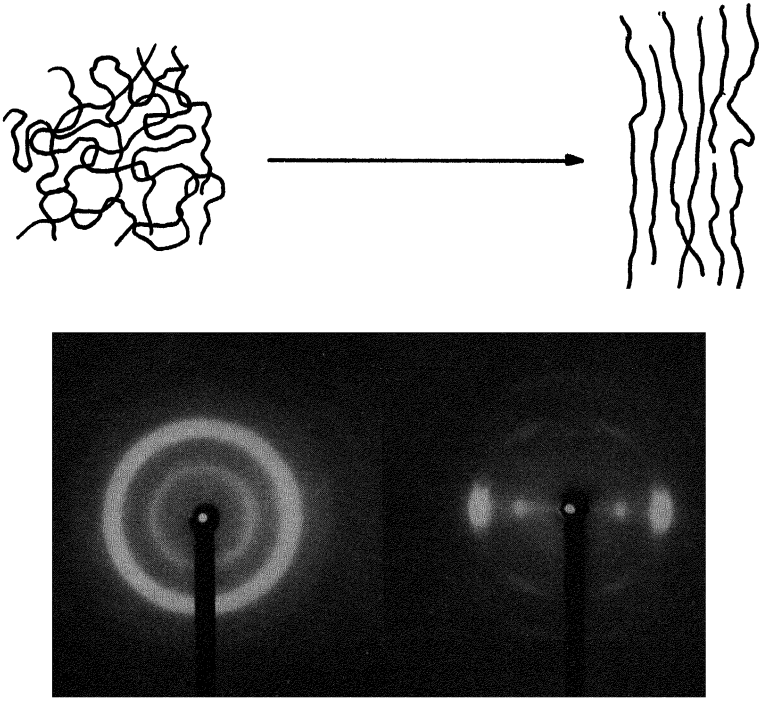

Fig. 74. Orientation of a linear polymer on stretching (Drawing of fibers and photos from Kruyt, *Colloid Science*, Vol. II, Fig. 83, p. 619.)
X-ray photographs of (a) an isotropic, (b) a highly oriented artificial cellulose filament.

Astbury found that the fibrous proteins can be divided into several configurational types. The collagen group of fibrous proteins, as found in tendons and cartilage, show a different diffraction pattern than hair or wool. The latter can be stretched on soaking

in hot water, and the X-ray spectrum is thereby changed. The distances of the repeating units have been determined, and important information was gained about the structure of these proteins. The stretching of proteins is usually accompanied by more complex changes than simple orientation. It could be shown that the *configuration* of the polypeptide chains may *change* on stretching. The same phenomenon occurs upon denaturation of globular proteins, in which case the unique pattern of the native protein is changed into the pattern of a fibrous protein (see Chap. 25–27).

The most difficult and complex problem is the interpretation and quantitative evaluation of the spot diagrams of single protein crystals. Considerable progress has been achieved in this field in the last decade. The names of Bernal, Bragg, Corey, Crowfoot, Huggins, Low, Pauling, and Perutz might be mentioned in this connection[16].

5. Atomic Distances and Bond Angles

It is possible to calculate the atomic distances and the spatial positions of atoms in molecules on the basis of X-ray analysis, electron diffraction, spectroscopy, and other experimental data. This information is important for the elucidation of the structure of macromolecules and micelles. It was found, for example, that the aliphatic carbon chain is not linear but instead has a zig-zag configuration. This chain has considerable flexibility, as indicated in several previous sections. The situation occurring in the linear arrangement of the glucose residues in cellulose is quite different in this respect. The flexibility is restricted here for constitutional reasons (and in solutions the stiffness is also due to a high degree of solvation).

Some data on atomic distances are compiled in Table 28, and X-ray identity periods of various linear polymers are presented in Table 29.

The bond angle of the zig-zag aliphatic carbon chains —C—C—C— is 111°. The angle is the same in the —C—O—C— linkage. In the aliphatic double bonds the angles are larger. It has also been established that the carbon atoms in benzene rings are arranged in perfect flat hexagons. The valency angle between the hydrogen atoms in water molecule appears to be 105°.

A certain correlation exists between the atomic distances (length of the bond) and the strength of the bond. According to the data

TABLE 28

ATOMIC DISTANCES OF LINKAGES IN MOLECULES[17]

Linkage	*Atomic distance in angstroms*(A)
C—C in aliphatic compounds	1.5
C—C in aromatic rings	1.4
C=C in olefins	1.3–1.35
C—H	1.0
C=N	1.3
C—O	1.4
C=O	1.13
N—H	1.0
O—H	1.0
O—H...O (hydrogen bridge)	2–3
Adsorbed layers (cohesive forces)	3–5

TABLE 29

X-RAY IDENTITY PERIODS OF SOME LINEAR POLYMERS

(from Schmidt-Marlies, *Principles of High Polymer Theory and Practice*, 1948, p. 169)

Polymer	*Mer*	*Fiber Period*, A	*Probable number of mers per period*
Polyethylene	—CH_2—	2.53	2
Buna rubber	—$CH_2CH{=}CHCH_2$—	5.0	1
Hevea rubber	—$CH_2C(CH_3){=}CHCH_2$—	8.2	2
Chloroprene rubber (neoprene)	—$CH_2CCl{=}CHCH_2$—	4.8	1
Polyisobutylene	—$CH_2C(CH_3)_2$—	18.6	8
Polyvinyl alcohol	—$CH_2CH(OH)$—	2.52	1
Polyvinyl chloride	—CH_2CHCl—	5.0	2
Polyvinylidene chloride	—CH_2CCl_2—	4.7	2
Glycol-dibasic acid polyesters:			
2–4 ester	—$O(CH_2)_2OOC(CH_2)_2CO$—	8.3	1
2–6 ester	—$O(CH_2)_2OOC(CH_2)_4CO$—	11.7	1
2–8 ester	—$O(CH_2)_2OOC(CH_2)_6CO$—	16.7	1
Cellulose		10.3	2
Cellulose acetate	as above, OH replaced	10.3	2
Cellulose nitrate	,, ,,	25.6	5
Nylon	—$HN(CH_2)_6NHOC(CH_2)_4CO$—	17.4	1
Silk fibroin	—HNCH(R)CO—	7.0	1

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of Table 28, the distances between the very strongly bound units, such as C—C, C—H, or O—H are only 1.0–1.5 A; the much weaker hydrogen bridge is 2–3 A long, and in case of adsorption the distance may be even 5 A. The primary bonds, like those of C—C or C—H have high dissociation energies of about 100–150 k.cal. per mole. The hydrogen bridge =O....H— is a weak bond, as the bond energy is only 4–8 k.cal. per mole. Even weaker are the cohesive forces (residual valency attraction); for example, the dissociation energy between the ...—$CH_2CH_2CH_2$—.... chains is only about 1 k.cal. per mole, calculated for a chain distance of 5 A, and assuming that each chain is surrounded by four others (see H. Mark and A. V. Tobolsky, *loc. cit.* ref. 17).

6. Infrared Spectroscopy[18]

Spectroscopy in the visible and ultraviolet regions is important in the study of small molecules and atoms. Infrared spectroscopy is capable of yielding extensive information on the structure of macromolecules and micelles. These relatively long waves have lengths between 8000 A and some millimeters. They do not originate from the vibrations of electrons but rather from the vibration of groups of atoms. The rays are produced e.g. by tungsten lamps, reflected by mirrors, and dispersed into a spectrum by prisms of sodium chloride or other non-absorbing materials. The spectrum is then analysed either photographically or by means of a thermocouple or bolometer[19]. Convenient instruments are offered by several companies.

Infrared spectroscopy was applied for the clarification of various structural problems of synthetic polymers and proteins. For example, it was shown that polyethylene absorbs waves which have the length of 0.0137 mm. This has been explained as being caused by the rocking of the —CH_2— groups of the chain[20]. Infrared spectroscopy has been helpful in the study of the structure, and degree of branching, of polychloroprene rubber[21]. In proteins, typical absorption maxima have been found at wave lengths of 0.003 mm, and 0.006 mm. The first maximum is due to the oscillations of the —NH— groups, the second of the carbonyl groups =CO of the peptide chains[22]. These data substantiate the current concepts of protein structure, and they disprove the contention of some authors that enolized peptide bonds —C(OH)=N— might be present in proteins.

7. The Optical Rotation of Natural Macromolecules

Proteins and polysaccharides are the most important naturally occurring macromolecular substances which exhibit optical activity. The experimental methods of measurement are very simple; however a quantitative correlation of the optical rotation data with the structural data is still to be accomplished. It was known long ago that the optical activity of dissolved organic substances was due to the presence of asymmetric carbon atoms in the molecules. The situation is relatively simple in small molecules containing only one asymmetric carbon, such as the majority of the natural amino acids or hydroxy acids. Several empirical rules have been found which correlate structural features and degree of ionization with the optical rotation data[23]. However the more complex the molecules the harder it is to correlate the optical rotation data with structure. The work of Greenstein[24] and Brand[25] has shown how complicated the relations become in amino acids and polypeptides containing two, three or more asymmetric carbons in the same molecule. Since protein molecules contain hundreds of such asymmetric carbons, the task may seem hopeless; however some progress has been made even with the proteins[26].

It has been shown that the optical activity of the synthetic optically active polyamino acids parallels the changes in the molecular configuration. The folded chains showed a lower optical activity than the extended chains[27]. The optical activity of polylysines varies regularly with the molecular weight or chain length of the polymers[28].

The polarimetric technique has been improved recently by increasing its sensitivity, as well as by applying well filtered monochromatic light, and using photoelectric cells for the comparison of the half-shade field. Such an improved instrument is presented in Fig. 75. Monochromatic light is polarized in the polarizing prism, passes through the tube containing the solution to be studied, and the degree of rotation is determined with the analysing prism and the photoelectric unit.

Since the optical rotation depends on the configuration of the atomic groups which are attached to the assymmetric carbon atoms, it reflects minor structural changes in the molecules, such as increased ionization of the carboxyl groups[29]. Aggregation of two or more macromolecules would not be reflected in any change of optical rotation, although intramolecular structural changes may cause a considerable change in the optical activity[30].

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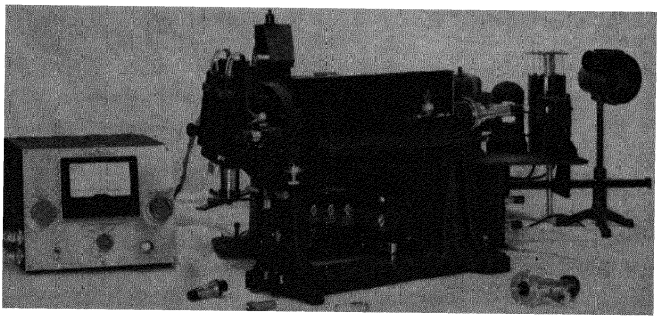

Fig. 75. A high precision spectropolarimeter.
(Courtesy of O. C. Rudolph & Sons, Optical Manufacturers, Caldwell, N.J.)

Optical activity is expressed as the *specific rotation* obtained by dividing the observed degrees of rotation α by the concentration and length of the tube, or

$$\text{Specific rotation } [\alpha] = \frac{\alpha \cdot 100}{c \cdot l},$$

c being the concentration in grams per 100 cc, and l the length of the polarimeter tube in decimeters. The specific rotation of an optically active substance depends very little on concentration[31]. The temperature dependence is not very pronounced either[32], although in several instances of organic macromolecules the change with temperature is significant. For example, serum albumin containing alcohol shows an increase in the specific rotation at higher temperatures. This may be interpreted as an expansion of the albumin molecules at a higher temperature[33]. (See also Chap. 25 and 26.) The most important parameter in the specific rotation is the *wave length* of the light employed. Most optical rotation measurements have been made with the yellow sodium light ($\lambda = 5889.9$ A). Formerly sodium chloride was placed in a small trough of platinum, and evaporated in a gas flame to obtain such a light source. Now sodium, mercury, cadmium, and other lamps are available, and definite wave lengths can be filtered out by light filters and monochromators. It was noticed long ago that the specific rotation decreases with increasing wave length.

The change of the optical activity with the wave length is known as the *rotatory dispersion*. Drude (in 1907) was the first who obtained

a satisfactory mathematical expression connecting optical rotatory power with the wave length of the light. According to Drude

$$[\alpha] = \Sigma K_i/(\lambda^2 - \lambda_i^2)$$

where $[\alpha]$ is the specific rotation, λ is the wave length, and K_i and λ_i are constants. Lowry, Hewitt, and others found later [34] that in many cases rotatory dispersion data can be fitted with a single term of the above sum of Drude:

$$[\alpha] = K/(\lambda^2 - \lambda_0^2), \quad \text{or} \quad 1/[\alpha] = \lambda^2/K - \lambda_0^2/K.$$

The latter equation expresses a linear relationship between the

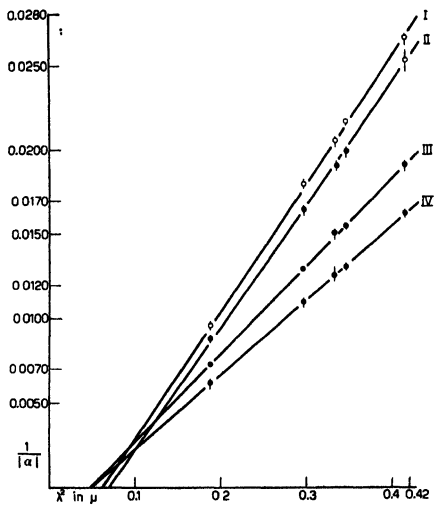

Fig. 76. The dependence of rotatory power on the wave length of light for four proteins. I – metal binding β globulin of human plasma; II – lysozyme of egg white; III – chymotrypsin (a proteolytic enzyme), and IV – chymotrypsinogen (a chymotrypsin precursor). (Data of B. Jirgensons.)

reciprocal of the specific rotation $1/[\alpha]$ and the squares of the wave lengths λ^2, as illustrated in Fig. 76. The rotation constant K which represents the slope of the straight lines appeared to be dependent on temperature, pH, and other experimental conditions. The dispersion constant λ_0 represents the intercept on the abscissa which was found to be independent of temperature and other parameters, but it is influenced by structural features[35]. The dispersion constant changes if some changes in the configuration have occurred[35].

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In spite of the fact that the correlations between the specific rotation and structure are not yet fully understood, the specific rotation is an excellent means for the *characterization* of natural macromolecules. Staudinger and Husemann substantiated by optical rotation data their conclusion that the glycogens recovered in polymer-analogous conversions from the triacetates are the same as the native glycogens[36]. Thus far single observations of the rotatory power of natural macromolecular substances have been chiefly used for the purposes of characterization. However, it has been shown recently that a much more powerful tool for such characterization is the determination of some optical activity function, such as the *dependence of the rotation on pH*[37], or the *dependence of the rotation on the wave length of the light*[38]. Finally, by observing the changes of the specific rotation with time, kinetic studies regarding changes of structure and configuration can be done conveniently[39].

Professor Doty and his associates (in Harvard University) have treated recently the dependence of the rotatory dispersion on configuration of large molecules from a different angle[40]. It could be shown that optically active polyamino acids exhibit strikingly different rotatory dispersion depending on the solvent. It was ascertained by light scattering studies that, depending on the solvent, the macromolecules of the polyamino acids may have the configuration of either rigid rods or kinked coils (see Chap. 25). Only the kinked coils exhibited the expected normal rotatory dispersion, whereas the rods showed abnormalities incompatible with the one term Drude's equation. These studies they later extended also to proteins. Since most of the proteins exhibit a normal rotatory dispersion, the conclusion seems likely that the ordinary albumins and other proteins do not possess very rigid macromolecules, but that the latter are flexible.

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CHAPTER 12

Swelling, Solubility, Solubilization

1. Swelling and Dissolution of Polymers

The solubility behavior of macromolecular solids is very different for various solute-solvent pairs. The following possibilities may be distinguished:

(1) the macromolecular solid is insoluble in a certain solvent;
(2) the solid swells, but has a limited solubility;
(3) the substance is soluble in the given solvent.

Examples of case (1) are: rubber in water, or albumin in ether. The space polymers do not dissolve in solvents without being chemically decomposed. There are also many examples of case (2), i.e. that a polymer swells and then is partly dissolved. Rubber swells in hydrocarbons and similar lipophilic solvents and dissolves in them more or less completely, depending on the solvent and on the particular rubber specimen. Gelatin swells in water; it dissolves in hot water completely, whereas cold water merely extracts some components of the gelatin. Complete and rapid dissolution is also quite common (case 3). For example, nitrocellulose is soluble in acetone, albumin in water, and polyvinyl alcohol in alcohol. If a macromolecular substance is soluble in some solvent, the solubility usually is very high.

It is possible to predict the solubility qualitatively on the basis of chemical composition of the solute and solvent. The basic rule is: "*like dissolves like*". Chemical and structural similarity between the solvent and the solute favor solubility. Rubber, polystyrene, and other polymeric hydrocarbons are dissolved by liquid hydro-

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carbons and similar nonpolar solvents. The polar macromolecules such as glycogen and albumin are insoluble in hydrocarbons, but are soluble in water.

The chemical composition alone, however, is an insufficient criterion for even a qualitative prediction of solubility. For example, starch and cellulose have the same chemical composition as glycogen; however, cellulose is insoluble, starch is difficultly soluble in cold water, whereas glycogen is highly soluble in water. The solubility is also determined by the *constitution* of the macromolecular substance. The following structural features are important in determining solubility: (1) *cross-linking* between chains, (2) molecular *size*, (3) molecular *shape*, and degree of branching, and (4) the degree of crystallinity of a polymer. As mentioned before, the space polymers are insoluble, since all the constituent atoms are linked by strong primary bonds. Cross-linked linear polymers are, in general, only slightly soluble, and as the degree of cross-linking increases, the polymer becomes more and more like a space polymer. A *cross-linked polymer*, such as vulcanized rubber, *swells* in proper solvents if the degree of cross-linking is low. The solvent, of course, must have some affinity for the polymer; it diffuses into the bulk, sticks to the macromolecules and increases the volume of the solid. It is, however, unable to cleave chains away from the bulk, since the primary bonds of the cross-links are too strong for the solvating liquid. This is illustrated in Fig. 77.

The molecular size, shape, and degree of crystallinity of the solid are all very important. The *solubility decreases with increasing molecular weight.* The high polymeric starches are more difficultly soluble than the lower molecular dextrins, which are degradation products of the starches. The molecular shape is also important; the linear polymer, amylose, is only slightly soluble; the branched starches (amylopectin) are more soluble, and the globular glycogen is the most easily soluble in water. When a substance of a given molecular weight is in an amorphous state, it is more easily soluble than if it were in a crystalline state.

All these facts may be easily understood on the basis of the following considerations. Suppose that no cross-linking is involved, and that the macromolecules in the bulk are held together only by cohesive forces. Dissolution will then occur if the *solvating affinity of the liquid is sufficiently great to overcome the cohesive forces acting between the macromolecules* in the bulk. The cohesive forces acting between the macromolecules depend on the constitution of the

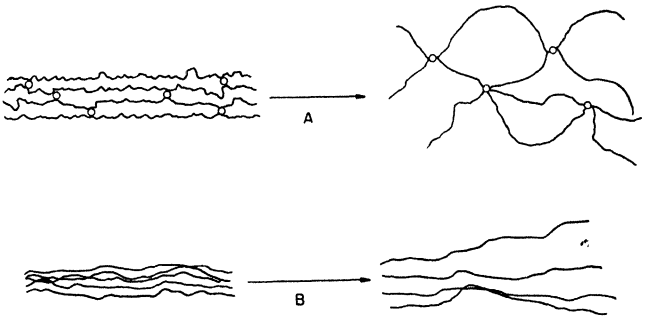

Fig. 77. Swelling and dissolution of polymers. A – swelling of a cross-linked polymer; B – dissolution of a linear polymer.

polymer and on its molecular weight. For substances of the same chemical composition, the *intermolecular cohesive forces are greater between linear chains than between ramified structures or coils of chains.* This is clear from simple geometrical considerations (Fig. 78). In closely aligned chains there are more points of contact than in the case of coiled or branched chains. The cohesive forces therefore, in the linear polymer cellulose are extremely great because of the geometry of the macromolecules. This is the reason that cellulose is so resistant to solvent attack. This is also why a crystalline (or predominantly crystalline) polymer is more difficultly soluble than an amorphous specimen of approximately the same molecular weight.

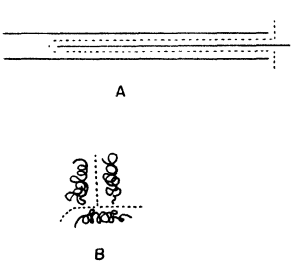

Fig. 78.

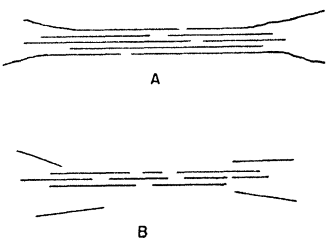

Fig. 79.

Fig. 78. Intermolecular cohesion between parallel chains (A), and between coils (B)
Fig. 79. Intermolecular cohesion and chain length. Short chains (B) are split off the bulk more readily than long chains (A).

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The influence of the molecular weight on the solubility can be explained by the same geometrical reasoning. Suppose we have two samples of a linear polymer. The molecules of sample A are twice as long as the molecules of sample B (see Fig. 79). The residual valency or cohesive forces are the same *per unit mer* (*residue*); hence in sample A two molecules will be held together by forces twice as strong as those in sample B. The solvent, penetrating the bulk by virtue of diffusion and Brownian motion, can, with the same solvating action, *liberate a short chain* (sample B) from the bulk, but *it cannot do so with the long chains*. An equal solvation effect will be able to loosen just one half of the chain; the other half will remain sticking at the solid mass. Consequently, a minimum solvating action is able to produce dissolution in a low molecular linear polymer, but it produces only a kind of swelling in a high molecular substance.

The dependence of the solubility on molecular weight, as mentioned before, has been treated quantitatively (see p. 130). The relationships are valid for separate polymer-homologous series. Noteworthy is the fact that it is very difficult to determine the solubility of polymers in pure liquids quantitatively, since the solubility usually is very high, and it depends on the amount of solid used for saturation (see next section). It is possible to determine the solubility more conveniently in solvent mixtures indirectly. According to Schulz[1] the sample, for instance a polymethacryl methylester, is dissolved in a good solvent (benzene), and by diluting with this solvent, a whole series of solutions of various concentrations are prepared. The diluted solutions then are titrated with another liquid which causes precipitation of the polymer. The appearance of turbidity at a certain mole fraction of the precipitant will indicate the solubility at this concentration of the polymer in a particular mixture of the liquids. If turbidity appears after adding v ml of precipitant to v_0 ml of the original solution,

$$\gamma = v/(v + v_0)$$

is the precipitability value. This, according to Schulz, is related to the solubility c in the particular solvent mixture by the simple relation:

$$\ln c = a - b\gamma.$$

As a and b are constants, the solubility c can be obtained readily from the precipitation titration data[2].

Data on compatibility and solubility of polymers is important in polymer technology. (The fact that the low molecular components are more readily soluble than the high molecular components finds practical application in fractionation.) For example, the low molecular components may be extracted by suitable solvents from the solid polymer. The solubility in mixtures of two or more good solvents, however, is not always predictable. For example, polyvinylpyrrolidone is soluble in water and also in dry acetone, but it is insoluble in certain water-acetone mixtures of low water content.

Examples of the solubility of polymers are compiled in Table 30.

TABLE 30

SOLUBILITY OF HIGH POLYMERS

(According to A. X. Schmidt and Ch. E. Marlies, *Principles of High Polymer Theory and Practice*, McGraw-Hill Publ., New York, 1948, p. 704)

Polymer	*Solvents*				
	Aliphatic hydrocarbons	*n-Butyl alcohol*	*Butyl acetate*	*Dioxane*	*Carbon disulfide*
Hevea rubber	D	N	D	D	D
Polystyrene	L	N	D	D	D
Polyvinyl chloride	N	N	L	D	L
Neoprene rubber	L	N	D	D	D
Saran	N	N	L	D	
Polymethacrylate	N	N	D		D
Polyvinyl chloride – polyvinyl acetate copolymer	N	N	D	D	L
Cellulose acetate	N	L	N	D	L
Cellulose nitrate	N	L	D	L	L

Keys: D – soluble; L – limited solubility; N – not soluble.

The ketones and esters are the most universal solvents for polymers. Monochlorinated aliphatic hydrocarbons have nearly the same solvent properties as hydrocarbons. Di- and trichlorinated aliphatic hydrocarbons are generally better solvents than monochlorinated hydrocarbons, e.g. the polymethacrylates are soluble in them but not in hydrocarbons and monochlorinated hydrocarbons. Aromatic hydrocarbons are more universal solvents than

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aliphatic hydrocarbons, e.g. the polymethacrylates dissolve in benzene and can be precipitated by hexane. The solubility, however, in all instances depends on the average molecular weight and on the possible cross-linking whenever it may occur.

2. The Solubility of Inhomogeneous Macromolecular Compounds

All of the synthetic, and most of the natural macromolecular substances are polymolecular, i.e. any specimen is a mixture of similar molecules of various sizes. As the smaller molecules dissolve more easily than the large ones, the material does not have a constant solubility, when various amounts of solid are put into equal amounts of solvent. The solubility increases with increasing amounts of the solid.

The study of solubility as a function of the amount of solid phase is sometimes used as a *test for homogeneity*. It is, of course, reasonable to apply this test only to essentially homogeneous materials. Another prerequisite for such a test is that the substance

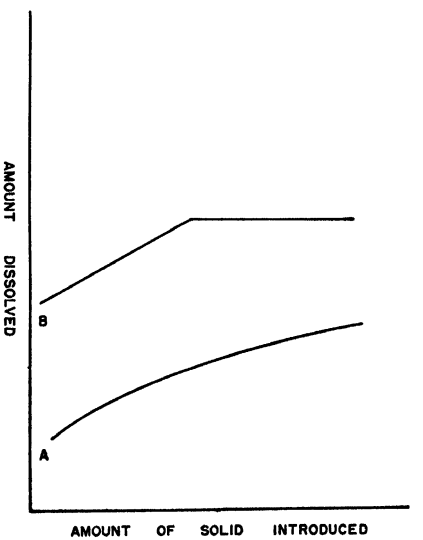

Fig. 80. Solubility and homogeneity. The solubility of a polymolecular solid increases gradually with the quantity of solid put into the solvent (A). The solubility of a homogeneous substance increases up to a certain point, and then remains constant (B).

to be tested must have a limited solubility in order to be able to determine the point of saturation. Such tests have been applied to the determination of homogeneity of proteins. Increasing amounts of the protein are placed in equal volumes of solvent, the mixture is equilibrated, and the amount dissolved is determined. The latter then is plotted against the amount of solid introduced for each determination. For an inhomogeneous solid a smooth curve is obtained (Fig. 80). The dissolved amount increases steadily with increased quantity of the starting material. For a homogeneous substance, however, the amount dissolved rises only up to a certain point, and does not increase beyond this point on further addition of the solid phase. Such cases have been found with some well purified, crystallized enzymes[3]. The homogeneous micromolecular substances, such as benzoic acid or carotene behave in the same way. The solubility criterion, however, is not an absolute one, as some proteins which were found to be homogeneous by the solubility test appeared to be inhomogeneous by other methods of testing.

3. Swelling Phenomena

Swelling can be considered as the first step toward dissolution. Swelling occurs only in solvents which are bound by the molecules of the solid. As a result of the solvating effect, the particles of the solid are pushed apart, and the volume of the solid increases. Whether or not a complete dissolution then occurs depends on two factors: the amount of solvent available, and the structure of the colloid[4]. If an excess of the solvent is available, the further fate of the system depends solely on the structure of the solid. In the case of cross-linked polymers the swelling is *limited* to a certain degree, and the major part of the solid does not dissolve; only a small portion of the lower molecular components may be leached out by the solvent. A spectacular example of such limited swelling is the swelling of polystyrene cross-linked by means of added divinylbenzene. The ordinary polystyrene dissolves in benzene easily. If, however, styrene is polymerized with a little of divinylbenzene

$$CH_2=CH—C_6H_4—CH=CH_2$$

added to the monomer, a cross-linked polymer is formed of the following constitution:

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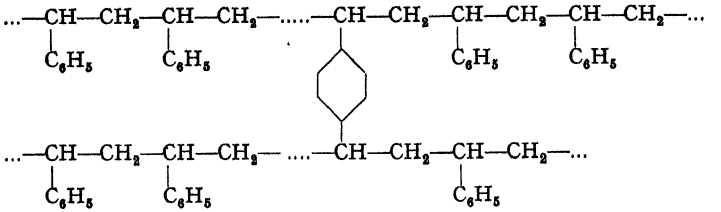

Such cross-linked polystyrene swells tremendously in benzene (Fig. 81), but it does not dissolve. The divinyl-benzene bridges are responsible for this. Because of them, the polystyrene chains are linked

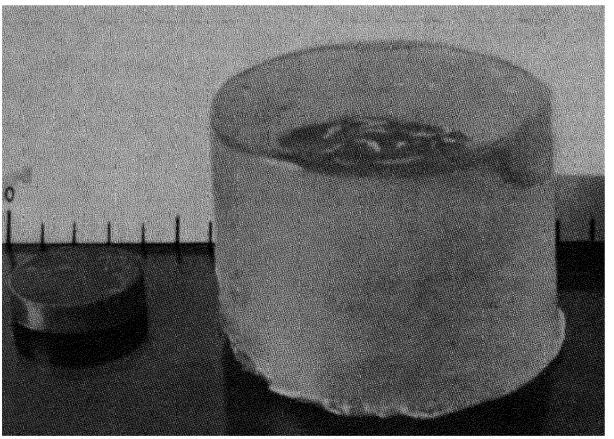

Fig. 81. Swelling of a piece of cross-linked polystyrene in benzene (after H. Staudinger).

into a loose meshwork capable of expanding and taking up large amounts of liquid[5]. In ordinary polystyrene, the chains are not interlinked by primary bonds, and they are liberated slowly from the bulk as more and more liquid diffuses into the intermolecular space. In a cross-linked polystyrene the chains are merely straightened out by the incoming liquid, but retain their position in the meshwork (Fig. 77, A). In the cases of *unlimited swelling* there are no cross-links between the colloidal entities in the solid, and it is dissolved slowly after the initial phase of swelling. If the cross-links

are weak, as is the case with gelatin, limited swelling occurs at lower temperatures, but at a higher temperature the swollen gel dissolves.

Although the volume of the swelling solid increases, the total volume of the system as a whole decreases. This indicates that a certain volume *contraction* has occurred. The contraction is due to the orientation of the molecules solvating the particles in the meshwork. These fixed molecules occupy somewhat less space than free molecules.

Swelling is a mildly exothermic process, i.e. heat is evolved on swelling[6]. This indicates that lowering of the temperature should promote swelling, i.e. at a lower temperature more of a liquid will be bound than at a higher temperature. The *rate of swelling*, however, increases considerably with increasing temperature. The rate of entry of the liquid is facilitated at a high temperature, since the molecular movements increase in intensity at higher temperatures. However, more of the liquid will remain bound in the meshwork after the equilibrium is reached at a lower temperature than at a higher one.

Swelling is studied either by measuring the *pressure* exerted by the swollen substance or by determining the increase of the weight or volume. The swelling pressure P, was found to be very great at the beginning of the process when the concentration c of the colloid is very high. The following relation holds, according to Posnjak (1912):

$$P = K \cdot c^n,$$

where K and n are constants. The value of n was found to be between 2.5 and 3 for most systems studied. The rate of swelling likewise is greatest at the beginning, and it decreases as the uptake of the liquid proceeds[7].

The swelling phenomena have been investigated by a variety of optical methods, such as light scattering and X-ray diffraction. If the swelling affects the amorphous parts of the solid only, the diffraction pattern does not change. If, however, the molecules of the crystalline portions are pushed apart, the X-ray diffraction pattern is affected considerably (*loc. cit* ref. 6).

The swelling of organic colloids is essentially reversible. The swollen gels shrink upon removal of the solvent, e.g. by keeping the swollen mass at a low vapor pressure. After such 'desolvation' the solid can be made to swell again. However, it was found that

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the reversibility is not complete[8]. The swelling ability decreases somewhat on repeated processing.

4. Degree of Swelling and Amount of Cross-linking

According to Staudinger (*loc. cit.* ref. 5) the amount of liquid bound on swelling is inversely proportional to the extent of cross-linking of the polymer. This is illustrated by the data of Table 31.

TABLE 31

THE SWELLING OF POLYSTYRENES IN BENZENE WITH VARIOUS DEGREES OF CROSS-LINKING
(according to Staudinger)

Number of molecules of divinyl-benzene per 100,000 molecules of styrene	*The number of times the volume increases on swelling*
10	12
5	26
3·3	50
2.5	78
2.0	181
0	unlimited swelling, dissolution

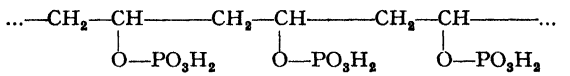
The result is comprehensible if we consider the fact that with increased cross-linking the freedom of expansion of the meshwork is greatly reduced.

5. Swelling of Polyelectrolytes

It was found long ago that the swelling of proteins depends on the pH of the solution and on the ionic strength and kind of salts present. There is a minimum of swelling at the isoelectric point of gelatin, while acids and alkalies promote swelling. Chlorides, nitrates, bromides, iodides and thiocyanates promote swelling in the order mentioned. Sulfates, tartrates, citrates, and acetates retard swelling.

The swelling of the muscle proteins is thought to be of major importance in muscular contraction. It is quite conceivable that contraction and relaxation of ionized fibrous materials may depend on the degree of ionization and degree of swelling. The increase in

net charge, as indicated before, results in mutual repulsion of segments and in expansion. Increase in net charge also involves more swelling, as more water will be bound due to the increased polarization at the ionized sites. This was demonstrated recently for a number of polymeric systems. Katchalsky and Eisenberg[9] found that polyvinyl phosphate swells reversibly, expanding and contracting in fractions of seconds, depending on the degree of ionization. The polyelectrolyte was prepared by phosphorylation of polyvinyl alcohol, and has the constitution:



In a nearly neutral solution ionization of the phosphate groups occurs, the segments of the fiber become highly charged and the polymer becomes highly swollen and expanded. If the ionization is suppressed, the material shrinks. Several other molecular mechanisms of contraction have been discussed[10] (see also Chap. 27 and 29). It was found by a group of Japanese workers[11] that some synthetic amphoteric polypeptides, such as polyglutamyl-lysyl-cysteine, exhibit reversible contraction. The polyamino acid expanded in alkali and acid, and it shrunk in neutral solution. The swelling behavior of cross-linked polymethacrylic acid, partially or wholly neutralized by mixtures of sodium and barium hydroxide, was studied recently by Katchalsky and Zwick[12]. At high degrees of neutralization and high barium content (depending on the conditions), the polymer either swells or contracts, and the dimensional changes depend on the ion exchange.

6. The Modes of Binding of a Solvent

A greatly swollen polymer may contain enormous amounts of solvent. Since the cohesive forces act only through a very short distance, a fiftyfold or even hundredfold increase in volume on swelling requires some explanation as to the possible means of binding of the liquid in the swollen substance. Some colloid scientists have implied that these facts indicate the existence of thick solvating layers and long range attractive forces. This opinion is not shared by all who have studied these problems. It seems more likely that the solvating layer is very thin, probably *monomolecular*. Numerous facts indicate this, such as molecular weight variation

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in various solvents, dielectric dispersion (p. 182), adsorption studies, and viscosity and sedimentation results. The free energy changes in swelling are very small, and the heat of swelling can be measured only in the first period, when the tightly bound monomolecular layer is formed. The rest of the liquid found in a swollen gel is bound very weakly. In many instances it can be squeezed out mechanically. It is now generally accepted that the greatest portion of the liquid found in highly swollen substances is *mechanically immobilized* by the solvated chains of the meshwork. In the case of polar systems, such as proteins swelling in water, *three* kinds of bound water can be considered: (1) tightly bound water of hydration, (2) water bound by cohesion and polarization, and (3) mechanically immobilized water. In non-polar systems there will be only two possibilities: (1) binding by cohesion, and (2) immobilization. It must be pointed out that an important condition for such immobilization is the *fibrous structure*. Spherocolloids, such as albumin or glycogen, dissolve without being swollen.

7. The Solubilizing Capacity of Micellar Colloids

The removal of grease and oily dirt from our hands by soapy water is a remarkable phenomenon. It is only recently that colloid chemical studies have given us an understanding of this process. The relationships found in these studies were also of great value in the solution of certain industrial problems.

It is interesting that the action of a detergent has a great deal to do with the fat and lipid transport in our blood stream. To take another example: the utilization of carotene and vitamin-A in tissues cannot be understood without simple colloid-chemical considerations. In each of the above mentioned cases, complex systems are involved, with *polar and non-polar* constituents. A simple soap micelle also has this double nature. A soap solution containing such micelles has both polar and non-polar loci. As a polar system it dissolves polar substances, such as glycerol. However, a soap solution is able to dissolve or *solubilize non-polar substances* also, such as hydrocarbons, and fatty oils. This is accomplished by the *lipophilic loci* of these micellar colloids. The internal part of the micelles in aqueous detergents is non-polar or lipophilic. (In oily detergents the internal part of the micelles is hydrophilic).

The term 'solubilization' was introduced by McBain (1937), and means spontaneous passage of a substance insoluble in water into

an aqueous solution of a detergent or other micellar colloid[13]. Extensive studies have been carried out to compare the solubilizing capacity of various micellar colloids, or on the ability to solubilize various non-polar substances by the same detergent. Most of these investigations have involved the solubilization of micromolecular hydrocarbons, e.g. benzene. The phenomenon was also studied by means of X-ray diffraction[14]. It was found e.g. that a 9% solution of sodium oleate in water is able to dissolve a certain amount of benzene, and that the long spacing thereby increases from about 78 A to 127 A. A layer of benzene has penetrated into the soap micelles, and is held there by the non-polar tails of the oleate Fig. 82).

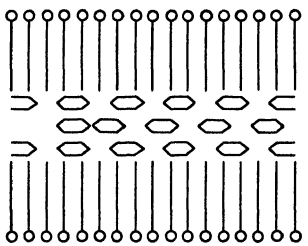

Fig. 82.

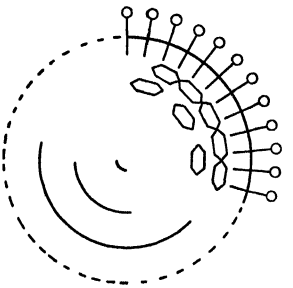

Fig. 83.

Fig. 82. Benzene molecules solubilized in a micelle of soap.
Fig. 83. Emulsion droplet of benzene stabilized with a layer of soap molecules. The polar heads of the soap molecules (drawn as rings) are turned toward the water phase.

It is hard to establish a borderline between solubilization and emulsification, and the term 'solubilization' has been used differently by various authors in this respect. A micellar detergent is able to absorb in its micelles large amounts of lipophilic substances insoluble in water. When the amount of the solubilized material is moderately great, the solution remains almost clear. Upon bringing more and more oily material into the colloidal solution of the soap, the oily core of the micelle becomes dominant in size, and the soap remains as a protecting layer around the relatively large droplets (Fig. 83). The system then looks opaque and turbid. It is now called an emulsion. Various monomeric starting materials which are insoluble in water are thus solubilized and emulsified for emulsion polymerization.

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Solubilization is also important in oily solutions of detergents, such as lubricating oils. When a detergent is dissolved in a hydrocarbon solution, micelles of the detergent are formed. However, in this case the polar 'heads' of the detergent molecules are turned inside the micelle, and the hydrocarbon 'tails' are turned toward the solvent. Such a micellar colloid has the capacity to solubilize water and water-soluble substances. This is of practical importance, for example in improving lubricating materials, since the added detergent promotes removal of sludge and acidic waste products of combustion; the water-soluble substances are taken inside the detergent micelles and washed away.

The micelle formation and solubilization in oily media was studied by a number of authors[15]. For example, di(2-ethyl-hexyl) sodium sulfosuccinate (Aerosol OT) forms micelles in *n*-dodecane. These micelles have spherical or asymmetric shapes, the core is hydrophile, and the exterior lipophile. The sol is able to solubilize water and water-soluble substances[16].

8. Solubility and Swelling from the Point of View of Thermodynamics

The dissolution of polymers can also be interpreted according to the principles of thermodynamics. The changes in any systems are *spontaneous* if the *free energy* F of the system *decreases*, i.e. if the free energy change ΔF *is negative*. This decrease is determined by three factors: the heat content of the system H, the temperature T, and the entropy S. According to the first and second laws then:

$$F = H - TS,$$

or, denoting by Δ any finite changes of the quantities involved:

$$\Delta F = \Delta H - T\Delta S.$$

In a spontaneous process of dissolution ΔF must be negative; this leads to the conclusion that $T\Delta S$ must be numerically larger than ΔH. In non-polar systems, such as rubber in benzene, the heat content change ΔH during the dissolution is very small, hence $T\Delta S$ must be numerically large. At a constant temperature (T), this will indicate a large ΔS. Consequently, the spontaneous dissolution of rubber in benzene[17] is due to a *large increase in entropy* ΔS. According to the Boltzmann relation $S = k \ln W$, k being the Boltzmann constant and W the probability. An increase in entropy

then means an *increase in disorder*, or an increased variety of molecular configurations. This is comprehensible if one assumes the increased flexibility of the fibrous molecules in solution, as compared with the conditions in the solid. It could be shown that ΔS in such cases is so great that a negative ΔF is granted even in the case of a small positive ΔH. It is also noteworthy that dissolution is promoted by the increase in temperature (T), as a large T will enhance the numerical value of the negative member $-T\Delta S$.

The conditions are somewhat different in dissolving a polar colloid in a polar solvent. In this instance ΔH has a definite negative value, i.e. the swelling and dissolution are *exothermic* due to the exothermic solvation effects. A negative ΔF thus seems to be always ensured. However, in these cases the conditions are more complex than in non-polar systems. First, in polar solids the intermolecular forces of attraction are more pronounced than in non-polar linear polymers. Second, the increase in entropy in polar systems is smaller than in non-polar ones. Because of the polarization and strong solvation, the order in the system may even increase on dissolution. Hence, even a *decrease of entropy* is not unusual in polar systems. This is especially true for the first stages in swelling, when the disordered solvent becomes more ordered in the process of solvation. Furthermore, the very polar macromolecules are often less flexible than the non-polar ones, which is a factor favoring the decrease of entropy. Consequently, a negative ΔF and spontaneous dissolution can be expected only in those cases when the process is strongly exothermic, and the entropy change is small. Micelle formation is another way of decrease in entropy, as the system becomes more ordered. In the case of soap, the spontaneous dissolution is due to the hydration of the polar heads which ensures a negative ΔH. Moreover, a considerable decrease in free energy is due to the fact that in the case of free soap molecules the interfacial energy of the hydrocarbon-water interface is very high, and it becomes reduced upon aggregation into micelles. The increase in temperature has an opposite effect, as the numerical value of the entropy member $T\Delta S$ then increases. Hence, a decrease in the temperature should favor micelle formation. This is in accord with experience and also with the molecular-kinetic considerations.

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CHAPTER 13

Flocculation and Gelation Phenomena

1. Flocculation in Non-polar and in Polar Systems

In the process of *flocculation* or *coagulation*, colloidal particles aggregate into larger units, which then usually precipitate (or float on top). The term '*precipitation*' is a broader one; micromolecular substances can also be precipitated. When applied to colloids, the term 'precipitation' can be used either to describe the whole course of flocculation, or only its final phase, in which case precipitation would be synonymous with sedimentation.

The first phase in any flocculation or coagulation is *aggregation*. If the latter happens to occur only to a very slight degree, the process is not called 'flocculation', but aggregation. An indescribable variety of these aggregation and coagulation phenomena are encountered in reality. As the particles are moving continuously (Brownian movement), they collide and occasionally stick together. Hence, a slow aggregation may occur in time. Such slow spontaneous aggregation is actually observed in many colloidal systems, and it is called *ageing*. It is conceivable that the collision of the particles of the various biocolloids in tissues is one of the causes of biological ageing. The colloidal structures are coarser in old organisms than in young bodies. The water content (hydration) in the former is lower than in the latter.

With respect to flocculation phenomena, all colloidal systems may be divided into two classes: *non-polar* and *polar systems*. Examples of the first are: the non-conducting solutions of rubber and polystyrene in benzene, of polyvinyl chloride in chlorobenzene,

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or of saran in dioxane. Examples of polar systems would be: nitrocellulose in acetone, glycogen in formamide, and albumin in water. It is desirable to classify the polar systems further into *non-ionic* and *ionic* solutions. Polar non-ionic solutions, such as nitrocellulose in acetone, or glycogen in water have several features common to purely non-polar systems. Micellar colloids may be also either non-polar or polar, e.g. soaps in hydrocarbon media are non-polar, whereas aqueous soap solutions are polar and ionic.

As indicated earlier, the stability of non-polar organic colloids is due to *solvation*. When a non-polar macromolecule is split off the bulk of the solid by a solvent, it is because of the solvation which overcomes the cohesive forces. The solutions thereby formed are very stable, especially when the degree of dilution is great. Flocculation or precipitation can be achieved by adding certain liquids which are non-solvents for the particular polymer and which are miscible with the solvent. By adding the non-solvent, the solvent is deteriorated, and the macromolecules will be pushed out of the solution. The precipitate formed may be either a rather compact solid or a gelatinous mass. The amount of the non-solvent needed for precipitation or flocculation depends on the following factors: (1) chemical properties of all components involved, (2) concentration of the polymer, (3) molecular weight (degree of polymerization) of the solute, and (4) the temperature. Regarding chemical properties: the more the precipitant differs in polarity from the solute the more powerful a flocculant it is. For example, methanol is the most powerful precipitant for rubber or polystyrene (which may be dissolved in benzene); the flocculating power decreases as the lipophilic part of the alcohol molecule increases. For polar polymers, such as acetyl cellulose, the reverse is true: methanol is the weakest precipitant (for acetyl cellulose in acetone), and the flocculating capacity increases with increasing molecular weight of the alcohols[1].

Several authors have observed the fact that stability maxima and minima occur when flocculation of polar colloids is carried out with polar non-solvents. For example, turbidity maxima have been observed with cellulose ethers in water-alcohol mixtures[2]. Ethylhydroxyethyl cellulose, for instance, has a tendency to flocculate in a water-propanol mixture containing 15–30% of *n*-propanol. The aggregation of the macromolecules at this propanol concentration is accompanied by a decrease in viscosity, which indicates that the originally fibrous macromolecules have aggregated into compact globular particles. The well known tendency of water and alcohol

molecules to associate may possibly provide an explanation of these facts. At some definite proportions of the water and alcohol there may be a minimum of solvation resulting in aggregation and coiling up of the linear macromolecules of the mentioned cellulose derivatives.

The influence of concentration and molecular weight was discussed earlier. The more concentrated a solution, the less of a non-solvent is needed in order to produce flocculation. The larger molecules from a polymer-homologous mixture thereby aggregate first, as their solubility in the particular mixture of the solvents is lower than that of the smaller molecules. A decrease in temperature facilitates aggregation in most cases.

Solutions of salts and other electrolytes as flocculants are of little importance for non-polar systems for the simple reason that they are insoluble in non-polar liquids. A few salts are soluble in lipophilic liquids, e.g. anhydrous aluminum chloride can be dissolved in ether or carbon tetrachloride. The electrolytic dissociation in such media with low dielectric constant, however, is insignificant. Such salt solutions have little effect on the stability of non-polar colloids in non-polar liquids. The same is true for polar but non-ionic systems, such as nitrocellulose in acetone, or starch in water. Their stability is not affected appreciably by electrolytes; for example, starch is not precipitated by salt solutions, acids or alkalies. Electrophoretic phenomena have been observed in non-ionic systems, though they are not as pronounced as in ionic conductors. Sometimes the electrophoretic mobility in such non-ionic solutions is caused by impurities (traces of adsorbed ions). Occasionally a few ionogenic groups may be chemical constituents of a macromolecular substance, e.g. some ionogenic end groups in a water-soluble polymer. The stability behavior in such instances is influenced very little by these groups, since they are vanishingly small in comparison with the size of the macromolecules. Solutions of polyvinyl-pyrrolidone in water are examples of polar and very weakly ionogenic systems. The polymer cannot be precipitated by dilute solutions of electrolytes, although flocculation can be achieved by adding a concentrated solution of ammonium sulfate. The higher polymeric fractions of this polymer need less ammonium sulfate for flocculation than do the lower molecular fractions[3], the concentration of the electrolyte being 0.7–0.9 *M*/l. The hydrosols of other similar polymers and polysaccharides can also be coagulated by concentrated solutions of ammonium sulfate and other salts[4].

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2. Flocculation of Lyophobic Colloids

The stability of lyophobic colloids is determined by the *charge* on the particles. Since electrical charges of the same sign repel each other, two similarly charged particles will remain separated on a close approach in their motion. This subject was treated recently from a theoretical point of view by Hamaker[5], Deryagin[6], Verwey[7] and Overbeek[8]. The interaction between two electrically charged

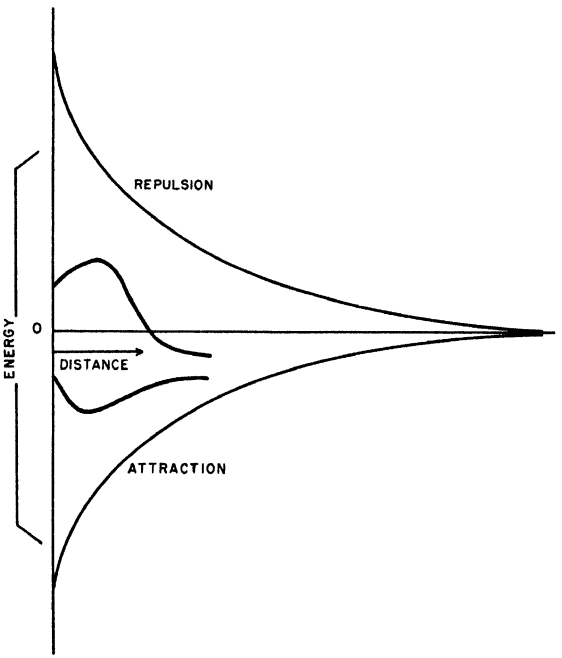

Fig. 84. Forces of attraction and repulsion near the surface of colloidal particle. Examples of potential curves (interaction energy versus distance).

particles is decided by two opposite forces: the Van der Waals-London *attraction,* and the electrostatic *repulsion.* Both forces decrease with increasing distance from the surface of the particle. This is shown graphically in Fig. 84. The energy resulting from the mutual interaction of both repulsion and attraction can be calculated for various cases. In order to keep the sol stable, the resulting potential curve must have a maximum at a sufficient height. Added

electrolytes shorten the range of the repulsive forces, and the attraction becomes predominant. From the point of view of the double layer theory, the added electrolyte decreases the thickness of this layer. The Van der Waals-London attraction is not influenced by adding an electrolyte to the colloid.

The theory was successful in substantiating the fact that bivalent ions must have about a hundred times greater flocculating power than the monovalent ions have. This fact was discovered long ago by Schulze, and much additional data on different colloids and electrolytes has been collected by Hardy, Freundlich and others. In the first place, it was observed that the *valency of the cation* is decisive in the coagulation of *negatively* charged particles, and that the valency of the *anion* is important in the flocculation of *positively* charged sols. Furthermore, it was noticed that the flocculating power of bivalent ions is not just twice that of the monovalent ones, and that the trivalent ions are not only 3/2 times more active than the bivalent ions. The *flocculating activity increases between ten and a hundred fold on increasing the valency of the acting ion by one.* This is known as the Schulze-Hardy rule.

The flocculation experiments are usually carried out in a series of test tubes. Equal amounts of a colloidal solution are introduced into each tube, and equal volumes of a flocculating agent are added, the concentration of the latter being varied stepwise from one tube to the next. After thorough mixing, the series is left alone, and the flocculation is observed after definite time intervals. The classical method for determining the flocculating value of an electrolyte was to estimate the *minimum concentration* which was able to produce turbidity or precipitation in the series, under the same conditions. For example, the concentration of sodium chloride added to an emulsion in a series may vary as follows:

Concentration in *M*/l: 0.20, 0.18, 0.16, 0.14, 0.12, 0.10, 0.0. If the emulsion is 'broken' with, say, 0.20 and 0.18 *M*/l NaCl, and if there is no change with 0.16 *M*/l NaCl, the flocculation value of NaCl in this case is 0.18 *M*/l. For a closer estimate another series could be run with concentrations between 0.18 and 0.16, e.g.: 0.18, 0.175, 0.170, 0.165, and 0.160 *M*/l NaCl, and it might be found that the more correct flocculation value is 0.170. Another method is to measure quantitatively the turbidities produced, plot them against the concentration of the electrolyte, and estimate the flocculation value by graphical extrapolation[9].

The flocculation values of mono-monovalent electrolytes (e.g.

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NaCl, KNO_3) are about 20–80 millimoles per liter of the sol-electrolyte mixture for the lyophobic colloids. The flocculation values of electrolytes with bivalent cations depend strongly on the charge on the flocculating particles. For sols and emulsions with negatively charged particles, the electrolytes with bivalent cations have small flocculating values of 0.3–0.9 millimoles per liter. The values for other cases are given in Table 32.

TABLE 32

FLOCCULATION VALUES FOR LYOPHOBIC COLLOIDS

Electrolyte type		*Negative particles*	*Positive particles*
Me^+An^-	(e.g. NaCl)	20 – 80 millim/l	10 – 80 millim/l
$Me^{++}An^-$	(e.g. $CaCl_2$)	0.3 – 0.9	5 – 15
$Me^{++}An^{--}$	(e.g. $MgSO_4$)	0.6 – 0.9	0.2 – 0.5
Me^+An^{--}	(e.g. K_2SO_4)	25 – 90	0.2 – 0 3
$Me^{+++}An^-$	(e.g. $LaCl_3$)	0.003 – 0.03	
$Me^{+++}An^{--}$	(e.g. $Al_2(SO_4)_3$)	0.009 – 0.05	

The smaller the *flocculating values*, the greater the *flocculating power* of the electrolyte. For the negatively charged colloids, the flocculating values of NaCl, $CaCl_2$ and $LaCl_3$ (or $AlCl_3$) are in the ratio of about 50 : 0.5 : 0.005. The flocculating power will be the reciprocal of these figures, or: 0.02 : 2 : 200. These figures may change markedly, depending on the system and method of evaluation. The fact remains, however, that for negatively charged particles, which are the most common, electrolytes with bi- and polyvalent cations possess much greater flocculating power than monovalent ions. Hence, for the flocculation of muds and for breaking emulsions, salts like calcium chloride or aluminum sulfate are the most effective reagents.

There are not many truly lyophobic systems among the organic colloids. The dispersions of finely ground space polymers and some emulsions have a lyophobic character. The very stable emulsions, however, possess a stabilizing lyophilic layer around the droplets, and the stabilizer renders lyophile properties to the system.

3. Flocculation of Electrically Charged Lyophilic Colloids

The variety of electrically charged lyophilic colloids is enormous. Many examples can be found of great stability toward the flocculat-

ing influence of non-electrolytes and electrolytes. However, there are also many biocolloids which are relatively unstable toward electrolytes. The behavior of a particular colloid, however, is more or less completely predictable, on the basis of the following considerations:

(1) The flocculating ability of a non-electrolyte for a colloid depends chiefly on the *solubility* of the colloidal solute in the non-electrolyte;

(2) The flocculating ability of electrolytes depends on the charge and valency, but to a lesser extent than in lyophobic sols;

(3) A *desolvating* activity of the electrolyte must be considered, as well as discharging effect.

As the stability of electrically charged lyophilic colloids still depends largely on solvation, the solubility relations of added non-electrolytes have a decisive influence on the state of aggregation. The addition of alcohol to a solution of gelatin and to a soap solution are illustrative examples of this point. The added alcohol causes flocculation of the gelatin, but the soap is affected in the opposite way—the micelles are dissolved and a micromolecular soap solution in the alcohol-water mixture is obtained. It is obvious that substances which are soluble in the particular liquid used as a precipitant will not be flocculated by it, and vice versa.

The influence of the electrolytes added to a lyophilic colloid is *twofold*: they act on both the *electrical* properties and on the *degree of solvation*. At low salt concentrations the charge is mainly affected, while at high salt concentrations both charge and solvation are influenced. Lyophilic colloids require more of the electrolytes for flocculation than are needed for lyophobic sols, but the differences between various colloids are considerable. The stability of amphoteric colloidal electrolytes depends on the acidity of the solutions (pH), and the stability has a minimum at the isoelectric point. Some proteins, such as casein, are precipitated at the isoelectric point. When skim milk is acidified with a dilute solution of an acid, flocculation of the casein occurs, if the pH is reduced to 4.7. In strongly acid solutions, however, a stable positively charged casein is obtained. In this instance the stability is determined chiefly by the charge on the particles. Many other proteins, however, are stable at the isoelectric point. Casein can also be coagulated by adding salts to the solutions, although the flocculation values are higher than for the unstable inorganic sols. The negatively charged casein particles can be coagulated by calcium chloride and

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other bivalent cations much more readily than by mono-monovalent electrolytes (with the exception of acids). The alkaline soaps also are easily coagulated by the heavy metal salts, although they are relatively stable toward neutral mono-monovalent salts.

The flocculation effects produced by bi- and polyvalent ions may be very complex: flocculation often occurs either at very low or very high concentrations, but at medium concentrations (of about 0.001–0.01 *M*/l) the sols are stable. The original negative charge of the particles in such stable sols then is reversed to a positive charge by the adsorbed cations. A whole group of proteins, the so-called globulins, have maximum stability in the presence of low and medium salt concentrations. They are precipitated on complete dialysis, or by very concentrated solutions of certain salts. Moreover, the stability of such colloids depends on the acidity (pH) of the solution. The closer the pH is to the isoelectric point of the protein, the less the stability (Chap. 26). If a globulin precipitates at its isoelectric point, it usually can be dissolved by adding moderate amounts of a salt solution. Such dissolution is often called *peptization*. It is explained as the result of adsorption of ions which render a charge to the particles.

Deuel and associates have thoroughly studied the flocculation of the pectins and other ionic polymers[10]. The pectins are composed chiefly of partly methylated polygalacturonic acid. The linear molecules can be considered as polyvalent units, since many carboxyl groups are bound to the chain. The colloidal solutions of the pectins can be coagulated by acids and salts, as well as by alcohol. About a hundred times more potassium chloride than calcium chloride is needed in order to produce flocculation. If 70% of the carboxyl groups of the polymer are esterified, the stability toward the electrolytes increases. Moreover, the greater the average molecular weight, the less stable are the colloids.

4. The Lyotropic Series and 'Salting Out' Effects

The salt concentrations needed for the precipitation of hydrophilic colloids are many times greater than those needed for the flocculation of hydrophobic sols. For example, the various proteins of blood plasma are precipitated with not less than 1.3–2.5 *M* per liter of ammonium sulfate. The action of the salt in this instance is not the discharge of the colloid but the deterioration of the solvent. The term *'salting out'* is used in such cases instead of flocculation.

One characteristic feature of the salting out reaction is the difference in the effectiveness of various ions of the same valency and sign. For example, the same concentration of a chloride is more effective in salting out than iodide. This effect was noticed long ago by Hofmeister (1887) who studied the coagulation of proteins by various salts. The ions can be arranged in series (Hofmeister series) of decreasing or increasing flocculating capacity. For the most common anions (for nearly neutral solutions of proteins) the series reads as follows:

$$\frac{\text{Sulfate}}{2} \text{—acetate–chloride–nitrate–chlorate–bromide–iodide–thiocyanate.}$$

$$\xrightarrow[\text{decreasing flocculating power}]{}$$

For the cations the series is less definite, and it runs as follows: (the anion being the same for all cations):

$$\text{Lithium–sodium–potassium–ammonium–}\frac{\text{magnesium}}{2}.$$

It was stated long ago that the order in each series depends on the acidity of the medium, and Loeb (1920) even disputed the validity of such series. The problem was carefully investigated by Gortner and his colleagues, and it was found that considerable difference in the flocculating capacity of the ions exist, even if the pH is carefully controlled[11]. The radius of the ions and their hydration seem to be responsible for these differences. The ionic interactions are in these cases not as decisive as the solvent effect, since the sulfates are also more potent than chlorides or bromides in the coagulation of such non-ionic colloids as polyvinylpyrrolidone (sect. 1).

Noteworthy is the fact that the stability of various colloids against salting out by one and the same salt depends on the *molecular weight* (particle size) of the colloid, if the series of colloids compared have *nearly the same chemical composition.* This was mentioned earlier in the cases of polyvinylpyrrolidone and pectin. The same is true for the proteins, if we disregard some of those of markedly different chemical composition. For example, the proteins of low molecular weight, such as myoglobin, require the highest concentration of ammonium sulfate for salting out; the medium molecular weight albumins require less; then follow the serum globulins, and finally fibrinogen, the salting out concentrations decreasing as the molecular weights of the proteins increase[12]. These facts indicate

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again that salting out is more similar to precipitation with non-electrolytes than the electrostatic discharge of lyophobic sols.

Finally, it ought to be mentioned that the ease with which lyophilic colloids can be coagulated depends also on the *particle shape*. The more asymmetric the particles are, the faster the flocculation, as the probability of collision is greater in a system with asymmetric particles than with corpuscular ones. However, this does not mean that any linear colloid is less stable than a spherocolloid of similar chemical composition. An extended chain in solution is more solvated than a coiled up chain, for purely geometrical reasons. Hence, the increased change of aggregation due to collision is compensated by the increased solvation. When, however, the solvating barrier is removed by a flocculating agent, asymmetric particles will flocculate faster than spherical ones.

5. Gelation of Lyophilic Colloids

Gelation is the transformation of colloidal solution into a gel or jelly. The dispersed phase does not separate out but the system gradually becomes more and more viscous and finally solidifies. Decrease of temperature or the addition of other substances may cause gelation or setting. Soap, gelatin, agar-agar, pectin, and many other colloids are soluble in hot water, and the solutions set on cooling. It can be shown by means of light scattering and other methods that a *partial aggregation* of the particles occurs upon gelation. However, the aggregation is less complete than in flocculation, and the loose, ramified aggregates *encompass the solvent,* thus forming a coherent system.

The fact that gelation is related to flocculation can be demonstrated by studying the influence of various electrolytes on the gelation. It was found that the same ions which are the best flocculants promote gelation, whereas those which are known as peptizing agents retard it. Gelation can be studied either by measuring the *changes in the viscosity*, or by determining the *gelation time.* The latter is determined by observing a series of solutions which are expected to set at a constant temperature. After definite periods of time, the containers with the solutions are gently and uniformly tilted; when the colloid is set it no longer pours. It was found in this way long ago that sulfates and chlorides promote gelation, but iodides and thiocyanates retard it strongly.

There is a certain correlation between the ability of a sol to set

and the *particle shape*. Spherocolloids do not set, except in some instances when their concentration is very high. The more asymmetric the particles, the lower the concentration of the colloid at which gelation might be expected. The pectins, starch, gelatin, agar-agar, rubber, and many other colloids which set easily have fibrous molecules. It is obvious that fibrous particles will be more capable of forming a coherent meshwork than globular ones. However, not all linear colloids are equally prone to gelation, even if the degree of asymmetry is the same. A 1% solution of gelatin sets easily but 4% nitrocellulose in alcohol-ether mixture (collodion) does not, although the nitrocellulose molecules are more asymmetric than the gelatin molecules. The problem is far from being solved, although it seems that some conditions regarding the possibility of partial aggregation of the participating molecules must be met[13].

6. Flocculation and Gelation in Systems containing Electrolytes and Non-electrolytes

A variety of phenomena have been observed in lyophilic colloids containing salts and non-electrolytes. Aside from plain flocculation or gelation, the colloid can separate as a liquid phase rich in the colloid. The liquid phase first appears in the form of small droplets which subsequently coalesce, and a layer of liquid is formed at the bottom of the container. Such separation of a liquid phase is called '*coacervation*'. For example, droplet formation can be observed in hot concentrated solutions of gelatin after the addition of a sufficient amount of alcohol. The separation of the colloid in the form of a viscous liquid layer is observed in many other cases with gelatin, polysaccharides, and synthetic polymers. Concentrated salt solutions may produce coacervation without or with a non-electrolyte added[14]. In many instances coacervation resembles flocculation and the colloid in the separated liquid phase is partly coagulated or jellied. Coacervation phenomena are observed chiefly with linear colloids, since they are prone to form viscous, gelatinous aggregates when the solvent is deteriorated by adding a substance which disturbs the equilibrium in the solution.

The addition of a non-electrolyte to a hydrophilic colloid containing salt may result in either a decrease or increase in stability. Several proteins, such as albumin, casein, and legumin have been studied from this point of view. It was found that the effect produced by the non-electrolyte depends on its chemical nature and

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on its concentration. Moreover, it depends also on the salts present, and on the properties of the protein. Surface inactive non-electrolytes, such as glycerol, the glycols, and sugars have little influence on the stability. If very large amounts of such organic substances are added, the stability often is somewhat increased because of the viscosity rise produced by these substances. Alcohols and other water-soluble surface-active non-electrolytes have a general tendency to flocculate the proteins. However, certain non-electrolytes are able to produce distinct peptizing effects, especially in the presence of sufficiently high salt concentrations. For example, casein can be dissolved in 40–60% propanol containing about 0.1–0.2 *M*/l calcium chloride or similar neutral salts[15]. This peptizing effect of

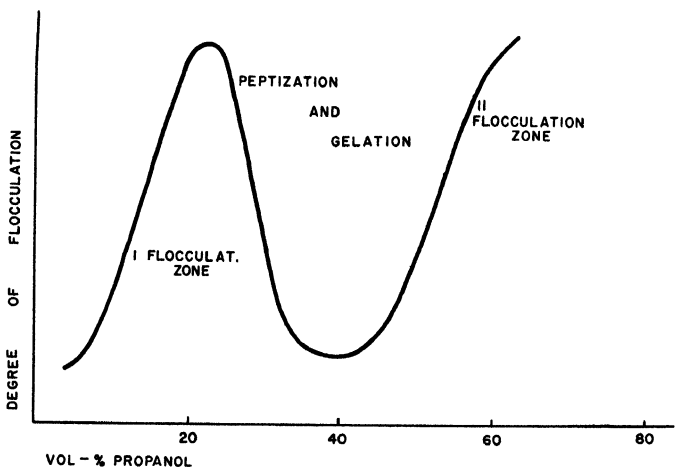

Fig. 85. Flocculation and peptization of albumin in propanol-water mixtures containing salt.

salts in certain mixed solvents can be attributed to complex formation between the proteins and the salt and solvent. It can be shown that the higher the concentration of the alcohol, the more salt is bound by the colloidal casein particles[16]. This peptizing action of mixed solvents containing salts is so strong that even feather keratin can be dispersed, if some thioglycollic acid is added to the mixture. These observations are of practical importance, as some cheap protein materials are useful in the manufacture of artificial protein fibers. In order to do this, the material must first be dissolved in a

proper solvent[17]. The anions which are the weakest flocculating agents, (iodides, thiocyanates) are the best peptizing agents.

Gelation phenomena also are described in mixed solvents. Egg albumin is a spheroprotein, and ordinarily it does not form gels (in egg white it is highly concentrated and mixed with fibrous proteins). However, when this protein is denatured by propanol, translucent gels may be formed under certain conditions. These conditions are: (1) the concentration of the colloid must be sufficiently high (about 2 %), (2) salt must be present, and (3) the propanol concentration must be 40–60 vol.-%. About 0.1–0.4 *M*/1 of a neutral salt must be present, and the gelation time in the presence of various anions corresponds to the order of the lyotropic series, i.e. the gelation time in the presence of a chloride is shorter than in the presence of iodide. The mixtures set to jellies only under these specific conditions; when they are not met, the colloid flocculates or coacervates[18]. Similar gelation phenomena have been observed with mixed solvents containing acetic acid[19] and albumin, and also with some other proteins. It is plausible that this gelation, instead of flocculation, is due to the same causes as the peptization in such mixed solvents (Fig. 85). Whether the mixture remains liquid or not is determined by the concentration of the colloid.

7. The Nature of Forces Involved in Aggregation

Flocculation is due to aggregation of the colloidal particles, and this aggregation can occur when the attractive forces overcome the stabilizing factors. Recapitulating the subject, it could be said that in the case of charged lyophilic colloids there are two stabilizing barriers to overcome: the solvating layer and the repelling charge. The stabilizing effects of the charge are eliminated by adding sufficient amounts of salt. The solvation problem is much more complex, because the colloids considered here are composed of both lipophile and hydrophile residues. Depending on the composition of the atomic groups which are on the surface of the particles, the colloid may be solvated by water only, or possibly more satisfactorily by an alcohol or alcohol-water mixture of proper composition. Gliadin, a protein of wheat, is more easily soluble in 60–70 % alcohol than in water. One of the casein components is also rather soluble in dilute alcohol solutions. However, the peptization in salt-containing propanol-water mixtures seems to be due as much to the salt as to the alcohol. Pfeiffer and his associates[20] have shown that

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those amino acids and peptides whose solubility is increased by certain salts are able to form complex compounds with these salts. A number of such complex compounds have even been isolated. However, no such compounds can be obtained with salts which do not enhance the solubility of the amino acids and polypeptides. Consequently, applying this reasoning to proteins, it seems plausible that solvated complex compounds of the proteins and salts are formed in the cases of peptization.

In flocculation, both the solvating layer and charge are so modified that the particles agglomerate in large clusters; in gelation the solvation is not reduced. If the opposite were true, the solvent would drain out of the gel. The formation of a coherent structure in a gel may be due to various kinds of forces. In gelatin and pectin jellies the fibrous molecules seem to be linked at the points of junction by *hydrogen bonds*. True primary bonds are present in the cross-linked gels of polymers. Such gels are stable, whereas the more weakly linked networks are less stable. For example, the albumin-propanol gels mentioned above are *thixotropic*, i.e. they can be liquified on shaking, and set again when left undisturbed. This indicates that the bonds must be very weak. In most gels of linear colloids the fibrous molecules are interlinked; but examples are also known where the particles are separate. Such examples are certain gels of tobacco mosaic virus. X-ray examination has shown that in some of these gels the virus particles lie about 300 A apart. The nature of the forces involved in these cases is not well understood[21]. It is possible that the water layer between the rod-like particles is strongly polarized, and that the oriented water molecules form a large number of *dipolar bridges*. Similar polarization effects may occur in the *soap gels*, as there cannot be any attraction between the negatively charged 'heads' of the soap micelles.

In non-polar systems, Van der Waals attraction is the only kind of force involved in flocculation and gelation. Since there are neither ions nor dipoles in non-polar colloids, the Van der Waals attraction is reduced to the quantum mechanical effects due to the fluctuation of electrons in atoms. This was studied theoretically by London, and is called the *London force*. The fluctuation of the electrons causes formation of rapidly fluctuating dipoles resulting in mutual attraction. This is a very short range attractive force, as it decreases with the sixth power of the distance between the attracting atoms[22]. The electrostatic dipolar attraction decreases with the square of the distance.

The London forces (also called dispersion forces) are important not only in the attraction between lipophilic particles in aggregation phenomena, but they are also important in considerations of solubility, swelling, chain flexibility, and in the mechanical properties of solid polymers. The effects caused by these forces also depend, among other things, on the geometry of the molecules involved. It was shown, for example, by De Boer[23], that benzene rings will tend to arrange themselves in a stacked up position rather than side by side (Fig. 86), since the force of attraction in the flatly

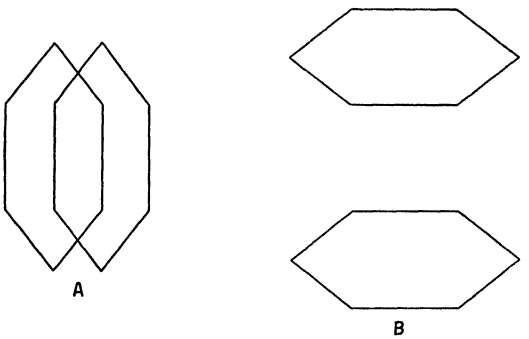

Fig. 86. Favorable (A) and unfavorable (B) spatial arrangement for intermolecular attraction between benzene rings (according to De Boer).

stacked arrangement is about three times greater than in side by side position. This determines to a certain degree the flexibility of polystyrene chains.

8. A Simple Treatment of Salting Out and Coacervation

Dervichian[24] and Lawrence[25] have shown that the separation of phases in many cases of concentrated colloidal systems do not involve specific interactions with the colloid, but the phenomena can be treated as in any partially miscible micromolecular systems. For example, it was shown that salting out of proteins or soaps, or the coacervations of gelatin with alcohol, are governed basically by the same rules as the unmixing of the system water-alcohol-salt. According to Dervichian, all three components in the system soap-water-potassium chloride play equivalent roles. When increasing amounts of the chloride are added to an aqueous soap solution, the

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soap (containing a certain proportion of the other components) separates as a new phase at a certain KCl concentration. This would be the ordinary 'salting out'. However, when soap is gradually added to an aqueous potassium chloride solution, at a certain concentration limit the soap precipitates the salt (or more correctly a phase very rich in KCl). The mentioned authors have even suggested to eliminate the term 'coacervation', as the phenomena can be understood as ordinary unmixing. There is no doubt that this simple treatment is applicable indeed to many concentrated mixtures containing a colloid, water, and a third component. The treatment is applicable even for concentrated mixtures of two colloids the particles of which carry the same charge, as will be shown in the following chapter. However, there are also many other instances when an added flocculant causes flocculation or gelation in specific interactions with the colloidal particles, as it was shown in this chapter.

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CHAPTER 14

Interactions among Colloids

1. The Stabilizing Effect of Hydrophilic Colloids

Hydrophilic colloids are capable of stabilizing various lyophobic sols. Such a stabilizing effect by soaps and similar detergents has already been mentioned. This *stabilization* or *protection* effect is especially important in the preparation of stable emulsions. The lipophilic 'tails' of the soap molecules become immersed in the oily droplets to be stabilized, and the polar 'heads' are turned into the solution. The droplets thereby are electrically charged as well as solvated. Proteins are also good protecting colloids, especially the very hydrophilic proteins, such as gelatin. This protein has proven extremely useful in the stabilization of the hydrophobic sols of gold and silver. An example of stabilization of a hydrophobic organic substance by proteins is seen in the rubber milk—the so-called latex. The rubber particles in latex are coated with a protecting layer of protein. The hydrophobic side chains of the protein are turned toward the rubber, and the polar groups toward water. Stabilization of Congo rubin dye by various hydrophilic colloids is another example of a similar phenomenon. Stabilization in lipophilic solvents is also of importance; for example, zinc and magnesium soaps are employed as protective colloids for dispersions in benzene. In this instance the polar 'heads' of the soap stick to the particle to be stabilized, and the lipophilic 'tails' are immersed in the solvent[1].

Attempts have been made to express the stabilizing power of various protective colloids quantitatively. The lyophobic colloids

of gold or Congo rubin have been chosen as examples of sols which could be protected. The more potent as a protective agent a given colloid is, the less of it is required for the stabilization of a certain amount of a gold or Congo rubin sol. The *gold number* is the weight of the protecting substance in milligrams which is just sufficient to prevent the coagulation of 10 ml of a red gold sol, when 1 ml of a 10% sodium chloride solution is added to it. The *Congo rubin number* was defined as the amount of the protective substance (in mg) which prevents color change in 10 ml of a 0.01% dye to which 0.016 g-equivalents of KCl are added. The gold numbers for gelatin are about 0.005–0.01, for gum arabic they are 0.1–0.2, and for starch 10–25. Hence, gelatin has a much greater protective capacity than the other two above-mentioned colloids.

It is obvious that such different substances as gold and Congo rubin dye would not be protected equally well by all hydrophilic colloids, since this protection must depend on the affinity between the colloidal partners. Williams and Chang[2] compared eleven hydrophilic colloids as protecting agents for both gold and Congo rubin. They found that great differences exist between the affinity of certain hydrophilic colloids to gold and to Congo rubin. The mechanism of protection was also investigated, and it was found that the lyophobic particles are covered by the hydrophile substance. The stabilizing layer seems to be monomolecular.

Some of the synthetic water-soluble polymers have been found to be good protective colloids. Polyvinylpyrrolidone was found very effective in the protection of silver colloids[3], in which case it is as effective as gelatin. The silver halide sols also can be stabilized by this polymer; however, it is ineffective for the stabilization of arsenous sulfide and similar unstable sols. The reasons for this difference are obscure. The effectiveness of various fractions of polyvinylpyrrolidone, which have different average molecular weight, was also studied. It was found that the fractions with mol. weight of about 10,000–30,000 are the most effective stabilizers. The higher molecular fractions were less effective than those just mentioned. This may be due to purely geometrical causes. A large molecular coil of the polymer is less likely to be able to form a protecting layer around a particle than several smaller coils (provided that the total mass of the stabilizer is in both cases the same). The large coil may stick at one side of the particle (Fig. 87), and the protection is then incomplete.It is interesting that the smaller molecules of dextran (a polysaccharide which also is used as a

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blood plasma substitute) are better adsorbed at activated carbon than the larger molecules[4]. The reason is essentially the same as that given above.

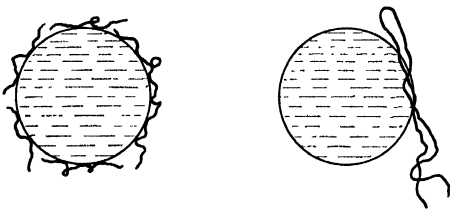

Fig. 87. Protection of hydrophobic colloidal particles by hydrophilic linear colloids. Left: good protection. Right: poor protection.

The binding of the stabilizer to the lyophobic surface may be due to Van der Waals attraction (and ionic bonds as well, if the components carry electrical charges). It ought to be mentioned that the interaction between a lyophobic and lyophilic component does not always lead to a more or less complete stabilization of the lyophobic component, but mutual flocculation may also occur. This will be discussed in the next section.

2. Mutual Flocculation of Electrically Charged Colloids

It was found long ago that flocculation occurs when a negatively charged colloid is mixed with a positively charged one. The flocculation was found to be nearly complete in those cases where the number of opposite charges was nearly the same. Such reactions have been observed with a variety of systems, and they can be illustrated briefly as follows:

$$(\text{Coll.})^{+}_{a} + (\text{Coll.})^{-}_{b} \longrightarrow \underline{(\text{Coll.})_a(\text{Coll.})_b} \downarrow$$

When two oppositely charged hydrophobic colloids react in this way, flocculation always occurs. The same phenomenon occurs when one component of the system is hydrophobic and the other is weakly hydrophilic, and when the latter is not in excess. For example, the positively charged particles of a ferric hydroxide sol are flocculated by negatively charged albumin, if the latter is not added in excess. Albumin in this case acts simply as a flocculating electrolyte[5]. Excessive albumin, however, will cover the ferric hydroxide particles, and they will be stabilized, and will take on a negative

charge. When both reacting components are strongly lyophilic, the interaction may result in a more or less complete aggregation rather than a complete flocculation. For example, when positive (acidic) gelatin is mixed with negative (alkaline) gelatin, no flocculation is observed. Flocculation occurs when the oppositely charged polyelectrolytes are less extensively solvated, or when they are large and very asymmetric, the number and distribution of the charges also being very important.

A great variety of mutual precipitation and coacervation phenomena have been described by Kruyt, Bungenberg de Jong and their associates[6]. Coacervation observed with oppositely charged lyophilic colloids was referred to by these authors as complex coacervation. Depending on the consistency of the precipitate, a complex coacervate may still be highly solvated, or it may be very similar to ordinary precipitates. However, strangely enough, coacervation was observed in certain systems by mixing two negative colloids, such as concentrated gum arabic with warm gelatin. Demixing into two layers occurs. In this instance the colloids do not combine mutually but in the one layer is found chiefly gelatin and in the other gum arabic. According to Dervichian[7], the separation of phases is due to the same reasons as the phase separation of any micromolecular systems, such as water-alcohol-KCl.

A very interesting phenomenon is the precipitation of tobacco mosaic virus by concentrated serum albumin[8]. As in the case of the coacervation of gelatin-gum arabic, both colloids have the same negative charge. However, in this case, the tobacco mosaic virus is forced out of solution by the albumin. The virus is precipitated and the albumin remains alone in the solution. This seems to be due to a competition for the solvent: albumin being more hydrophilic than the virus.

Apart from these phenomena which are determined predominantly by solvation effects, the mutual interaction of charged colloids can be explained as due to electrostatic interactions. The interactions between the following groups of colloids have been studied by various authors:

(1) Polyvalent macro-anions with polyvalent macro-cations;
(2) Macromolecular polyelectrolytes with the amphoteric proteins;
(3) Soaps and detergents with proteins.

Many other combinations are possible, and actually have been studied, but the three groups mentioned have been chosen because of their exceptional importance. Moreover, the mechanics of these

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interactions is relatively clear. The interactions between different proteins are also important and will be treated later.

3. The Flocculation of Polymeric Acids by Polymeric Bases

This group of interactions was studied by Deuel and associates[9], among others. An example is the flocculation of pectin by polyethyleneimine. The pectin contains the carboxyl anions of the galacturonic acid residues, and the polyethyleneimine the $—NH^+_2—$ cations. It was found that there is maximum flocculation when the anions and cations are in equivalent amounts, and the reaction can be visualized as follows:

$$\underset{\text{Pectin}}{...—(C_5H_7O_4COO^-)_n—...} + \underset{\text{Polyethyleneimine}}{...—(CH_2—CH_2—NH_2^+)_n—...} \rightarrow \underset{\downarrow}{\text{Precipitate.}}$$

When an excess of pectin or of the polyethyleneimine is present, the precipitate either does not appear or dissolves on adding the excessive component. The precipitate is dissolved also on adding neutral salts. In solutions containing an excess of one component, the excessive component *stabilizes* the particles containing the minor ingredient, and the stabilized particles are similar to simple complex compounds. If, for example, the ratio of the pectin to polyethyleneimine is 2:1, each molecule of the latter will be surrounded by two molecules of pectin, and the complex will be negatively charged (Fig. 88).

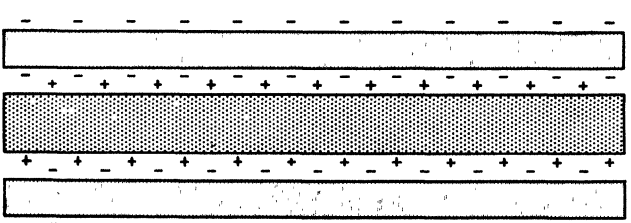

Fig. 88. A complex particle composed of a positively charged component and two negatively charged particles of another colloid.

Since the galacturonic acid residues in the pectin can be esterified to various degrees, this is an excellent example of the effect of the number of charged loci on the extent of interaction. With each carboxyl group esterified, the valency of the macro-ion will decrease

by one. It was found by Deuel and associates that the amount of polyethyleneimine needed to produce flocculation of pectin decreases with increased degree of esterification of the pectin. This indicates that the flocculation is of electrostatic nature. When the pectin chains contain only a few carboxyl ions, one poly-cation can bind many such chains.

4. Reactions Between Polyelectrolytes and Proteins

Macromolecular polyelectrolytes may either precipitate or peptize proteins depending on the conditions of the experiment. As might be suspected from the previous discussion, precipitation will occur when both colloids are oppositely charged and when they are in approximately equivalent amounts. The prediction has proven true in many observations.

The precipitation of bovine serum albumin by four synthetic polyelectrolytes was studied by Morawetz and Hughes[10]. The influence of polymer/protein ratio, degree of polymerization of the resin, as well as the importance of pH and ionic strength were studied. Polymethacrylic acid, and a maleic anhydride–styrene copolymer were taken as examples of polymeric anions, polyvinylamine was used as an example of a poly-cation, and a diethylaminoethyl methacrylate–methacrylic acid copolymer was used as an amphoteric polyelectrolyte for the flocculation studies. Albumin is a macromolecular ampholyte (see Chap. 10); the molecules possess a negative net charge in neutral and alkaline solutions, and a positive net charge in acidic solutions of pH below 4.7. Hence, as expected, the polymethacrylic acid precipitates albumin in acidic solutions. The polymeric anions combine with the cations of the albumin. With an excess of the polymeric anions, more or less complete peptization occurs. The cationic polyvinylamine, on the contrary, precipitated albumin best in alkaline solutions. This precipitation with the polyvinylamine could be called coacervation, since the separated phase was liquid and highly viscous. Either sodium chloride or alcohol strongly promoted this coacervation. The ampholytic polymer used interacted much less completely with albumin than the poly-anions or poly-cations, and the flocculated components could be separated very easily. The stability of the albumin-polymer compound was great in the case of albumin–maleic anhydride–styrene copolymer, when the components could not be separated and recovered. Separation, however, was possible with

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the polymethacrylic acid–albumin complex. This variety in the stability of the macromolecular compounds seems to be due to stereochemical effects. The degree of polymerization was found to be of little importance in these flocculation phenomena.

The interactions of various polyacids with proteins is especially interesting at pH values which are close to the isoelectric point of the proteins. It was found by Greenstein and associates that sodium desoxyribonucleate can prevent heat coagulation of albumin solutions at pH 5.5[11]. This pH is just a little on the alkaline side of the isoelectric point, hence the albumin molecules at this pH carry a negative net charge. Since the polyanion also is negative, this stabilization cannot be due to electrostatic interaction. It has been found recently[12] that the same stabilizing effects can be achieved with various other poly-anions, such as heparin, dextran sulfate, alginic acid, and bacterial polyglutamic acid. The molecular weight of the protecting agent is not important, as stabilization was observed with the very high molecular desoxyribonucleate (mol. wt. about 10^6) and also with the relatively low molecular dextran sulfate (mol. wt. 8000). The stabilization effects take place when each molecule of the albumin is balanced by about two anion valencies of the polyacid. The mechanism of this stabilization is obscure, especially considering the fact that the polyacid is present in very low concentration (only one part by weight against 100–300 parts of albumin).

Of considerable interest are the interactions of some basic polyelectrolytes with the viruses and bacteria. It was found that polylysine, a synthetic poly-cation made up of lysine residues, is able to inhibit the infectivity of tobacco mosaic virus[13]. In this instance the polyelectrolyte combines with the virus, and the efficacy of the poly-cation depends on its molecular weight[14]. The polylysine is also able to inhibit bacterial growth, and it may cause agglutination of cells[15]. In all these phenomena the poly-cation reacts with the anionic groups of the biocolloids. It is also interesting that this inhibition of bacterial growth can be reversed by the addition of poly-acids. All this is comprehensible on the basis of the views outlined in this chapter. It must be pointed out that lysine, which is the monomeric material of which the polylysine is built, is ineffective.

5. Interactions of Micellar Electrolytes with other Colloids

Interactions among micellar electrolytes and proteins are predictable on the basis of the same considerations as for the previously mentioned systems. The most important factors are (1) the sign of charge of both interacting components, and (2) the ratio of the components. The anionic soaps are the simplest examples of micellar electrolytes, and *excess* of soap has a stabilizing effect on protein particles, as well as on grease, oil, carbon particles, etc. If the other component of the system has electrical charge opposite that of the soap, precipitation occurs provided that the soap is not present in excess.

The interactions between synthetic detergents and other colloids have been studied extensively. The investigations have been of interest due to the importance of the problems of detergency, stabilization of emulsions, and the antibiotic effects shown by most of the cationic detergents. The colloid-chemical reactions between cationic detergents and polyacrylic acid also have been studied[16].

The precipitation of proteins by anionic detergents was studied by Bull, Neurath, and others[17]. It was found that in homologous series of sulfates and sulfonates the salts containing more than eight carbon atoms per molecule can be good precipitants for proteins. This is approximately the same hydrocarbon chain length at which micelle formation begins. The flocculation is incomplete, when a large excess of an oppositely charged protein is present, and there is no precipitation whatsoever, if the detergent is in large excess. Anionic detergents can precipitate proteins only at a pH which is acidic in comparison with the isoelectric point. Hence flocculation series with about equal amounts of protein and detergent at various pH can be used for the determination of the isoelectric point of proteins.

Soaps and synthetic *anionic* detergents do not precipitate most proteins from neutral or weakly alkaline solutions, since then the charge of the protein is the same (negative) as that of the micellar detergent. The *cationic* detergents, such as octadecyl-dimethyl-benzyl ammonium chloride, however interact with the proteins. It was shown recently that this cationic detergent can be used for the fractionation of proteins[18]. The fractionation can be carried out in neutral or weakly alkaline solutions in the presence of sodium chloride.

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The interaction of the anionic detergents with proteins has also been studied by means of electrophoresis. It was found that complexes between egg albumin and alkylbenzene sulfonates were formed even at pH values which were alkaline to the isoelectric point[19]. When the protein was in excess, the weight ratio of the protein and detergent was 3 : 1 in the complex. The anions of the detergent presumably combine with the cations ($—NH^+_3$) of the protein, as such cations are present, even when a negative net charge prevails on the amphoteric protein. Moreover, it is plausible that the anionic detergent must have affinity to the other sites of the protein which have an electropositive character. A certain affinity and Van der Waals attraction may exist also between the non-polar sites of the detergent and the protein.

Observations on other colloidal interactions support the view that electrostatic interactions of charges of opposite sign are not the only decisive factors in these phenomena. Legumin, a protein of legumes, is insoluble in water, but it is soluble in sodium dodecylbenzenesulfonate detergent solutions. Upon treating legumin with nitrous acid, the free amino groups of the protein are replaced by OH groups, and this deaminated legumin is soluble in the detergent solution. This indicates that interactions between protein and anionic detergent can occur even if the amino groups of the protein are removed[20].

6. Compatibility of Polymers

Investigations regarding the compatibility of polymers are of great practical importance. With respect to the miscibility of two or more solutions, gels, pastes or solids, the same rules hold as do for solubility. Like dissolves like; and like is compatible with like. Hence, it can be predicted that a solution of rubber in benzene will be compatible with a solution of polystyrene in benzene. The benzene solutions of these polymers however will precipitate, when a solution of nitrocellulose in an ether-alcohol mixture (collodion) is added to either of them. The flocculation is caused chiefly by the alcohol and ether, which are non-solvents for hydrocarbon polymers. In the preparation of polymeric mixes for molding and other applications, the final preparations frequently contain very little solvent, or even no solvent at all. In such cases the compatibility is determined by the *chemical composition* of the macromolecules and the substances added. Of the physical aspects, the *polarity* is

the most important property which determines miscibility and compatibility. Non-polar polymers are compatible with other non-polar substances, and polar macromolecules are compatible with other similar compounds. Examples of compatibility are presented in Table 33.

TABLE 33

COMPATIBILITY OF VARIOUS SUBSTANCES WITH HIGH POLYMERS

Substances	*Polymers*				
	Rubber	*Poly-styrene*	*Polymeth-acrylate*	*Cellulose nitrate*	*Cellulose acetate*
Mineral oil	C	C	N	N	N
Benzene, naphthalene, and homologues	C	C	C	L	N
Halogenated cyclic hydrocarbons	C	C	C	L	N
Cetyl alcohol	L	N	N	N	N
Benzyl ether	C	C		C	C
Ethylene glycol monobenzyl ether (benzyl cellosolve)	N	N		C	C
Butyl stearate	C	C		C	N
Butyl acetyl ricinoleate	C	L	L	C	N
Diethylene glycol dihydro-abietate (Staybelite ester 2)	C	C	L	C	N
Butyl phthalate	C	C	C	C	L
Tetrabutyl urea		C	C	C	N
Tricresyl phosphate	N	C	L	C	L

Key: C – compatible; L – limited compatibility; N – not compatible.

The compatibility of polar dispersions with non-polar substances can be achieved to a certain extent by means of detergents, as the latter are able to solubilize lipophilic substances.

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CHAPTER 15

Gels and Membranes

Most colloid chemists use the term 'gel' in a very broad sense. Some apply this term to practically any semi-solid or solid disperse system, such as charcoal, cellulose fiber, grease, etc. McBain suggested distinguishing gels from jellies wherein the term 'gel' would be restricted to the more or less solid crystalline forms, and the term 'jelly' would denote a solidified sol. This suggestion has not been accepted by all colloid chemists. In this treatise the term *gel* is used as McBain used the term jelly[1]. It is difficult to find justification for calling charcoal, silk, or any semi-solid grease 'gel'. Gel is any coherent colloidal system rich in liquid. If the amount of liquid is smaller than that of the solid, the term *'xerogel'* (dry gel) could be used. Jelly may also be used without any confusion for the transparent gels very rich in liquid. In the following discussion, the terms gel and jelly will be used interchangeably and they will be applied only for coherent colloidal systems rich in solvent.

It is interesting that all semipermeable membranes are gels. As the water content of membrane gels is low, dry membranes belong to the xerogels.

1. The Formation of Gels

Some of the ways in which gels or jellies are formed have been mentioned before, especially in Chapters 12 and 13. Recapitulating, we might state that gels can be formed in the *setting* of certain colloidal solutions, and also in the *swelling* of solid colloids (or xerogels). Cooling of hot solutions of gelatin, agar-agar, starch, or

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many other colloids is a very common way of making gels. Direct swelling usually produces gels which contain less liquid than those obtained on cooling hot solutions. Exceptional in this respect are the cross-linked polymers which are able to absorb astoundingly large amounts of solvent.

Besides swelling and gelation, organic gels can be formed in the following ways; (1) mutual *coagulation* of two or more colloids, (2) flocculation with salts or non-solvents, (3) certain *chemical reactions* involving changes in particle shape, and (4) by *evaporating* certain colloidal solutions. In the flocculation phenomena observed with organic colloids, the phase which separates out is often a very voluminous gel. This is due to the highly solvated state. Voluminous, viscous gels appear particularly in flocculating linear colloids, since the fibrous aggregates are prone to occlude large amounts of liquid.

The formation of gels by chemical reactions associated with changes in molecular shape is important in the case of proteins. The cooking of egg white is an example. In this case the proteins of egg white are denatured, some uncoiling of the globular molecules occurs, and new secondary (probably also some primary) links are formed in the system. Another important example is blood clotting. The spontaneous polymerization of styrene and certain other monomers into solid gels can also be included in this category. The long fibrous macromolecules always encompass some liquid, unreacted monomer, so that the polymer appears as a gel.

One of the simplest ways to prepare gels is to liberate the colloidal solution from the excess of solvent by evaporation. This method is particularly suitable when the solvent is volatile. For example, collodion is a solution of nitrocellulose in a mixture of alcohol and ether; when this colloid is left in an open bottle or dish, a nitrocellulose gel is obtained. On a very complete evaporation of the solvent, a dry xerogel in the form of a solid residue will remain. Nitrocellulose *membranes* of various average pore diameters can be prepared in this way, when a layer of the solution is allowed to dry out on a surface. The more complete the drying the more compact the meshwork of the fibrous macromolecules, and hence the smaller the pores.

2. The Structure of Gels

Recent studies on gels by X-ray structural analysis and electron microscopy have established the concept that only fibrous particles

are able to form a coherent meshwork which can hold large amounts of liquid. Generally speaking, all the *liquid-rich gels* containing about 95–99% (or even more) of the solvent are built of very *asymmetric, fibrous macromolecules.* These macromolecules build a meshwork or skeleton which adsorbs and immobilizes the liquid. There are numerous examples in the realm of plants and animals *of gelatinous appearance,* and they all are due to fibrous structures containing a surprisingly large amount of water. Let us consider the simple fact that a cucumber or water melon contains more water than milk, although milk is liquid whereas cucumber or water melon are solids. The hydrated fibrous structures are responsible for this high water content. Upon removing most of the liquid of such gels, tough xerogels are obtained. Electron micrograph of a xerogel is presented in Fig. 89.

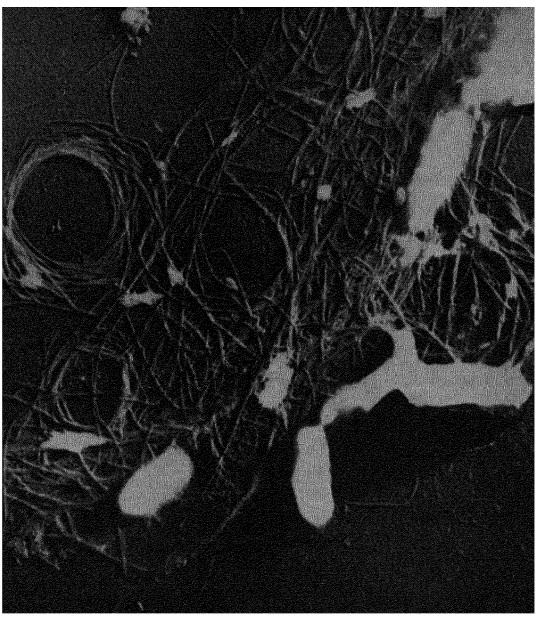

Fig. 89. The structure of bacterial cellulose, according to Muehlethaler. Electron micrograph of a cellulose xerogel. Magnif. 11,000 ×.

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The less asymmetric are the macromolecules or micelles, the higher must be their concentration in order to produce a gel. There are no gels of globular particles which contain as much as 94% liquid. However, gels with moderately asymmetric particles are known, e.g. the soap gels are composed of cylindrical or laminar micelles. In this instance the asymmetry of the constitutive entities is smaller than in agar-agar, pectin, or fibrin gels, and the water content is correspondingly less. Particles of moderate asymmetry often become aligned into end to end aggregates which then form some type of skeleton in the gel[2]. The older concept that gels are composed of spherical particles holding thick hydrate layers around them has now been abandoned.

The nature of forces and linkages responsible for the coherent structure of gels is not understood in all details. It is generally accepted that in most gels the parts of the macromolecular or micellar framework must be linked either by a few primary bonds or many secondary valencies. There is no doubt that *primary bonds* are involved in all gels of cross-linked polymers, such as the previously mentioned gel of swollen polystyrene which is cross-linked by divinyl-benzene[3], or swollen vulcanized rubber. In the gels of gelatin, pectin, nitrocellulose and many other linear colloids, however, such interlinking by primary bonds is not probable. The gelatin gels may be liquified by slight warming. Many gels of highly diluted linear colloids are thixotropic, i.e. they can be reversibly liquified upon vigorous shaking. This indicates that primary valency forces hardly could be involved in this inter-linking. It is more likely that hydrogen bonds and other kinds of residual valencies are involved in the formation of the coherent structures. In the case of very asymmetric macromolecules of non-polar, linear colloids, mechanical entanglement of the molecules may also be of some importance in the gel formation.

The concept that the framework in a gel must be coherent is supported by a number of experiments. For example, a concentrated dispersion of graphite does not conduct electricity in a liquid state, but becomes a conductor after it sets. This proves that many contact points between the graphite particles are formed upon setting. Coherent structures are directly visible in the electron microscope, although the objection may be raised that the specimen observed may differ in some respects from the original gel.

Apart from the directly bonded structures, there are gels the framework of which is somewhat similar to an expanded crystal.

Although the liquid content of these systems is relatively much lower than in the gels of linear colloids, they may also be classified as gels. The specimens of swollen tobacco mosaic virus, and some soap gels belong to this group. According to the X-ray diffraction data, the rod-like particles of the tobacco mosaic virus are arranged in parallel arrays. The distances between the rods are considerably larger than should be required for direct interlinking with the short side chains of the protein molecules (sect. 7 in Chap. 13).

The degree of crystallinity or order in gels may be very different in various cases. In the case of tobacco mosaic virus the degree of order is very high. A relatively high order is found also in stretched gels of linear colloids, as revealed by the X-ray analysis. A qualitative evaluation of the degree of alignment of the fibrous constituents can be done simply by the appearance of the gel. The highly translucent, non-opalescent jellies have a very amorphous, completely disordered framework. The opalescent and turbid gels, on the other

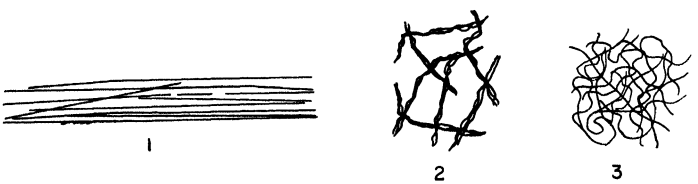

Fig. 90. Schematic presentation of several gel structures.

hand, are composed of coarse, partly aggregated entities. In the case of linear colloids, such aggregation will always be more or less lateral, resulting in an increased order. The various possibilities of structures are presented schematically in Fig. 90.

3. Mechanical Properties and Structure of Gels

Rigidity and tensile strength are important mechanical properties of gels, which can be correlated with the structure. Rigidity is defined as the ratio of shearing stress (force per unit area) to strain (angular deformation observed). The greater the stress and the smaller the deformation, the greater the rigidity of the gel. This is essentially the same property as viscosity, and it may be studied by subjecting a gel to torsion between two concentric cylinders in an apparatus like the Couette viscometer (p. 140). Another method of measuring rigidity is to determine the amount of sagging of the

gel under its own weight. Furthermore, blocks of gels can be stretched or pierced. Various apparatus have been designed for such experiments[4]. Table 34 shows three examples of gels with different degrees of rigidity which were determined by three different methods.

TABLE 34

MECHANICAL PROPERTIES OF SOME GELS
(from Deuel, Solms and Altermatt, ref. 10, Chap. 13)

Gel	*Rigidity by the concentric cylinder torsion*	*Amount of sagging, %*	*Relative tensile strength*
Pectin gel, 0.4%	1.2	24.8	51
Agar-agar, 0.5%	1.5	15.6	16
Gelatin gel, 4.5%	2.8	11.5	48

The more rigid the gel the less the sagging. The tensile strength, however, does not always vary in the same way as the rigidity measured by the torsion effects between concentric cylinders. It is also conspicuous that the pectin of low concentration (0.4% solid) has approximately the same tensile strength as a much more concentrated gelatin gel (4.5% solid material). The reason for this is a difference in the *molecular shape*. The pectin macromolecules are more asymmetric (less kinked) than the macromolecules of gelatin.

The rigidity of gels increases with the *concentration* of the solid component of the gel (for the same components). It was also found that, for constant weight concentrations, the rigidity increases with the average *molecular weight* of the macromolecular substance. In all these comparisons an important parameter is the *temperature*, which must be constant. The rigidity is inversely proportional to the temperature. The gelatin gels have been thoroughly investigated by Ferry (*loc. cit.* ref. 2), and the dependence of the rigidity upon the mentioned factors is shown in Figs. 91 and 92.

The decrease in rigidity with increased temperature is comprehensible, since the weak junctions of the strands will be disrupted upon strong molecular impacts. The melting points of gels are important characteristics for those gels which melt. The rigidity and melting point of gelatin gels depends very little on pH. For a 3% gelatin gel, the rigidity did not change within the pH limits

of 4.6–8.2 (Gerngross, 1926), and the melting points, too, are practically constant in these limits.

Most gels are *elastic*, i.e. they recover their original shape after removal of the stress. There are, however, various degrees of elasticity in various gels. Contrary to the elastic, quivery gels of rubber, gelatin, or pectin, polyvinyl chloride in cyclohexanone forms relatively plastic systems which do not recover their shape upon

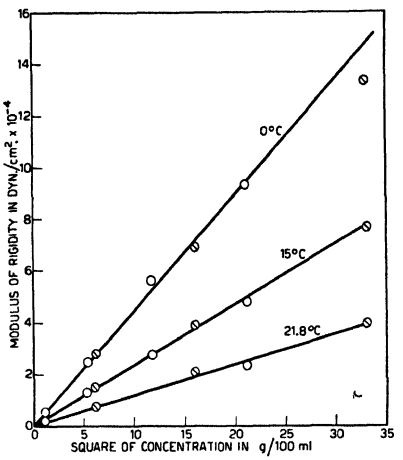

Fig. 91. Rigidity of gelatin gels plotted against square of concentration at three different temperatures. Data of J. D. Ferry (1948). (From *Advances Protein Chem.*, 4 (1948) 1.)

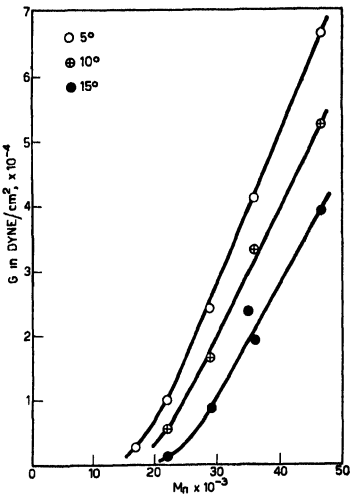

Fig. 92. Rigidity of gelatin gels plotted against number average molecular weight. Concentration – 4%. Data of J. D. Ferry (1948). (From *Advances Protein Chem.*, 4 (1948) 1.)

removal of the stress. According to Ferry[2], the elasticity is due chiefly to the mode of linkage between the strands. When there are few points of junction at widely separated points, the gel will be more elastic than in the case of weak attraction at any point along the chains. In the latter case, the macromolecules will glide along each other upon deformation, and become interlinked at any new position or state of deformation.

4. Syneresis of Gels

The structural stability of various gels is very different, depending on the structure, concentration, and the affinity of the colloidal

structural elements for the liquid. Most stable are the chemically cross-linked gels holding a liquid which has strong affinity for the macromolecules. For example, the highly swollen cross-linked polystyrene-benzene gels of Staudinger[3] have been known to be stable for years. The gels with secondary valency junctions are less stable, although they may maintain their structure for weeks or even longer. The less asymmetric the particles, the less stable the gel. Moreover, an important structural property which decides the stability is the degree of dispersion or *coarseness* of the strands. The coarser the meshwork the more easily the liquid will be liberated from the gel. Such spontaneous liberation of the liquid from a gel is called *syneresis* (meaning: taking together). The 'perspiring' of cheese, which is a complex gel of casein, is an example of syneresis. Diluted gels of sodium soaps also expel liquid easily. The coarser the structures the less strongly the liquid is held inside the gel. This can be demonstrated best with a series of denatured albumin gels in water-propanol mixtures containing salt (*loc. cit.* ref. 18, Chap. 13). The gels with 2% albumin, 50 vol.-% propanol and 0.3 *M*/l $CaCl_2$ are clear and stable. The moderately asymmetric particles of the denatured protein seem to be linked by weak secondary bonds, since the gels are thixotropic. When the salt concentration is only 0.1–0.15 *M*/l, the resulting gels are coarse, turbid, and separate out the liquid. Likewise, the coarseness may be increased by taking 35–40 vol.-% of propanol (or 65%) instead of 50, and in all cases of turbid, coarse gels, strong syneresis was observed.

Syneresis is the reversal of swelling. Various ions promote syneresis in the same order as they promote gelation, and in the same order as they retard swelling (lyotropic series), i.e. sulfate is more active than chloride, and the iodides and thiocyanates are least active. Hence, syneresis can be regarded as flocculation, or as a result of flocculation of some gel components. It can be assumed that only part of the colloidal particles are linked into a coherent framework, and that some particles are still relatively free. Because of Brownian movement, they eventually collide and cling together, exactly as it happens in solutions. Because of this aggregation, the coarseness increases, the size of the intermolecular space also increases, and the liquid can spill out. Various intermediate stages between clear, stable, highly disperse jellies on the one hand, and coarse, fast synerizing gels on the other are known. The same continuity exists between liquid coacervates and gels of various degrees of consistency.

5. Diffusion and Chemical Reactions in Gels

Small molecules and ions spread in gels easily. The diffusion rate of micromolecular substances in jellies rich in water is the same as in water. This is not surprising, when we consider the fact that such jellies are composed of 99% or more liquid. The ability of small ions to diffuse in gels is the same phenomenon as their ability to pass the semipermeable membranes in ultrafiltration and dialysis. Conductivity of ions also is not impeded in highly diluted jellies.

This property of gels of not retaining small molecules and ions makes them convenient media for the study of diffusion and some chemical reactions. Colored substances, of course, are the most convenient to observe. The only important condition for successful experimentation, then, is that the gel must be chemically indifferent to the substances studied. The ordinary gelatin gels can be used in many cases, for example, to demonstrate the difference between the diffusion rate of colloids and micromolecular substances. A high quality commercial gelatin can be used. The weighed gelatin (the amount being calculated for a 1% solution) is first washed with distilled water for several hours. The swollen colloid is then dissolved at 80–90° C, and diluted to the desired volume. (Boiling is not advisable, as it may reduce the ability to set.) The hot solution is then distributed to several test tubes, which are filled to about 2/3 with the colloid. The solutions are placed in a refrigerator, where they set within a certain time. The diffusion of various colored solutions may now be compared, if they are poured on the gel and observed after several days. For example, one can use the colored copper salts or chromate ions, and discover that they diffuse much faster than hemoglobin.

The formation of precipitates in gels can be observed, for instance in U-tubes containing a gel, by adding the reagents on top of the gel. Another method is that of dissolving the one component together with the colloid, and placing the other component on the gel. The precipitates formed in gels often have a periodic structure. Silver chromate is an example of precipitates which appear in periodic layers, but many organic substances are also precipitated in similar periodic layers, e.g. the banded structures in gallstones. Formation of supersaturated solutions and even colloids of the precipitating material were considered in an effort to explain the formation of the rings (called Liesegang rings, after the discoverer of the phenomenon). Stabilization of the temporarily formed col-

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loidal precipitate by adsorption of some gel components onto the particles formed was also found to be important. Periodic structures of precipitates may be formed even in the absence of gels, and the structures depend largely on diffusion and super-saturation. In gels the processes definitely are influenced by the gel itself[5].

Gels have also been used recently as supporting media for electrophoretic separation and identification of colloidal electrolytes, such as proteins (see also Chap. 10). When a drop of a protein solution, containing a mixture of proteins, is introduced into a slit cut in a starch gel containing buffer, and an electric potential applied, the proteins travel a certain distance depending on their charge density. The presence of the proteins at various distances from the origin can be detected by cutting the gel and staining it with reagents which reveal the presence of proteins. It was shown that a sharp spectrum of the various protein components can be obtained in this way[6]. The framework of the starch gels, if they are not too dry and dense, does not much affect the electrophoretic mobility of the protein molecules. The very large molecules of some proteins, such as fibrinogen, however, do not enter the gel.

6. Membranes as Gels

All natural and artificial semipermeable membranes are gels or xerogels. According to Elford and Ferry[7], the transparent membranes of collodion have an average pore diameter of 100–400 A. It must be pointed out that channels in a membrane are not of uniform width, and the macromolecular skeleton in a membrane is a meshwork (Fig. 90). There is a variety of pores even within one section of a membrane. A particle which tries to penetrate the membrane has to struggle through a kind of chaotically built grating. The path an ion will have to follow in a dense grating will be much longer than in a loosely built meshwork, in which case micromolecular ions will not be hampered in their migration. This will be the case with the opalescent membranes of collodion (nitrocellulose), since, in such membranes, the average pore diameter is about 400–1200 A. The semicolloids which have a particle size of 5–15 A will be able to pass such membranes, although the dialysis rate will be very slow. The reason for this is that 400 A is not the narrowest channel the particles will have to pass. Four hundred or more A is just an average figure, and there may be sections in the membrane where the space between the strands is only some

few angstroms wide. This is the reason that colloidal particles with diameters of 20–200 A (and more) are unable to pass the membranes which have an average pore size of 100–400 A. The opalescent membranes with pore diameters of 400–1200 A are penetrated only by the smallest of the colloidal particles, and the diffusion rate in the membrane is very small. The chemical nature of the substances involved is also of great importance, as will be shown later.

The diffusion of small ions in membranes of various average pore diameters was thoroughly studied by Manegold[8]. He found that the diffusion is unrestrained only at a certain minimal pore size; for sodium chloride in aqueous collodion this pore size is about 130 A.

Since the membranes are gels, their liquid content is very important. The more swollen a membrane the larger is the average pore size. The commerical cellulose foils used for dialysis (cellophane) are xerogels which swell when placed in water, and the water content of the membrane increases up to 30, 50 or even 80%. Such aqueous membranes will be permeable only for substances which are soluble in water. (The ordinary nitrocellulose membranes are also aqueous, since the alcohol and ether, which has remained after drying, is replaced by water upon washing the membrane with water.) The membranes which are saturated with water cannot be used for dialysing lipophilic systems. However, the water in the meshwork can be replaced by alcohol (or any other solvent miscible with water) on washing the membrane with alcohol for a sufficiently long time. A further exchange of the alcohol can be accomplished upon placing the alcoholic membrane in benzene or similar lipophilic solvent. Such cellulose membranes impregnated with benzene (or a similar lipophilic non-polar solvent) can then be used for liberating any lipophilic colloid from micromolecular components.

Not only the liquid held in the membrane is important, but the chemical nature of the framework also plays a part in these phenomena. Cellulose membranes are chemically rather indifferent, but the polyelectrolyte membranes may show striking selectivity in what they will allow to pass. As examples, the ampholytic protein membranes of animal bladder or the anionic pectin membranes can be mentioned. The skin of apples contains the anionic pectins, and is *selectively permeable* for cations[9]. At a very low concentration of the dialysing electrolyte, only cations will enter the membrane. At a higher concentration, ions of both signs will fill the meshwork (Fig. 93). Exchange of the counter-ions also occurs. Since the cations will pass the membrane faster than the anions, a potential

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gradient will be formed across the membrane. Uneven distribution of ions due to selective permeability causes *bioelectric potentials.* These phenomena may occur also with the amphoteric membranes of proteins in animal body. Some membranes may possibly be composed of acidic proteins (excess of carboxyls over free amino groups), others may have an excess of the basic amino groups. The former will acquire a negative charge, and they will be selectively permeable for cations. The membranes with excessive amino groups, on the contrary, will be positively charged, and will be selectively permeable for anions. Assuming an equal number of acidic and basic groups, the *net charge* of the membrane will depend on the pH of the medium. In such a case, *the selective permeability of a certain*

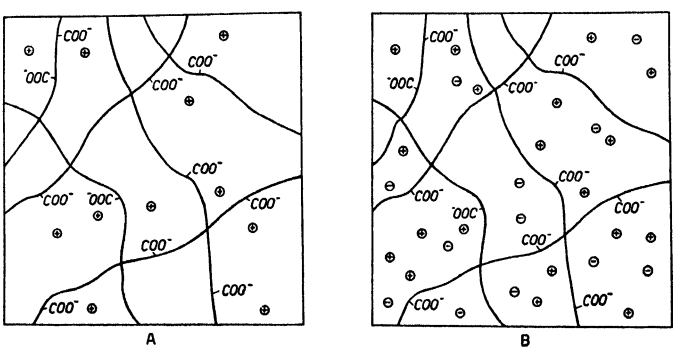

Fig. 93. Selective permeability of ions. A – cation-permeable membrane in a dilute solution of an electrolyte. Mobile cations only are present. B – the same membrane in a concentrated solution of an electrolyte Mobile anions also are present in the membrane. (After K. H. Meyer, *Natural and Synthetic High Polymers,* Interscience Publ., 1950, p. 812.)

membrane will change, depending on the degree of acidity. The membranes in tissues and organs are even more complex, as they contain lipids and other ingredients (Chap. 29).

The electrochemical nature of the membranes is important also in electrodialysis. Sollner and associates[10] have shown that electrodialysis is greatly facilitated by taking a positive membrane at the anode and a negative one at the cathode.

7. Donnan's Equilibrium

The dialysis of *colloidal electrolytes* involves the interesting fact that only the counter-ion is able to pass the membrane, whereas the

colloidal ion is retained. When an aqueous colloidal electrolyte, e.g. sodium polyacrylate or sodium pectate, is placed in an osmotic cell, osmosis will occur, and a certain osmotic pressure will be produced. This pressure will be larger than that expected from the colloidal anions, as the anions require the presence of counter-ions. All sodium ions cannot leave the colloidal solution, because the potential gradient thereby produced will pull the cations back. Those sodium ions which managed to pass the membrane must be replaced by some other cations in order to compensate the charge of the colloidal anions. In water only the hydrogen ions are available; they will pass into the membrane in the direction to the colloid. The result will then be a certain *hydrolysis*, since the acidity of the colloid in the cell will increase. The only reason for this hydrolysis is the inability of the colloidal anion to pass the membrane.

This distribution of ions through membranes in cases of colloidal electrolytes was studied by Donnan and his associates (1911 and later). A mathematical theory was elaborated which enables us to calculate the distribution of ions capable of dialysis, as well as to predict the effect this distribution will produce on osmotic pressure, hydrolysis, and membrane potentials[11]. Let us denote by R^-Na^+ the colloidal electrolyte, by c the concentration of the anions, and by x the concentration of the hydrogen ions which have been exchanged for the sodium ions. Then, at the *start*, assuming that R^-Na^+ is an ideal electrolyte,

Na^+	R^-
c	c , and $x = 0$.

At the *equilibrium* state:

Na^+	R^-	H^+
$c-x$	c	x .

As the hydrogen ions are taken from the water by displacing the ionic equilibrium of water dissociation, an equivalent amount of OH^- ions will make the water outside the cell alkaline.

If the colloidal electrolyte R^-Na^+ is placed against sodium chloride Na^+ Cl^-, an uneven distribution of the diffusible ions will occur. Some of the chloride ions now will diffuse into the cell, and they will be accompanied by an equivalent amount of sodium ions in order to preserve electrical neutrality. Denoting by c_1 the initial concentration of R^-, and by c_2 the initial concentration of Cl^- outside the colloid, the following changes in concentration will take place:

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At start, inside the cell: outside the cell:

R^-	Na^+	Na^+	Cl^-
c_1	c_1	c_2	c_2

At equilibrium, inside the cell: outside the cell:

R^-	Na^+	Cl^-	Na^+	Cl^-
c_1	c_1+x	x	c_2-x	c_2-x.

For very dilute solutions, and assuming the volumes of the solutions inside and outside are equal, the theory predicts that:

$$\frac{[\mathrm{NaCl}]\ \text{inside}}{[\mathrm{NaCl}]\ \text{outside}} = 1 + c_1/c_2.$$

This conclusion is practically important for the osmotic pressure measurements, and for correct evaluation of the data for molecular weight calculations. The osmotic pressure of colloidal electrolytes is always greater than expected, because of the effects produced by the counter ions. But this distortion of the results can be largely compensated by measuring the pressure against a salt solution. The most correct result will be obtained when the concentration of the diffusible electrolyte inside and outside is the same, i.e. when the ratio of the concentrations c_1/c_2 is very small (see the above equation). This will be the case when the concentration of the colloidal electrolyte c_1 is very small, and when c_2 is relatively large. For this reason, the osmotic pressure of colloidal electrolytes is not measured against water but against a salt solution of moderate ionic strength.

Donnan effects must be considered also in studies of the swelling of colloidal electrolytes, and for the permeability of charged membranes. Generally, they are important in all cases when restrained colloidal ions are present.

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CHAPTER 16

Properties of Highly Consistent Macromolecular Substances

1. Mechanical Behavior of Macromolecular Substances

The mechanical properties of polymers are of extreme practical importance. According to the mechanical behavior, the polymers have been classified into *elastomers* and *plastomers*[1]. Elastomers, or elastics, are the rubbery elastic materials. Plastomers, or *plastics*, are macromolecular materials which do not show much recovery on deformation. There are two extreme ways to respond to a deformation. (1) In an ideal *elastic* deformation the changes take place instantaneously, and the body *returns* to its initial state instantaneously upon removal of the stress. (2) In a *plastic* deformation the body *flows*, and the deformation is *permanent*. The force acting on the body is called *stress*. The change in the geometrical state of the material is known as *strain*.

It was shown by Hooke (in 1676) that certain solids exhibit ideal elasticity, and that strain is proportional to stress. If S is the stress or tension, and ΔL the change in the geometrical state, then, according to Hooke,

$$S = E\Delta L,$$

where E is the proportionality constant called the *modulus of elasticity*. When the stress is plotted versus strain, a straight line results which crosses the origin, and has the slope E (Fig. 94). A linear proportionality, however, can be observed only up to a certain limit of tension. At a still higher tension the material begins to flow, the molecules slip along each other, and the material is deformed permanently.

There are three practically important ways of deforming a solid: (1) shear, (2) tension, and (3) compression. Shear is applied in measuring the consistency of liquids, semisolid pastes, and gels. A material is sheared when subjected to torsion between two coaxial cylinders (p. 140). The shear strain is defined as the tangent of the angle of shear. The tensile strain is defined as the increase in length Δl divided by the length l, or $\Delta l/l$. Rods of materials to be tested are subjected to pull in special testing machines. For compression the strain is defined as $\Delta V/V$, where ΔV is the volume decrease of the material being compressed. The stresses in a strained material are defined by calculating the force per unit area, and they are expressed usually in kg per cm^2.

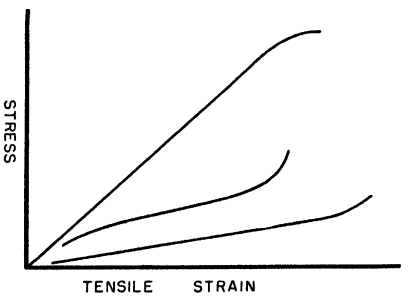

Fig. 94 Curves illustrating the dependence of the tensile strain on stress.

The following other tests are performed in order to characterize a solid polymer: (1) impact strength or shock resistance, (2) hardness (measured by determining the weight needed to make a permanent impression by a steel ball), and (3) fatigue tests, which are carried out by bending a test piece frequently, and determining the number of deformations necessary for break.

Data for a number of practically important polymeric materials are compiled in Tables 35 and 36.

The mechanical properties of polymeric materials, as indicated before, depend on several factors, namely: (1) the *degree of polymerization* (molecular weight, length of the chains), (2) *cohesive forces acting between the chains*, (3) degree of *crystallinity*, (4) *flexibility of the chains*, and (5) their *branching* and *cross-linking*. Moreover, the strength depends on the primary bonds of the linear polymers, although in carbon chains these energies are nearly the same for various carbon compounds.

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TABLE 35

PROPERTIES OF PLASTOMERS (Houwink, *loc. cit.* ref. 1)

Material	*Spec. weight*	*Tensile strength kg/cm²*	*Modulus of elasticity 10^4 kg/cm²*	*Impact strength*
Phenol-formaldehyde, sawdust base, molded	1.4	560	8	7
Phenol-formaldehyde, paper base, laminated	1.3	840	3–21	30
Urea-formaldehyde, cellulose filler, molded	1.5	500	8	7
Polyethylene	0.9	140	0.1	
Polystyrene	1.0	420	3	250
Polymethyl methacrylate	1.2	560	3	20
Cellulose acetate, molded	1.3	350	1.5	25
Cellulose nitrate with camphor, formed	1.4	450	2	150
Casein – formaldehyde	1.3	700	4	30
Nylon, molded	1.1	700	2	140
Nylon, fiber	1.1	3500	2–4	
Hard rubber, molded	1.1	700	10,000	15
Steel (for comparison)	7.8	12,500	200	

TABLE 36

PROPERTIES OF ELASTOMERS (Houwink, *loc. cit.* ref. 1)

Material	*Spec. weight*	*Tensile strength*	*Elong. at break, %*	*Fatigue 10^3*
Natural rubber, crude	0.91	30	1000	
Natural rubber, vulcanized	0.96	310	750	700
Natural rubber, vulcanized, 25% carb.	1.12	310	600	140
Butadiene-styrene rubber, vulcanized, 30% carbon black	1.12	210	580	21
Buna-S rubber	1.15	225	550	1500
Chloroprene rubber, vulcanized	1.32	300	950	2400
Polyvinyl chloride, +50% plasticizer	1.3	170	325	1500

The *degree of polymerization* of linear polymers is a very important factor. Polymers of molecular weight of only several thousands are unable to form foils and fibers, and a block of such material has low tensile strength. Foils and fibers can be obtained only from macromolecular compounds which are colloids, and which have the molecular weights of over 10,000, or a degree of polymerization of at least 1000. Tensile strength and modulus of elasticity of elastomers increases with increasing degree of polymerization. The industrially important polymers which are used for molding and similar purposes all have average molecular weights of 15,000–500,000, and higher.

The *cohesive forces acting between the macromolecular chains* also are of great importance. Materials in which the chains are interlinked by hydrogen bonds are stronger than those with non-polar cohesive attraction. Interlinking by primary bonds, such as found in space polymers, increases the mechanical properties even more favorably. Data about specific molar cohesion (for chain length of 5 A, and assuming that each chain is surrounded by four others) are presented in Table 37.

TABLE 37

INTERMOLECULAR ATTRACTION BETWEEN CHAINS OF POLYMERS

(from H. Mark and A. V. Tobolsky, *Physical Chemistry of High Polymeric Systems*, 1950, p. 150)

Substance	*Groups responsible for lateral attraction*	*Specific molar cohesion, cal.*
Polyethylene	CH_2	1000
Polyisobutylene	CH_2, CH_3	1200
Rubber	CH_2, CH_3	1300
Polychloroprene	CH_2, CCl	1600
Polyvinyl chloride	CH_2, CCl	2600
Polyvinyl acetate	CH_2, $COCH_3$	3200
Polystyrene	CH_2, C_6H_5	4000
Polyvinyl alcohol	CH_2, OH	4200
Cellulose acetate	OH, $COCH_3$	4800
Cellulose	OH, COC	6200
Polyamides (nylon)	CH_2, CONH	5800
Silk fibroin	CHR, CONH	9800

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The last three macromolecular substances are known as common materials for high quality fibers and fabrics, and the specific molar cohesion for these materials is the highest. It can be shown that rupture upon tension is not associated with the breaking of the chains themselves, but it is due to slippage of the chains. Hence, the tensile strength and other mechanical properties are due partly to this intermolecular attraction. As other factors also are involved, a strict parallelism between the molar cohesion and tensile strength, of course, cannot be expected.

The following simple relation between tensile strength S, degree of polymerization n, and molar cohesion (per mer) a holds (as a crude approximation);

$$S = Kna,$$

where K is a proportionality constant[2]. The equation says that *tensile strength is proportional to the degree of polymerization and intermolecular cohesion.*

The degree of *crystallinity* of the polymer also determines the mechanical properties. The intermolecular attraction depends on molecular orientation, and the chain length also determines the attraction, as long chains can be more easily oriented than short ones. The better oriented the chains the greater the cohesion surface (Fig. 78). A striking example of this importance of *orientation* is the difference in the tensile strength between not oriented and extruded nylon (see Table 35). There are various intermediate states between partial orientation and crystalline lattice structure. It is obvious that *branching* and the presence of *bulky side groups* are unfavorable factors for orientation and intermolecular attraction. The well oriented highly crystalline cellulose which has great tensile strength, and the branched mechanically weak starches are examples of the effect of branching. Moderate cross-linking of chains improves the mechanical properties (vulcanization of rubber!) but extensive cross-linking changes a deformable plastic or elastic polymer into a hard and difficultly moldable, non-elastic space polymer.

The Italian polymer chemist Natta[3] has recently published several important papers on the crystallinity, configuration, and mechanical properties of polymers. He and his associates succeeded in synthesizing several highly crystalline vinyl polymers, such as polypropylene, polybutylene, and even polystyrene. This was accomplished by using certain catalysts under specific conditions of polymerization. A mixture of the polymers of various degree of orientation is formed,

and the crystalline fractions can be separated from the amorphous components by means of solvents. The crystalline polymers are less soluble, and they have higher melting points than the amorphous components. Most important was the discovery that the highly crystalline, so called *isotactic* polymers produced fibers of high tensile strength and other excellent mechanical properties. This is due to the highly ordered, smooth configuration of the chains. Some polymer chemists have envisaged a great future for these materials.

The *flexibility of chains* is one of the essential factors of rubbery *elasticity*. Disorderly, *amorphous* arrangement of the flexible, long macromolecules are other factors involved. Whether flexible chains will mutually arrange into oriented crystallites or to amorphous masses, is determined largely by the *constitution* and *spatial configuration* of the atoms in the constituting residues (mers). The temperature also appears to be an important factor.

The reversible elastic deformation of rubber and rubber-like materials can be considered from the point of view of energetics as follows. According to the first and second laws, the free energy change ΔF depends on the heat content (enthalpy), ΔH, and the entropy term $T\Delta S$:

$$\Delta F = \Delta H - T\Delta S, \tag{1}$$

where T is the absolute temperature. It is obvious that upon stretching or compression of an elastic body the potential energy of such a deformed system increases, as the body is able to do work on recoiling. If T or S were zero, the changes of free energy would be reflected only in the change of enthalpy H. In fact, however, the second term in the equation (1) $T\Delta S$ never can be neglected. On the contrary, it may become dominant. Experiment shows that at moderate temperatures the tension is proportional to the absolute temperature. Thermodynamic analysis of these data leads to the conclusion that in the reversible elastic deformation of rubber the change of the heat (internal energy) content is insignificant, and that the free energy change depends solely on the second term $T\Delta S$. The free energy change at a constant temperature then is due only to the change in entropy of the system ΔS. *The entropy decreases on tension and it increases on recoiling.* From a molecularly-kinetic point of view, this decrease of entropy is due to *orientation* of the flexible macromolecules of rubber. Upon recoiling, when the system performs work, the macromolecules become disoriented.

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i.e. they attain a more probable state of disorder, and the entropy thereby increases[4].

A very important property in highly elastic polymers is a certain amount of *cross-linking* between the linear macromolecules[5]. If the chains are permitted to slip along each other, the material will flow, and it will not recover its initial shape. However, excessive fixing, a high degree of orientation, and crystallization of the material is equally unfavorable for rubbery elasticity, since then any deformation is restricted.

2. Thermal Properties of Polymers

It is important to note that the mentioned mechanical properties of certain macromolecular substances appear only in definite limits of temperature. The mentioned cases were thought to be at the ordinary temperatures of about 0° C to 50° C. At a certain high temperature many polymers become soft. A polymer which is hard and brittle at room temperature may acquire certain elasticity at a higher temperature. Those polymers, however, which are soft and elastic at the ordinary temperatures may become hard and brittle at certain low temperatures. These temperatures at which a profound change of mechanical properties occurs are called *transition temperatures*. Some data are presented in Table 38. Because of

TABLE 38

TRANSITION POINTS* AND BRITTLE POINTS OF POLYMERS

(after Houwink, *Fundamentals of Synthetic Polymer Technology*, Elsevier Publ., 1949, p. 49)

	Transition point °C	*Brittle point °C*
Polyisobutylene	—74	—50
Natural rubber	—73	—58
Polyvinylidene chloride	—17	
Polyvinyl acetate	+28	
Nylon	+47	
Cellulose nitrate	+53	
Polyvinyl chloride	+75	+81
Polyvinyl alcohol	+85	

* The low-temperature transition points of rubber are the *second order* transition points. The *first order* transition point is a higher temperature at which the polymer softens and melts.

the variety of polymers, and also depending on the method of testing, these data do not have the same significance as e.g. the melting points of micromolecular substances. Some authors have introduced the terms *brittle point* which differs from the transition point.

The reason that rubber becomes brittle at a low temperature is understood to be a consequence of restrained molecular motion. The molecules are absolutely motionless only at the absolute zero. At a certain higher temperature they oscillate and rotate, these movements being restricted by the size, shape, and mutual interlinking of the large molecules. At a relatively low temperature of the hard, glassy state the oscillations are very limited, hence the short range cohesive forces hold the molecules into a highly consistent mass. The higher the temperature the more intense the oscillations will be. Both the distances between adjacent chains and between the segments of the same chain thereby will increase. Consequently, the intermolecular and intramolecular attractive forces will decrease. Finally, at the transition point the macromolecules will be able to stretch out and slip along each other under stress, the degree of elasticity being determined by the constitutional characteristics of the particular substance. Some authors believe that the changes in mechanical properties at the transition temperatures are due to the onset of free rotation of some segments of the chains and groups attached to the chains[6].

For testing of polymeric materials, the *softening* or *heat distortion point* (first order transition) is important. The temperature is determined at which the material shows a notable flow. For highly consistent solids pressure is applied on heating the specimen, for example a bar. Continuous resistance to heat and inflammability also are important characteristics. The continuous resistance to heat measures the resistance of the material to high temperatures, and it is usually determined by measuring the loss of weight of the polymer after keeping it at a high temperature for a certain time. Some data of these properties are presented in Table 39.

There are other thermal properties which are of importance in the evaluation of plastics and fibers[7]. *Thermal conductivity* is one of these properties. Polymers which are good electrical insulators, such as all macromolecular hydrocarbons, are also good thermal insulators. The thermal conductivity of all plastics is much lower than that of metals, and this is one reason for the wide utility of plastics in such items as handles of cooking utensils, doors, and inside

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TABLE 39

THERMAL PROPERTIES OF PLASTICS
(after Houwink, *loc.cit.* ref. 1)

	Softening point (heat distortion) °C	*Continuous resistance to heat °C*
Phenol-formaldehyde resin, cast	40–80	215
Phenol-formaldehyde resin, molded, wood-flour filler	138	130
Urea-formaldehyde, molded, cellulose filler	132	82
Polyethylene	50	100
Polystyrene	75	70
Polyvinyl chloride–acetate copolymer	63	55
Polymethyl methacrylate	88	70
Cellulose acetate, molded	70	82
Cellulose nitrate with camphor	63	60
Casein-formaldehyde, formed	150	155

trimmings of automobiles. In the instances when heat dissipation is desired, the conductivity can be increased by using conducting fillers in the mixtures before molding the plastic. *Thermal expansion* (and shrinkage) of plastic materials is another important thermal property which deserves consideration in many applications. This is important for example in applying plastic parts in aircraft, automobiles, and similar constructions which may be exposed to considerable temperature variations. The thermal expansion coefficients of organic polymers are generally higher than those of metals.

Thorough studies have been done lately on the dependence of the thermal and mechanical properties of pure polymers on the chemical composition and constitution. Especially elucidating are the cases in which linear, not branched and not cross-linked macromolecules are investigated. The work of Batzer on the influence of substitution on the properties of polyesters deserves attention[8]. The importance of this work is enhanced by the fact that the linearity of the macromolecules of the polyesters studied was wholly established, and the complex effects of branching and cross-linking thereby were eliminated.

The polyesters of hexanediol $HO{\cdot}CH_2CH_2CH_2CH_2CH_2CH_2{\cdot}OH$ and sebacic acid $HOOC{\cdot}CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2{\cdot}COOH$ were investigated, and the influence of the alpha disubstitution (α, α') by butyl residues in the sebacic acid on the properties of the polymers was studied. The average molecular weights, softening temperatures,

and other properties of the unsubstituted and substituted polyesters are shown in Table 40.

TABLE 40

PHYSICAL PROPERTIES OF SUBSTITUTED AND UNSUBSTITUTED POLYESTERS

	M	*Soft. P. °C*	*Properties*
Polyester of hexanediol and	6000	liquid	sticky, oily, viscous
dibutyl sebacic acid	26,000	liquid	sticky resin
Mixed condens. with sebacic and dibutyl sebacic acid in the ratio 2 : 1	6000	42–44	sticky, not plastic
	27,000	41–43	the same
Mixed condens. with sebacic acid + dibutyl sebacic acid in the ratio 5 : 1	6700	52–53	somewhat sticky powder
	31,000	50–51	plastic, foils, fiber
Sebacic acid	8500	61–62	powder
	28,000	63–65	foils, fibers
	85,000	71–73	strong foils and fibers

The results indicate that the more highly substituted the chains the lower the softening point will be. This is due to the fact that the butyl groups prevent alignment of the chains, and reduce the intermolecular forces of attraction acting between the chains. In such substituted chains the softening points are independent of the molecular weight. Foils and fibers can be formed only when there is very little of the substitution, and the best (mechanically strongest) materials can be obtained with the unsubstituted sebacic acid. In this instance the softening points increase with the molecular weight of the polyester[9].

3. Chemical and other Properties of Polymers

One of the main goals of organic colloid chemistry is to correlate the various properties with structural characteristics, in order to predict and plan any desired modifications in the properties. So far the mechanical and thermal properties have been mentioned. The chemical properties, such as resistance to solvents, acids, alkalies, oxygen of air and moisture, are other practically important properties. The general rule holds that the saturated non-polar

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compounds are chemically very resistant against acids, alkalies and oxygen. Non-polar polymers however may be attacked by non-polar solvents. The electrical properties are important when a polymer is considered as an insulator. Adhesive power and wettability by water are other properties which may be of interest. In Table 41 some of the most important properties are correlated with the structural characteristics. This, of course, is a very generalized correlation which does not hold strictly for all possible cases.

TABLE 41

RELATION BETWEEN PROPERTIES AND STRUCTURAL CHARACTERISTICS OF POLYMERS (mainly after H. Mark, *Ind. Eng. Chem.*, 34 (1942) 1343)

Property	*Mol.wt.*	*Cross linkage*	*Polarity*	*Flexibility of chains*	*Degree of orientation of chains*
Modulus of elasticity	+	+		—	+
Tensile strength	+	+	+	—	+
Elongation at break	+	—	—	+	—
Surface hardness	+	+	+	—	+
Resistance to temperature	+	+	+	—	+
Electrical resistance		+	—		
Resistance to swelling	+	+		—	+
Adhesive power		—		+	—
Resistance to creep	+	+	+	—	+
Resistance to acids and alkalies		+	—		+

A plus sign indicates that the property under consideration increases; a minus sign means that it decreases. For example, modulus of elasticity increases with the increase in molecular weight (+ sign); adhesive power decreases with increased cross-linking (— sign). In cases not marked the relation is little investigated, or it varies too much among various polymers.

The adhesive power, wettability by water, and the swelling and solubility properties are the most interesting for a colloid chemist. The *adhesive power* appears to be determined very little by the molecular weight, although the latter must be above a certain minimum value. Micromolecular monomers are not sticky, but semi-colloidal polymers are. The adhesive power depends also on the amount of *branching* of the macromolecules. Polymers built of

smooth chains are less sticky than branched polymers. For example, cellulose is not sticky, but the solutions of the branched starch are. A high adhesive power have also the dextrins which are obtained in degradation of starch. When bulky atomic groups are hung at the chains, the adhesive power is enhanced. For example, the polyesters of sebacic acid (Table 40) and hexanediol are not adhesive, but the polyesters made of sebacic acid substituted with butyl residues are sticky.

The wetting, swelling, and solubility properties depend chiefly on chemical composition and constitution of the macromolecular substance, as already outlined in Chapter 12. Non-polar surfaces are not wetted by water, and non-polar macromolecules do not swell in polar solvents. Chemical properties of polymers are important also when dyeing of yarn or fabric is considered.

The properties of polymers manufactured for various practical purposes are always modified by various means, such as copolymerization, addition of softening and plasticizing agents and fillers, and by treating the material at various temperatures.

The *optical properties* of macromolecular solids also deserve attention. Translucent, glass-clear polymers are amorphous. Small crystalline regions of the solid have a different refractive index from that of the amorphous parts, and the material therefore will appear opaque. Examples of amorphous polymers are: the polymethacryl methacrylates, polystyrenes, and all copolymers. (As mentioned before, the bulky side groups and uneven arrangement of the chains prevents crystallization.) Plexiglass is a polymethyl methacrylate product. The ordinary polystyrenes also are glassy. The polyethylene resins, on the contrary, are more or less crystalline, and they are opaque.

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PART TWO
SELECTED GROUPS OF ORGANIC COLLOIDS

CHAPTER 17

Macromolecular Hydrocarbons

The organic macromolecules which are built of carbon and hydrogen atoms only are the simplest chemically. A great variety of macromolecular hydrocarbons are known, despite this chemical simplicity. The carbon chains may be of various lengths, they may be branched to various degrees, and there can be complete or incomplete saturation with hydrogen. We shall consider those groups of macromolecular hydrocarbons of the most interest and practical importance: (1) the polyethylenes, (2) the polyisobutylenes, (3) the polystyrenes, and (4) the natural and synthetic rubbers. In addition, a few other polymeric hydrocarbons and their derivatives will be mentioned briefly, because of some special interest they have.

1. The Polyethylene Resins

The polyethylenes have been mentioned in the preceding chapters, and the formula $(CH_2)_n$ was shown to be a characteristic for the chemical composition. It is an incomplete and oversimplified formula, since it represents only the basic portion of the constituting residues. The methylene residues, $—CH_2—$, comprise the major part of the molecules, but other groups also are present in the polymer. Moreover, the end groups differ from the chain residues.

Several reactions are known in which polyethylenes or polymethylenes can be obtained[1]. However, only two methods are of industrial importance: (1) the polymerization of ethylene at very high pressures, and (2) the catalytic polymerization at low pressures (the Ziegler-process). The high pressure process employs pressures

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over 1200 atm., and temperatures of about 200° C[2]. The Ziegler process operates at ordinary pressures using catalysts[3]. The high-pressure polyethylene process is of older date, and the polymers have been investigated very thoroughly. Some properties of these polymers, produced by Union Carbide and Carbon Corporation, are presented in Table 42.

TABLE 42

PROPERTIES OF POLYETHYLENE RESINS[4]

Designation	*Mol. wt.*	*Melt viscosity at 130°C, poises*	*Softening temp. °C*	*Description*
DXL-1	1,000	0.4	37.5	Grease
DYDT	4,000	5.2	93.0	Wax
DYGT	7,000	120	98.0	Soft resin
DYJT	10,000	800	100.0	Resinous
DYLT	12,000	$3 \cdot 10^4$	104.0	Resinous
DXL-14	14,000	$1.4 \cdot 10^4$	104.0	Resinous
DXM-16	16,000	$5 \cdot 10^4$	106.0	Resinous
DNF	19,000	$4 \cdot 10^5$	108	Resinous
DYNF	21,000	$1 \cdot 10^6$	110	Resinous
DYNJ	23,000	$1.5 \cdot 10^6$	111	Resinous
DXH-28	28,000	$3 \cdot 10^7$	112	Resinous
DXH-34	34,000	$1 \cdot 10^8$	112	Resinous
DXH-38	38,000	$3 \cdot 10^8$	112	Resinous
88A-1008	38,000		140	Resinous

The polymers produced for most applications have molecular weights of 10,000–40,000. They are flexible and tough, and become soft at 100–120°, at which temperature the material can be formed into any desired shape. The Table shows that the melt viscosity increases very rapidly with increasing molecular weight, but the softening temperature becomes constant above a definite molecular weight. Great differences, however, can exist between various specimens of nearly the same average molecular weight, depending upon the polymerization. This is demonstrated by the difference in the softening point between the last two specimens in the Table.

Spectrophotometric studies with infrared light have shown that, beside the —CH_2— residues, which comprise the major part of the macromolecules, there are other groups present, notably —CH_3. The number of methyl groups is greater than two for each macromolecule, indicating that the macromolecules are branched[5]. The

degree of branching, however, is not extensive, so that polyethylenes can be called essentially linear. Moreover, the branching decreases as the molecular weight increases. Some data are presented in Table 43.

TABLE 43

THE AMOUNT OF BRANCHING OF POLYETHYLENE RESINS[6]

Resin	*Number average molecular weight*	$\%CH_3$	*Branches per 100 C atoms*
DXL-1	880	10.4	6.6
DYDT	3300	6.1	4.8
DXL-6	4000	5.3	4.2
DYJT	5700	4.3	3.5
DYLT	6700	3.4	2.8
DYNH	9100	1.8	1.3

A small number of double bonds was also found in the polyethylenes. Data are shown in Table 44. The great difference between the viscosimetric and number average molecular weights indicates polymolecularity of the specimens.

TABLE 44

DOUBLE BONDS IN POLYETHYLENE RESINS[6]

Resin	*Viscosity Mol. wt.*	*Number average molecular weight*	% $C=C$	*Double bonds per molecule*
DXL-1	1,000	880	2.8	1.03
DYJT	10,000	5,700	0.25	0.59
DXL-14	14,000	6,700	0.17	0.47
DYNH	21,000	9,100	0.09	0.34

The double bonds found were of the type RHC=CHR and $RHC=CH_2$, the latter being end groups ($—CH_3$ are other end groups). The amount of unsaturation decreases as the molecular weight increases. In the ordinary polyethylenes any one molecule out of 3 or 4 contains one double bond.

According to X-ray analysis, the polyethylene resins are composed of crystalline and amorphous regions[7]. There may be some correlation between the amount of amorphous material and branch-

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ing[8]. Moreover, it has been shown that the crystalline component is the one which is important in determining the softening point and hardness. Variations in the properties can be governed by variations in the pressure and temperature of polymerization.

The new method of Ziegler (*loc. cit.* ref. 3) of producing polyethylene at normal pressure seems to have a great future, as this process is cheaper than the older method. Many new plants are under construction in various countries at the present time, and the output of this material is increasing steadily.The polyethylenes which were created by Ziegler and his associates in the Max Planck Institut für Kohlenforschung (Mülheim, Germany) differ somewhat from the high-pressure polyethylene. The Ziegler resin is more crystalline than the ordinary one and it is practically unbranched. It has a higher tensile strength than the high-pressure resin. Its modulus of elasticity is 5500–11,000 kg/cm^2, whereas that of the high-pressure resin is about 2000–5000 kg/cm^2. Being a largely crystalline material, it is harder and more resistant toward various solvents and chemicals than the more amorphous high-pressure polymer[9]. The only property in which the Ziegler resin seems to be inferior to the high-pressure product is the optical translucency. The highly crystalline resins are less translucent than the more amorphous materials.

The increase in the production of polyethylene resins (polythene, Lupolen) is due to several reasons. The starting material is cheap, and the polymerization can be well handled and modified. The polymer is very light, has good mechanical properties, is very resistant toward chemicals and solvents (even hydrocarbons are able to make it swell and dissolve only at higher temperatures, and then only slowly), and it is an excellent electrical insulator. Moreover, it is water-repellent. Tubing, bottles and other containers, wrapping material, and insulating materials are examples of applications.

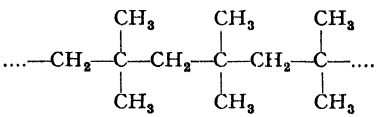
Although the arrangement of carbon atoms in a paraffin chain is well known from X-ray analyses, the structure of polyethylene has not been solved in all its details.

A new, interesting way to modify the properties of polyethylene and other polymers is to expose them to the high energy radiation of nuclear reactors. Two effects were demonstrated: (1) chain degradation, and (2) initiating of cross-linkage[10]. Irradiation of polyethylene produces an increase in cross-linking, and a decrease in crystallinity in the material. The polymer becomes more flexible,

despite the increase in cross-linkage. This indicates that crystallinity is the decisive factor in stiffness, since breaking up the crystalline structure reduces the hardness. At very high radiation doses the crystallinity may be destroyed almost completely, and a flexible material, somewhat transparent at room temperature, is obtained. The mechanical properties of this material, however, are poor. Paraffins are changed by such irradiation into non-melting gelled materials, because of the formation of cross-links. The viscosity of lubricating oils (which are composed of shorter paraffin chains) is increased upon such irradiation, since larger, branched molecules are produced by the increase in cross-linking. Chain degradation does not occur in these instances. The latter effect was observed with other macromolecular substances, as will be shown in some of the following sections.

2. Polyisobutylene

Polyisobutylene is another example of a synthetic macromolecular hydrocarbon of both scientific and industrial interest. It is prepared by polymerizing butylene in the presence of catalysts ($AlCl_3$, BF_3) at low temperatures. If very large molecules are desired, the chains are grown slowly at temperatures of – 20° or even – 80° C. Polymeric mixtures possessing average molecular weights of nearly one million can be obtained this way. The polymer has the structure



It differs from the polyethylene chain structure in that it has methyl groups attached to the saturated chain. The methyl groups make the chain bulky, and intermolecular cohesion and orientation are hampered. The chains of polyisobutylene are twisted and partly coiled, and the material possesses a high degree of elasticity. The polymers which have very large molecular weights are rubber-like and they can be extended reversibly to about ten times their original length. The highly stretched polymer shows a fiber diagram; the unit cell is rhombic, the dimension along the fiber axis being 18.6 A[11]. At very low temperatures of about – 50° C the material becomes hard and brittle. Like other polymeric hydrocarbons, polyisobuty-

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lene is light, has good electrical insulating properties, is water-repellent, and it can be dissolved in hydrocarbons. It is used as an additive to improve lubricating oils and greases. The molecules in solution appear to be flexible, and coiling occurs to various degrees depending on the interaction with the solvent.

Extensive studies on the viscosity and osmotic pressure of a large number of sharply fractionated polyisobutylene specimens have been made by Flory[12]. The intrinsic viscosity was determined in diisobutylene, which is a good solvent for this polymer. The osmotic pressure was measured in a good solvent—cyclohexane, and in a poor solvent—benzene. The results of this work are very instructive, and a portion of these data is presented in Table 45 (see also Table 21, p. 150).

TABLE 45

NUMBER AVERAGE MOLECULAR WEIGHTS AND INTRINSIC VISCOSITIES OF FRACTIONATED POLYISOBUTYLENE (according to Flory[12]).

Specimen	*Intrinsic viscosity (in diisobutylene)*	*Solvent for osmotic p.*	*Conc. g per 100 ml*	M_n
C(3–3.3)	2.29	C_6H_6	1.0	970,000
			0.50	970,000
C(3.5–4)	1.87	C_6H_6	1.00	625,000
			0.50	608,000
C(4.5–5.5)	1.35	C_6H_{12}	0.50	408,000
			1.00	378,000
C(5.5–6.5)	1.12	C_6H_{12}	0.50	304,000
			1.00	308,000
C(6.5–8)	0.83	C_6H_{12}	0.50	193,000
			1.00	213,000
C(10–12)	0.551	C_6H_{12}	0.50	98,400
			0.50	99,200
			1.00	98,800

It was found that in cyclohexane, a good solvent, the reduced osmotic pressure values increase rapidly with concentration, because of the increased interaction. In a poor solvent (benzene) the reduced osmotic pressure values did not change much with concentration. The extrapolated values (to zero concentration), however, were the same. When the logarithm of the number average molecular weight (obtained by osmotic pressure measurements) was plotted against

the logarithm of the intrinsic viscosity, a straight line was obtained. This had the slope of 1.56, and the intercept 5.378, or:

$$\log M_n = 5.378 + 1.56 \log |\eta|, \quad \text{or}$$
$$|\eta| = KM_n^a = 3.60 \cdot 10^{-4} \cdot M_n^{0.64}.$$

There is one more constitutional difference between the polyethylenes and polyisobutylenes. The former are branched, the latter are not. This may be due to the method of polymerization. When the chains are allowed to grow slowly, at moderate or low temperatures, there is little or no branching in the case of bifunctional molecules. Branching occurs under drastic conditions at high temperatures and pressures when various side reactions can occur by virtue of the high activation energy available under such conditions. An excellent example is the low and high pressure polymerization of ethylene. The catalytic low pressure process produces straight chains, whereas the high pressure method yields branched chains. The molecular weight decreases and the branching and unsaturation increase with increase in polymerization pressure and temperature.

The linearity of polyisobutylene molecules also follows, according to Flory, from the fact that even the very large molecules (mol. wt. over one million) are completely soluble. The solubility property indicates indeed that there is no cross-linking among the chains, although branching would favor solubility. The ease of solubility seems to be due to the weak cohesive forces acting between the twisted, ill-aligned chains of this polymer, and to the absence of any cross-linking.

The effect of high energy nuclear irradiation of polyisobutylene is to break the chain rather than to form cross-linkages (*loc. cit.* ref. 10).

3. Polystyrene

Polystyrene is now one of the most indispensible materials for electrical appliances, mostly as an insulating material. A great variety of polystyrenes are commercially available in the form of sheets, fibers, or powder, which can be molded at a temperature of 100–180° C. The material is known under a variety of trade names (Trolitul, Styron, Lustron). The polymer is light (density 1.05), resistant to moisture and air, sufficiently strong, and resistant to most chemicals, except some lipophilic solvents. Polystyrene becomes plastic at higher temperatures, when it flows under stress,

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although cross-linked polystyrenes are relatively stable even at 250° C and higher. The material is amorphous, hence it is transparent, if sufficiently pure. Its specific resistance is 10^{18} ohm cm, which is one of the highest of any material known (polyethylene has a value about $5 \cdot 10^{17}$, rubber 10^{16} ohm cm). Its dielectric constant is 2.5.

The mechanical properties, swelling, solubility, and also the softening point depend on the molecular weight (degree of polymerization), the degree of branching, and especially on the degree of cross-linkage. Staudinger, Schulz, and their associates have prepared polystyrenes under different conditions of polymerization[13]. It was found that the polymers prepared at lower temperatures are of higher molecular weight and are less branched than those prepared at higher temperatures. Cross-linking is accomplished by adding a trace of divinyl-benzene, as mentioned before (Chap. 12). Addition of inhibitors (such as quinone) and modifiers results in the production of shorter chains than by using pure styrene with only a trace of a catalyst. The dependence of properties on degree of polymerization for not cross-linked, slightly branched polystyrenes is shown in Table 46.

TABLE 46

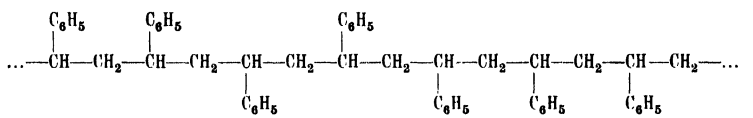
PHYSICAL PROPERTIES AND AVERAGE DEGREE OF POLYMERIZATION (P) OF POLYSTYRENE RESINS (after Staudinger[14])

P	*Appearance*	*Solubility in non-polar solvents*	*Viscosity of dil. sol.*	*Applications*
2–10	Liquid or fragile solid	Easily soluble	Low	
10–100	Brittle solid	Swells and dissolves	Medium	Lacquers
100–500	Tough, glassy solid	Swells and dissolves slowly	High	For thermoplastic molding
500–15,000	Very tough, glassy, elastic at high temp.	Difficultly soluble, strong swelling	Very high	For foils, and fibers

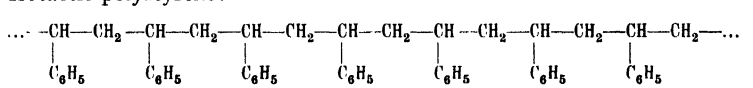
The *isotactic polystyrenes* of Natta[15] are impressive examples of new possibilities in modifying the properties of simple polymers. These new polystyrenes which were synthesized by means of specific catalysts in slow polymerization appeared to be *crystalline*, and

their mechanical properties differed strongly from those of the usual polystyrenes (see p. 270). The crystalline polystyrene appeared to be a very tenacious polymer, and fibres drawn of it possessed exceptional tensile strength. The softening temperatures of the crystalline material are higher than of the ordinary polymer. The only reason for these striking changes in the properties is molecular geometry, spatial arrangement of the atomic groups of the chain. In the ordinary polystyrene the benzene residues are sticking out at random in different directions. In the isotactic polystyrene, which exhibits beautiful X-ray diagrams of crystalline material (Fig. 95), the benzene side groups spiral orderly round the main chain. The macromolecules thus become highly oriented, and they can assemble easily in higher order structures. A great future has been predicted for the plastics and fibers manufactured from the isotactic polystyrene and other isotactic hydrocarbons, especially polypropylene and polybutylene. The configuration of the ordinary and the isotactic polystyrene is crudely illustrated with the formulas below.

Ordinary polystyrene:



Isotactic polystyrene:



The long, linear molecules of polystyrene in solution are coiled to various degrees, depending on the solvent. The intrinsic viscosity relationships have been treated recently by Flory and associates[16], evaluating the solvent influence from thermodynamic data. The intrinsic viscosities were correlated with the molecular weights of the polystyrene specimens on the basis of the equation

$$\log M = (\log |\eta| + 4.013)/0.74,$$

which is valid for solutions of polystyrene in benzene. It is interesting to note first of all how the polymerization varies with variation in the conditions of polymerization. Four batches of styrene were polymerized in bulk at 60° C in the presence of various amounts

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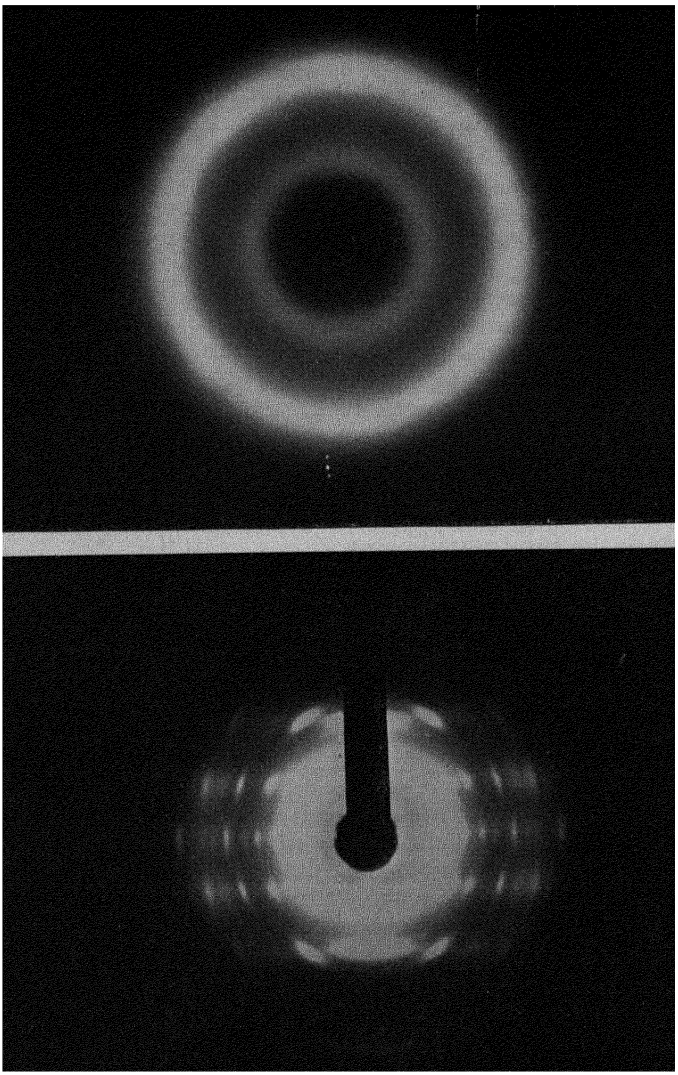

Fig. 95. X-ray diffractionphotographs of ordinary amorphous (above) and of isotactic (below) polystyrene. (From J. W. S. Hearle, *Research*, 9 (1956) 461.)

of benzoyl peroxide. The differences in the average molecular weights of the material are striking, as is shown in Table 47.

TABLE 47

VISCOSITY AVERAGE MOLECULAR WEIGHTS OF POLYSTYRENE DEPENDING ON THE AMOUNT OF CATALYST AND MODIFIER (Fox and Flory[16])

Specimen	*Mol % peroxide*	*Mole % dodecyl mercaptan*	*Extent of reaction, %*	M *of the polymer*
18A	0.006	0	14	800,000
14A	0.04	0	26	350,000
11A	0.5	0	22	150,000
3D	1.0	0.055	28	45,000

The average molecular weight decreases as the amount of added catalyst is increased. On fractionation of the material, seven fractions were obtained, the lowest average molecular weight being 43,600 and the highest 1,270,000. The influence of the solvent on the intrinsic viscosity can be seen from Table 48.

TABLE 48

THE INTRINSIC VISCOSITY OF POLYSTYRENE IN VARIOUS SOLVENTS (according to Fox and Flory[16])

Solvent	*Temp. °C*	$\lvert\eta\rvert$ *observed*	$\lvert\eta\rvert$ *calculated*
Specimen 18AF1B, M = 1,270,000			
Cyclohexane	34	0.89	0.90
	45	1.14	1.17
	55	1.43	1.41
Benzene	30	3.20	3.18
	45	3.17	3.14
	55	3.13	3.12
Specimen 18AF5, M = 360,000			
Cyclohexane	34	0.47	0.48
	45	0.56	0.56
	55	0.66	0.66
Benzene	30	1.26	1.28
Toluene	30	1.21	1.11
Specimen 3DF2, M = 92,000			
Cyclohexane	34	0.23	0.24
	45	0.25	0.26
	55	0.28	0.29
Benzene	30	0.455	0.495

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The fourth column of the Table contains the intrinsic viscosity values calculated according to the theory of Flory which evaluates the polymer-solvent interactions from thermodynamic considerations. The agreement between the experimental and theoretical values is impressive, and it establishes the validity of this approach. It is also interesting to note two more facts: (1) the strikingly large differences between the intrinsic viscosities in a poor solvent (cyclohexane) and a good solvent (benzene), and (2) the increase in the intrinsic viscosity with rising temperature in a poor solvent. Both facts are due to the flexibility of the macromolecules, differences in coiling, and solvation. In cyclohexane the polystyrene chains are strongly coiled; in benzene the coils are very loose and voluminous, which results in a high viscosity in this solvent. Solvation is also greater in benzene, as the latter has an affinity for the benzene rings of the polymer. At a higher temperature the adsorption of benzene to the polystyrene chains will decrease. In a poor solvent (cyclohexane) the temperature effect is different, and more difficult to explain. An extensive study on polystyrenes which were prepared by different methods was published recently from Staudingers' laboratory[17].

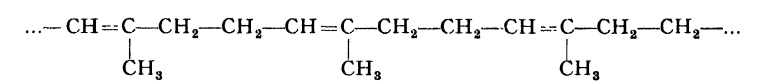
The dimensions of the molecular coils were also calculated by Fox and Flory, using the viscosity and light scattering data of Outer, Carr and Zimm[18] in these evaluations. The distance (a mean value) between the ends of a kinked chain was found to be 730 A at 25° for a polystyrene of $M = 10^6$ (in the absence of interaction with the solvent). In a good solvent (benzene) where solute-solvent interaction is strong, the mean distance for chains of the same size was estimated to be 1100 A. The fact that the same value of 1110 A for the same size polystyrene molecules was found by Debye and Bueche[19] is noteworthy. Comparison of polystyrene with polyisobutylene of the same degree of polymerization showed that polystyrene molecules are more extended than polyisobutylene molecules. This seems to be due to steric hindrance exerted by the benzene residues suspended on the chain. These residues are more bulky than the methyl groups of polyisobutylene. Consequently, the coiling of the latter can be more complete than the kinking of polystyrene. For the same reason, polystyrene is soluble, and solid ordinary polystyrene is amorphous.

Melville and his associates have studied the influence of chain branching on the physical properties of polystyrene. Branched polystyrene was prepared by brominating polystyrene photochemically

and subsequently irradiating the brominated product in the presence of fresh monomer[20]. The latter then polymerizes on the activated loci of the chains, and side chains (branches) thus are grafted on the linear macromolecules of the polymer. The effects of branching were studied by the light scattering method.

4. Natural Rubber[21]

Polyethylene, polyisobutylene and polystyrene are all synthetic macromolecular hydrocarbons. They can be regarded as saturated compounds, as the degree of unsaturation in the polyethylenes is small, and the aromatic double bonds of benzene in polystyrene are relatively unreactive. Natural rubber is an unsaturated macromolecular hydrocarbon of the composition $(C_5H_8)_n$. Depolymerization of rubber by dry distillation yields isoprene, $CH_2{=}C(CH_3){-}CH{=}CH_2$, which has the same elementary composition as rubber. Oxidative decomposition, hydrogenation, and other chemical facts support the view that natural rubber is a polyisoprene of the following constitution:



There is one double bond per isoprene residue in the chains.

Rubber or caoutchouc is produced by several species of plants, notably by *Hevea brasiliensis*. Plantations of this "rubber tree" are now found in many tropical countries, especially in Southeast Asia. The milky sap of the rubber plants is a dispersion of rubber in water. The soft, spherical particles of the *rubber milk* or *latex* are not rubber molecules, but they are aggregates of many rubber molecules which are stabilized by a layer of proteins. How the plants make rubber is not known. However, it is likely that the mode of formation of the macromolecules in the cells of plants is different from the polymerization reactions performed in chemical laboratories.

Rubber is separated from latex either by evaporating the water over a flame (or by allowing thin layers of latex to dry) or by precipitating the rubber with dilute acids. The precipitated material then can be purified by washing it with water and other solvents. Traces of proteins, carbohydrates, lipids and inorganic substances thus can be almost completely removed. Such purification is not neces-

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sary for most technological products. The manufacture of rubber for practical applications involves two main procedures: (1) *kneading* or *mastication*, and (2) *vulcanization*. Rubber is kneaded between warm rolls, and a number of additives are kneaded into the mass at the same time. These include: (1) about 2–4% (or more) sulfur, (2) reinforcing fillers (carbon black, zinc oxide, magnesium carbonate), (3) pigments, (4) accelerators and other catalytic agents, (5) protectors against ageing, and (6) softeners. Not all of the agents mentioned are included in all rubber mixes, and their quantities vary widely, depending on the purpose. For example, a rubber tire mix may contain 50 parts of carbon black to 100 parts of rubber, whereas surgical rubber gloves contain no carbon black. Zinc oxide, the fatty acids, and some organic sulfides and thiazoles serve as accelerators to facilitate vulcanization. Various antioxidants are added as protectors against ageing, e.g. aromatic amines of the naphthalene series (0.25–1.5%). As the rubber chains are relatively sensitive to oxidative decomposition by air, these antioxidants have proven to be very important. The softeners have the function of facilitating mastication by acting as a kind of lubricant. Mineral and vegetable oils, waxes, pine tar and similar materials are used for this purpose. The second and final step in the manufacture is the molding and vulcanization. Fine rubber articles are often vulcanized at ordinary temperatures, by immersing the molded object in a dilute solution of sulfur monochloride S_2Cl_2. Tires are vulcanized at high temperatures.

Pure, unvulcanized rubber is a colorless or yellowish highly elastic material which swells and dissolves slowly in benzene, carbon disulfide, and other lipophilic solvents. Solution, however, is not quite complete, an indication that rubber is partly cross-linked. In some cases the insoluble portion can be brought into solution by adding small amounts of organic bases. This indicates that in these cases not primary interlinking of chains but some polar acidic groups are involved in a kind of secondary bond interlinking. Small amounts of such polar groups may be formed on oxidation of the chains during isolation and processing. The presence of some impurities may also be responsible for this apparent cross-linking. When rubber is milled or kneaded, its solubility increases, and the viscosity of the solutions decreases. It can be shown that rubber reacts with oxygen of the air, and partial degradation (chain cleavage) occurs as a result of such oxidation[22].

It was shown by Charlsby[10] that cross-linking increases upon

irradiation with high energy nuclear radiation. The elastic modulus increases with radiation doses up to a certain maximum, and decreases when the material is exposed to still higher doses. It is assumed that in the first stage, up to the maximum, radiation produces some activation at certain points of the chain, and the cross-links are formed on mutual saturation of these activated loci. On further irradiation, cleavage of the chains becomes predominant.

The infrared spectral analysis of natural rubber and its derivatives was performed recently by Salomon and associates[23]. Some interesting conclusions on the shift of double bonds in certain reactions of rubber, as well as on the presence of some polar groups can be made. The number of polar groups in natural rubber is very small, and they arise from the impurities which were not removed in the process of isolating rubber from latex.

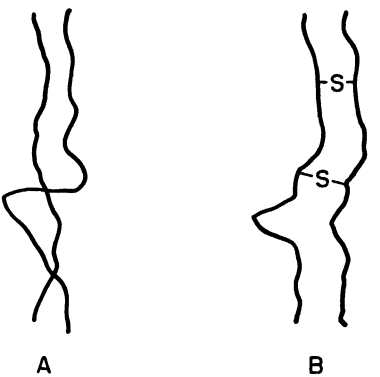


Fig. 96. Hydrocarbon chains in unvulcanized (A) and in vulcanized (B) rubber.

The molecular weight of native rubber is about 150,000–300,000[24], and after strong mastication it decreases to 30,000–60,000. Upon vulcanization the partly broken chains are interlinked by strong primary cross-links, and a cross-linked polymer of indefinite, very large molecular weight is formed. The changes are illustrated in Fig. 96.

Extensive studies have been made on the structure of stretched rubber by means of X-ray analyses[25]. Unstretched rubber is quite amorphous, but the spectral pattern of a crystalline substance appears after stretching. It has also been shown by means of X-ray analyses that balata and gutta-percha, two other polyisoprenes

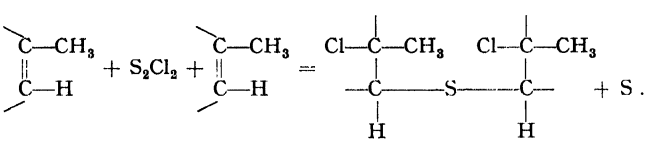
produced by plants, differ from rubber only in the spatial configuration of the chains. Rubber is a cis isomer, and gutta and balata are trans isomers. Both of the latter polymers are much less elastic than rubber at ordinary temperatures. The softening point of unvulcanized rubber is at about 25° C, whereas the softening point of gutta is 50–65° C.

The viscosity of rubber solutions was studied thoroughly by Staudinger and his associates[26]. By the polymer-analogous conversion of rubber into hydrogenated rubber, Staudinger produced strong evidence that rubber is a macromolecular substance. He also showed that the chains are not cleaved if oxidative decomposition is avoided. Many other researchers have extended these studies toward a more precise characterization of rubber specimens.

The molecular weight and intrinsic viscosity are correlated by the equation[27]

$$\log M = 5.85 + 1.5 \log |\eta| .$$

The vulcanization of rubber can be accomplished in several ways. Two methods are very important: (1) cold vulcanization with sulfur chloride, and (2) hot vulcanization with sulfur. The mechanism of cross-link formation is not completely explained, and it is possible that it is different in different cases, depending on the temperature and the additives. According to Meyer and Mark (*loc. cit.* ref. 25), sulfur monochloride reacts with two adjacent chain segments in cold vulcanization as follows:



It can be shown[28] that in this case the bound sulfur is present in the vulcanized rubber as thioether sulfur, C—S—C. High temperature vulcanization may involve reactions with some of the other additives, such as zinc oxide, and bridges like —C—S—Zn—S—C have been assumed. The interlinking of the chains by attachment of the sulfur to the alpha-methylene carbons, without involving the double bond, has also been considered.

The composition of rubber mixes for highly elastic articles and for reinforced tire rubber, as well as some mechanical properties of the vulcanized products are listed in Table 49. The reinforcing

carbon black probably forms a kind of skeleton in the rubber. Reinforcing effects can be achieved also by adding various condensation polymers to the rubber mix. The reinforcing effect of the added resins is due to chemical bonding between the resin and the rubber molecules[29].

TABLE 49

COMPOSITION AND MECHANICAL PROPERTIES OF VULCANIZED RUBBER
(K. H. Meyer[25])

Highly elastic articles		*Automobile tires*	
Pale crepe rubber	100	Smoked sheet rubber	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	Carbon black	50
Vulcanized for 20–30 minutes at 140°C		Sulfur	3
		Mercaptobenzothiazole	1
Tensile strength, kg/cm²	200–240	Vulcanized for 60 minutes at 134°C	
Modulus of elasticity, kg/cm² at 500% elongation	20–28	Tensile strength, kg/cm²	273
Breaking extension	825–850	Breaking extension, %	535

Vulcanization with large amounts of sulfur at high temperatures produces highly cross-linked, hard rubber. This material is a space polymer possessing very little elasticity, and it is amorphous, even on deformation. A *moderate amount of cross-linking* must be produced in vulcanization in order to make a highly elastic yet mechanically strong and durable material.

Since rubber is an unsaturated macromolecular hydrocarbon, many derivatives of it can be obtained easily. Chlorinated rubber, and hydro-rubber are examples of such products.

Latex, being a concentrated dispersion of stabilized rubber (35%), can be used directly, for instance for coatings. Vulcanizing agents, accelerators, and other ingredients are added directly to latex, and the objects to be coated are immersed in the dispersion and warmed.

5. Synthetic Rubbers

The first synthetic rubber was made nearly fifty years ago, by polymerizing isoprene. However, nothing was done on an industrial

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scale at that time. The synthetic rubber problem became acute in Germany during the first World War, as Germany was cut off from sources of natural rubber by the blockade. There were two chief problems to solve: (1) to find a suitable large scale method for synthesizing isoprene, and (2) to elaborate methods for improving the qualities of the polymer. In spite of the tremendous amount of effort, the first large scale synthetic rubber produced was of low quality. Its elasticity and other mechanical properties were unsatisfactory. It could be shown later that the products formed on polymerization of isoprene and dimethylbutadiene were far from having the same constitution as that of natural rubber.

The problem of finding more suitable ways for synthesizing rubber-like materials was studied extensively and successfully solved in 1925–35 in Germany, the U.S.A. and some other countries, and the production of synthetic rubber reached its peak during the second World War in Germany and the U.S.A. Three major methods are now available for the manufacture of synthetic rubbers. The materials obtained by these methods differ considerably from each other both chemically and in their physical properties. They all resemble more or less natural rubber, and the best synthetic rubbers now even surpass natural rubber in some mechanical properties[30].

One way to manufacture synthetic rubber is to polymerize butadiene, $CH_2{=}CH{-}CH{=}CH_2$, in the presence of metallic sodium. The starting material was made available by several synthetic procedures, for instance from acetylene and formaldehyde according to Reppe[31]. Depending on the conditions of polymerization, butadiene forms more or less branched chains of various length. The resulting polybutadiene contains one double bond per residue, and the material can be vulcanized, thereby improving the mechanical and other properties. The chains have a structure largely as follows:

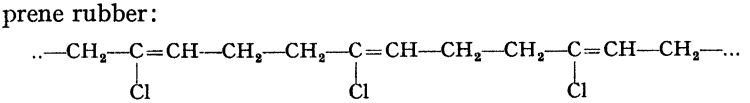
$$\ldots{-}CH_2{-}CH{=}CH{-}CH_2{-}CH_2{-}CH{=}CH{-}CH_2{-}CH_2{-}CH{=}CH{-}CH_2{-}$$

The chains are smooth, and crystallization might be expected to a certain extent. However, the stretched butadiene rubbers (buna rubbers) are less crystalline than natural rubber. This is explained by the extensive branching of buna. The specimens polymerized at a relatively high temperature of 50° seem to be more branched than those obtained at a low temperature[32]. This is in accord with the observations made on other polymers, such as polyethylene and polystyrene.

The *copolymers* of butadiene and styrene or of butadiene and acrylonitrile are of even better quality than a pure polybutadiene. The butadiene-styrene copolymers are called buna-S or GR-S; the copolymers with acrylonitrile are called perbunans. They have both been and are being produced on a large scale. Other monomers, such as isoprene, also are added for certain purposes. The polymerization is carried out in emulsions using peroxide catalysts and modifiers, such as dodecylmercaptan. The following composition can be mentioned as an example: (K. H. Meyer, *loc. cit.* ref. 25) Butadiene 75 (parts by weight), styrene 25, potassium persulfate 0.3, fatty acid soap 4.2, dodecylmercaptan 0.6, and water 180. The polymerization is performed at 50° C, and it is interrupted after 15–17 hours, when 77% of the monomers are converted into the copolymer. The dodecylmercaptan is incorporated into the chains, one residue of it being detected for each macromolecule. Since this rubber is a copolymer, with uneven chains with benzene residues attached to them, the material does not crystallize. Both GR-S and perbunan can be vulcanized with sulfur, thereby improving their properties. The vulcanized materials are strong and elastic, and they are very resistant to abrasion. This was ascertained in road tests comparing tires of various types of rubber. The best quality buna S tires proved to be more resistant toward wear than the tires made of vulcanized natural rubber. Perbunan contains the polar nitrile groups CN in the molecules which make it resistant toward hydrocarbons.

Extensive viscosimetric studies on the degree of branching of polybutadiene-styrene rubber were made by Cragg and Fern[33]. The emulsion polymerization of the butadiene-styrene mixtures was performed at various temperatures, and the copolymers were carefully fractionated. It was concluded that branching in a diene polymer of this type is repressed as the temperature of polymerization is decreased. When the polymerization was carried out at 15° C, no branching could be detected in any fraction of the copolymer.

The third important type of synthetic rubber is the polychloroprene rubber:



It was developed by Carothers in the early thirties in the laboratories of Du Pont de Nemours and Co. at Wilmington[34]. The start-

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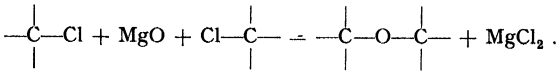
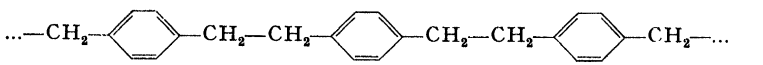
ing material is 2-chlorobutadiene or chloroprene which is prepared synthetically from acetylene and hydrogen chloride. The polymerization of chloroprene is accomplished in emulsions in the presence of suitable catalysts and modifiers. The degree of polymerization and the properties of the polymer depend on the conditions of the polymerization. The polymer is elastic, and the macromolecules become oriented on stretching. The polychloroprene rubber (neoprene) can be vulcanized with oxides of metals (MgO and others), and sulfur accelerates this process. It seems that the oxides react with the chlorine atoms of adjacent chains, and ether bridges are formed[25]



Natta succeeded recently in synthesizing crystalline 1,2-polybutadienes which have high melting points and excellent mechanical properties[35]. Structural studies by means of electron and X-ray diffraction and infrared spectroscopy permitted calculation of the dimensions of the elementary unit cell, as well as the coordinates of the individual carbon atoms of the cell. It appears that the chains are almost planar, and they have a zig-zag structure; the vinyl group substituents are arranged in the 1-3 sequence on both sides of the plane. Natta has proposed to call this type of spatial arrangement of crystalline polymers a *syndyotactic* arrangement.

6. Other Polymeric Hydrocarbons and Their Derivatives

Among the many other macromolecular hydrocarbons obtained in various polymerization reactions, poly-*para*-xylylene is of considerable interest. This polymer was prepared by Szwarc[36] in Manchester (England) by pyrolytic dehydrogenation of *p*-xylene. The probable structure of this polymer is:



The material is very tough, highly crystalline, its softening temperature is very high (over 360° C), and it is insoluble in ordinary hydrocarbon solvents. It dissolves slowly in biphenyl at 254° C, and in some other molten hydrocarbons at high temperatures. The polymer is known in an opaque and in a transparent modification and both are crystalline; moreover, the transparent modification

shows a negative birefringence. The polymer can be sulfonated, the sulfonated product swells but does not dissolve in water. According to Mark and associates[37], the polymer is partly cross-linked, although the chains seem to be highly oriented. This is not surprising, as they are very smooth. The high melting point and resistance to solvents may be due to both a high degree of crystallinity and cross-linking. The material is of technical interest mainly because of the resistance to heat and solvents, and also on account of its good dielectric properties. The chief disadvantage of the high-melting polymers is the difficulty of shaping them. Because of this intractability their utility is limited[38] although methods might be found to overcome this.

Polytetrafluoroethylene (Teflon) is another example of such highly resistant polymers[39]. It has the constitution

$$\ldots\text{—}CF_2\text{—}CF_2\text{—}CF_2\text{—}CF_2\text{—}CF_2\text{—}CF_2\text{—}CF_2\text{—}CF_2\text{—}CF_2\text{—}\ldots.$$

It softens only at temperatures over 320° C, is insoluble, and resistant to oxidation and strong acids. The polymer was developed by the Du Pont company, and it is prepared by polymerization of tetrafluoroethylene under pressure, and in the presence of catalysts. It is non-inflammable, because of its high fluorine content.

Much more easily workable are the halogen derivatives of polyethylene which contain chlorine and fluorine (e.g. polychlorotrifluoroethylene of Union Carbide and Carbon Corporation), or the incompletely chlorinated polyvinylidene chlorides. The well known Saran of the Dow Chemical Company is composed chiefly of polyvinylidene chloride:

$$\ldots\text{—}CCl_2\text{—}CH_2\text{—}CCl_2\text{—}CH_2\text{—}CCl_2\text{—}CH_2\text{—}CCl_2\text{—}CH_2\text{—}\ldots$$

The material is resistant to water and most chemicals, it has good mechanical properties, and its softening temperature is high (210–220° C). It is insoluble in organic solvents at room temperature. Copolymers and mixtures of polyvinylidene chloride with other polymers are used in order to improve workability. The polymer is used for coatings, foils, and fabrics.

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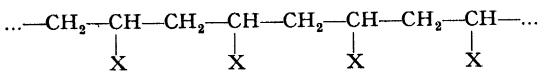
CHAPTER 18

Macromolecular Vinyl Derivatives

A great variety of synthetic vinyl polymers is known. Some of these have been mentioned previously, and other important examples will be described in this chapter. Comprehensive monographs are now available for those who are specifically interested in this field[1].

The general formula for a vinyl monomer is $CH_2{=}CH{\cdot}X$, where X is a substituting group or element. In the polymerization reactions the double bond is opened, and the radicals line up, thereby forming long, saturated chains of the following constitution:



In the instance of polyethylene, X=H; in the case of polystyrene, $X = C_6H_5$. Other examples will be presented in the following sections.

1. Polyvinyl Chloride

Polyvinyl chloride is one of the most important synthetic polymers of this group. In this instance X = Cl. The polymer is obtained by polymerizing vinyl chloride ($CH_2{=}CHCl$) under pressure. (The monomer is synthesized from acetylene and hydrogen chloride.) Benzoyl peroxide acts as a catalyst, and the degree of polymerization depends on the amount of catalyst and modifiers, as well as

on the pressure and temperature. Its molecular weight commonly ranges between 30,000 and 150,000. The material is amorphous, but partial crystallization occurs upon extension. The polymer softens above 85° C, and begins to decompose at still higher temperatures. In order to facilitate molding (without decomposition), the polymer is plasticized by the addition of tricresyl phosphate, dibutyl phthalate, or similar plasticizers. Tubes, foils and threads of such plasticized material are insoluble in water, alcohol, and saturated hydrocarbons, and are extremely resistant to acids. The specific gravity of the plasticized material is 1.2–1.6, the tensile strength is in the range of 80–600 kg/cm^2. Copolymers of vinyl chloride with other vinyl derivatives are also used extensively. Since they have lower softening points, they are more easily workable. Flexible sheets of plasticized polyvinyl chloride or its copolymers are used as leather substitutes for shoes, belts, seat covers, and similar items; they are used also instead of rubber for raincoats, shower curtains, gaskets, and many other articles. The resin can be colored by including various dyes in the material before forming[2].

Polyvinyl chloride swells and dissolves slowly in benzene, acetone and in esters. The molecules in solution are highly flexible, and are coiled to various degrees depending on the solvent. The viscosity-molecular weight relationships of polyvinyl chloride have been studied extensively[3]. The elastic properties of polyvinyl chloride gels have been studied recently[4]. The rubber-like elasticity of highly plasticized polymers seems to indicate that there may be some similarity between the structure of rubber and plasticized polyvinyl chloride. The plasticizer possibly binds the chains in a network, not by the primary bond cross-linking as in vulcanized rubber, but interlinking by residual valencies between the chains and the plasticizer. The resulting plasticized polymer is somewhat similar to a very viscous solution with a certain internal structure.

Interesting studies on the substitution of the chlorine atoms by aromatic residues have been made by Teyssié and Smets[5]. These Friedel-Crafts type reactions occur in the presence of aluminum chloride as a catalyst, and linear or branched substitution products are obtained. The original macromolecules thereby are partially split, as confirmed by osmotic and viscosimetric measurements. Moreover, the reaction is not a simple substitution, but it proceeds as follows:

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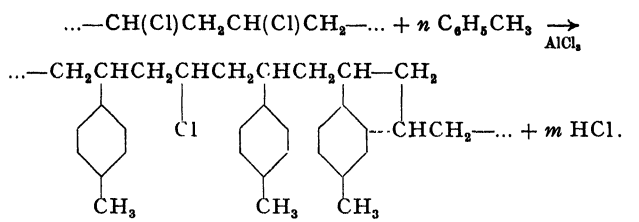
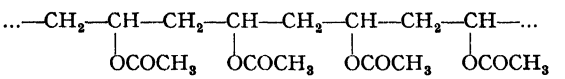


2. Polyvinyl Acetate

In this instance X = $OCOCH_3$, and the chains of the polymer have the constitution



The macromolecules are formed by the polymerization of vinyl acetate, $CH_2{=}CHOCOCH_3$, the latter being synthesized from acetylene and acetic acid. This polymerization may be induced by light or by peroxides.

The mechanical properties of the polymer depend on the degree of polymerization, which may be very high, in which case the polymer is incompletely soluble. At higher temperatures the material becomes soft and exhibits rubber-like elasticity. According to the X-ray analysis, only incomplete orientation of the chains takes place upon stretching, and no definite crystallites are formed even upon maximum extension. This is probably due to the bulkiness of the acetate groups hanging on the chains.

Polyvinyl acetate is chemically much less resistant than polyvinyl chloride. However, the copolymers of vinyl chloride containing 12–15% vinyl acetate have the same chemical resistance as polyvinyl chloride, but are more easily workable than the latter. These copolymers also have good mechanical properties (Vinylite of Union Carbide & Carbon Corporation), and may be formed into sheets, foils and fibers for various practical uses.

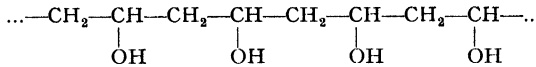
The relatively low-molecular specimens of polyvinyl acetate (mol. wt. 2,000–15,000) are brittle, and become sticky even at room temperatures. They are easily soluble, and are used as adhesives, as additives to varnishes, and for coatings. The polymer is soluble in alcohols, ketones, chlorinated hydrocarbons, and esters, but it is insoluble in aliphatic hydrocarbons, fats and waxes. Specimens of

large molecular weight swell in solvents such as chloroform, and are incompletely soluble. The macromolecules of the polymer may be linear or branched. The viscosity of diluted solutions of various polyvinyl acetates has been studied by several authors[6].

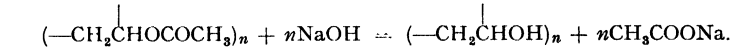
Because of its high adhesive power, polyvinyl acetate with a medium degree of polymerization is included in dispersions used for coating leather, cardboard, wood, metals, etc. The dispersions are prepared by emulsion polymerization of mixtures containing vinyl acetate, acrylic esters, and other monomers. Soaps and other detergents are used as stabilizers, and peroxides as catalysts for the polymerization.

3. Polyvinyl Alcohol

Polyvinyl alcohol is a water soluble polymer of the following constitution



Contrary to the previously mentioned polymers, this substance is not prepared by polymerization of a vinyl alcohol (since a stable vinyl alcohol is not known), but by means of a polymer-analogous reaction from polyvinyl acetate. The acetate must be saponified as follows:



These reactions have been thoroughly studied by Staudinger and his associates[7]. It could be shown that the degree of polymerization remains the same after saponification, if a pure polymer is used. This is shown in Table 25 (p. 189). Consequently, the carbon backbone in the macromolecules of polyvinyl acetate and polyvinyl alcohol is the same. The results with the very high molecular commercial polyvinyl acetates, however, were somewhat different. The average degree of polymerization after saponification was the same only for the specimens with medium large molecules (mol. wt. about 50,000–100,000), whereas the degree of polymerization of the very high polymeric fractions decreased on saponification (Table 50). The decrease is to the extent of one half to one third of the original degree of polymerization (P). Hence, the very large molecules of the polyvinyl acetate seem to appear in solutions as dimers or

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trimers. The bonds between the macromolecules are relatively strong, although they are split by saponification. That there may be some cross-linking in these polymers is substantiated by the swelling and partial solubility. The fact is noteworthy that the large molecules of polyvinyl alcohol obtained in the saponification reactions could be reacetylated without changing the degree of polymerization (Table 50).

TABLE 50

POLYMER-ANALOGOUS REACTIONS WITH POLYVINYL ACETATE AND POLYVINYL ALCOHOL (after Staudinger[7])

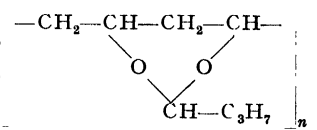
Polyvinyl acetate in acetone (Average degree of polymerization, P)	*Polyvinyl alcohol in water*, P	*Reacetylated polyvinyl acetate in acetone*, P
650	700	650
5000	2400	2450
5000	2450	2600
7500	2200	2250

The solubility of polyvinyl alcohol in water is due to the high polarity and large number of OH-groups in the macromolecules. Hence, polyvinyl alcohol is a hydrophilic colloid. It has a certain protective ability, and is coagulated by highly concentrated salt solutions. Strangely enough, coagulation occurs also upon heating the solution. Another interesting property of aqueous solutions is the formation of blue compounds with iodine. In this, polyvinyl alcohol resembles starch. Gelation of polyvinyl alcohol solutions occurs in the presence of azo dyes of the Congo red type[8]. The dye molecules probably produce interlinking of the linear polymer molecules by some type of secondary bond formation.

Contrary to polyvinyl acetate, the solid polyvinyl alcohol is strongly oriented upon stretching, and the X-ray spectrum reveals periodicities compatible with the structural formula presented above. The unit cell is traversed by two chains which are stretched like paraffin chains, and the identity periods along the fiber axis are 2.5 A. Intermolecular linking between adjacent chains is indicated by a spacing of 2.7 A, which is assumed to be hydrogen bonding between two OH.....OH groups[9]. It may seem surprising why this polymer is soluble in water when other linear polyhydroxy com-

pounds, such as cellulose, are insoluble. The reason for this seems to be the much higher degree of crystallinity of cellulose in comparison with that of polyvinyl alcohol. Because of this very high degree of orientation, the other constitutive groups contribute to the intermolecular cohesion more than in polyvinyl alcohol, although the hydrogen bonding in the latter is significant in the intermolecular attraction. The dissolution of polyvinyl alcohol in water is an exothermic reaction. This indicates that the energy liberated upon hydrate formation with the OH-groups of the polymer is larger than the energy needed for cleaving the chain linking hydrogen bonds. The stability of aqueous solutions of this hydrophilic colloid is due to the solvation of the hydrophilic OH-groups by water. This hydration decreases as the temperature increases. At a certain high temperature there is so little hydration that the macromolecules stick together on collision due to cohesion and H-bonding between chains, producing a flocculation of the colloid.

Many derivatives of polyvinyl alcohol are known, since the alcoholic hydroxyls react readily with many reagents. The starting material for these derivatives usually is polyvinyl acetate which is hydrolyzed with acid in the presence of the reagent. Polyvinyl butyral is one of the most important derivatives in this group. It is prepared by hydrolyzing polyvinyl acetate with mineral acid and condensing the polyvinyl alcohol with butyraldehyde[10]. Polyvinyl butyral has the following constitution:



This polymer is used as a component in very adhesive mixes, for example in multilayer safety glass or in corrosion protecting metal paints[11].

4. Polyacrylic Acid and its Derivatives

Acrylic acid CH_2=CHCOOH and acrylonitrile CH_2=CHCN are important monomers which can be polymerized readily by ordinary methods. Methods for synthesizing these monomers from cheap starting materials (e.g. ethylene and hydrocyanic acid) are available, and both monomers and polymers are prepared industrially in large quantities. The properties of the polymers depend to a large extent

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on the conditions of polymerization. Slow polymerization at lower temperatures and with small amounts of catalyst renders very large molecules. The very high polymeric materials are less soluble than the lower molecular products. The viscosity of the solutions is high, and the intrinsic viscosity increases with increasing molecular weight[12]. Since the macromolecules contain the highly polar and ionogenic carboxyl groups, the polymer is soluble in water, and insoluble in hydrocarbons. Polyacrylic acid is a typical polyelectrolyte, a polybasic acid which exhibits the usual anomalies in osmotic properties, and in viscosity behaviour, as mentioned before[13]. The viscosity of the acidic solutions increases upon neutralization with a base, reaching a maximum near the equivalence point, and decreasing in the presence of excessive base. Maximum viscosity occurs at maximum ionization since the segments repel each other, and the chains tend to stretch out. Moreover, the electrostatic interactions between the large polyions and counter-ions produce a certain structuration of the whole system. Addition of salts or excessive alkali disturbs the electrostatic equilibrium of the systems, as does any flocculating agent (see Chap. 13).

It is interesting to compare the viscosity of the ionized polyacrylic acid and its salts with the viscosity of the non-ionized derivatives, e.g. esters of the acid. Staudinger and Trommsdorff have prepared esters of polyacrylic acid[12], which they dissolved in butyl acetate in order to determine the intrinsic viscosities. The esters then were saponified, the polymer-analogous salts were dissolved in sodium hydroxide, and the viscosity of these solutions was measured. The results are presented in Table 51.

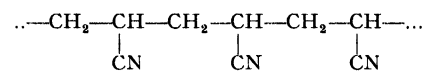
TABLE 51

INTRINSIC VISCOSITY OF POLYACRYLIC ESTERS (IN BUTYL ACETATE) AND SODIUM POLYACRYLATE (IN AQUEOUS 2*N* NaOH) (according to Staudinger)

Average degree of polymerization	*Intrinsic viscosity of the ester*	*Intrinsic viscosity of salt*	*Ratio (salt : ester)*
100	0.047	0.071	1.5
150	0.080	0.115	1.4
250	0.118	0.178	1.5
300	0.150	0.250	1.6
550	0.276	0.426	1.5
850	0.429	0.626	1.5

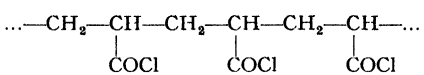
It is obvious that the viscosity of the polymer-analogous salts is approximately 1.5 times greater than that of the esters. Although the solvents are different, the constancy of the intrinsic viscosity ratio is significant. It indicates that the carbon backbone of the macromolecules has not changed in the polymer-analogous transformation.

Polyacrylic acid and its salts have limited practical applications. More important are the acrylic esters, and especially *polyacrylonitrile*. Acrylonitrile is now synthesized directly from acetylene and hydrocyanic acid ($C_2H_2 + HCN = CH_2{:}CHCN$). The monomer can be polymerized easily, whereby a difficultly soluble polyacrylonitrile is obtained. It has the following constitution:



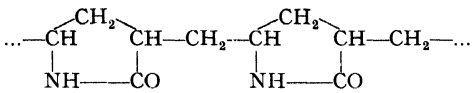
The high resistance of this polymer to solvents may be due to very strong intermolecular attractions which depend on the presence of the highly polar CN groups, and may also be due to some cross-linking. The polymer can be dissolved in dimethyl formamide. The intrinsic viscosity-molecular weight relationships of the polymer have been studied recently[14]. The fibers which can be spun from these solutions (Orlon) have outstanding mechanical properties, and are being manufactured in increasing amounts for mixed fabrics (with wool, nylon, rayon and other materials).

As the nitrile group is rather reactive, polyacrylonitrile can be converted into various derivatives of acrylic acid, such as esters, amides, amines, and the acid itself, by means of the ordinary procedures of organic chemistry. The polymer-analogous reactions, however, must be performed in a nitrogen atmosphere in order to avoid oxidative cleavage of the chains, as pointed out by Staudinger. Many interesting transformations in a long and complex sequence have been accomplished by Vrancken and Smets[15]. For example, polyacroyl chloride was prepared by polymerizing pure acroyl chloride, $CH_2{=}CHCOCl$, under nitrogen atmosphere using ultraviolet irradiation; the polyacroyl chloride obtained,

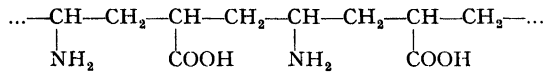


was dissolved in dioxane and treated with sodium azide. A series of

complicated reactions now follow. By reaction of the R·COCl with the azide N_3Na, acroyl azide groups are formed, which rearrange to isocyanate groups R·NCO after losing a nitrogen molecule. The isocyanate residues interact with the neighboring (unreacted) acroyl chloride residues, whereby a cyclic derivative is formed which can be isolated. Upon the hydrolysis of this cyclic polymer, HCl and CO_2 are split off, and a polylactam is obtained which has the constitution



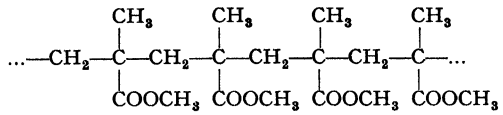
The rings can be opened by treating the polymer with alkali, whereby a polyamino acid



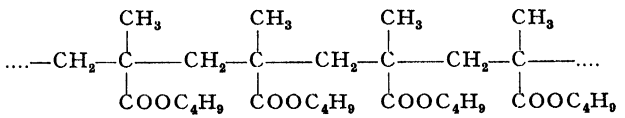
is formed. Remarkable is the fact that the same ampholytic polymer was obtained by treating polyacroyl chloride with hydroxylamine. Unstable seven-member rings are formed along the chain in this reaction. On hydrolysis of this intermediate, the same polylactam and the same polyampholyte are formed as in the reaction with sodium hydrazide. A number of further derivatives have been prepared from these synthetic β-polyamino acids, and the conclusions were substantiated by analytical data.

5. The Polymethacrylates

The polymethacrylates are of great practical importance, especially because of their excellent optical and mechanical properties. These completely amorphous polymers are being manufactured in steadily increasing amounts, chiefly for the production of synthetic organic glass (Plexiglas, Lucite). The most important monomer in this series is the methyl ester of α-methacrylic acid $CH_2=C(CH_3)\cdot COOCH_3$, which is synthesized in several ways, e.g. from acetone and hydrocyanic acid. The monomer can be polymerized easily, and long chain polymethacrylates are formed which possess the constitution:



The example presented is the methyl ester of polymethacrylic acid. Various other radicals can be introduced instead of methyl in the carboxyl group. Polybutyl methacrylate is an important derivative. It is produced by polymerizing the butyl ester of methacrylic acid $CH_2=C(CH_3)\cdot COOC_4H_9$, and has the constitution:



The esters of polymethacrylic acid differ from the esters of polyacrylic acid in that the former are harder and mechanically stronger than the latter. Moreover, the softening temperatures of the polymethacrylates are higher than those of the polyacrylates. The softening points in both series decrease as the molecular weight of the alcoholic residue of the ester increases, as shown in Table 52. The recorded temperatures pertain to polymers of approximately the same average degree of polymerization.

TABLE 52

SOFTENING POINTS OF POLYACRYLIC AND POLYMETHACRYLIC ESTERS

(after H. T. Neher in Houwink's *Elastomers and Plastomers*, Elsevier Publ., Vol. 2, 1949, p. 157)

Polyacrylic esters		*Polymethacrylic esters*	
Methyl	+ 3° C	Methyl	+ 125° C
Ethyl	— 25° C	Ethyl	+ 65
n-Butyl	— 45	*n*-Butyl	+ 33
sec.-Butyl	— 10	*sec.*-Butyl	+ 62

All of the esters are transparent, glassy plastics which are completely amorphous. The polymethacrylic esters of very large molecular weights become elastic and rubber-like at their softening temperatures; the lower molecular weight specimens on melting are converted into viscous liquids.

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The hard and high melting methyl ester of polymethacrylic acid is the chief material for the production of organic safety glass. There are several reasons why this material is preferable to ordinary inorganic glass. (1) Organic glass is much lighter than ordinary glass; (2) it has a high impact strength, and does not collapse and split into sharp splinters; (3) it is much more easily workable than inorganic glass. The lightness is especially important in aircraft. The properties of polymethyl methacrylate glass are compiled in Table 53.

TABLE 53

PROPERTIES OF CAST POLYMETHYL METHACRYLATE
(after H. T. Neher in Houwink's *Elastomers and Plastomers*, Vol. 2, Elsevier Publ., 1949, p. 158)

Light transmission	91–92%
Scattering effect	Practically none
Refractive index	1.49
Specific gravity	1.18–1.19
Hardness, Mohs units	2–3
Tensile strength at 30° C	407–562 kg/cm²
	5800–8000 lb/sq. in.
Impact strength	6.8–7.2
Modulus of elasticity	0.21–0.35 times 10^5 kg/cm²
Coefficient of expansion	0 00008/°C
Water absorption in 24 hours	0 6%

Above 125° C the material softens, and can be formed easily into various shapes. Chemical decomposition begins at very high temperatures of about 300° C, when the large molecules split into the monomer (depolymerization). It is true that the surface of Plexiglas can be scratched more easily than that of ordinary glass. This may be eliminated by multilayer glass in which two thin glass sheets are stuck together by means of a layer of Plexiglas. Such "sandwich glass" is still considerably lighter than ordinary glass, its surface is that of ordinary glass, and it does not form sharp, dagger-like splints and edges upon breaking.

Large, massive blocks and sheets of Plexiglass are made by block polymerization. For injection and compression molding the polymethacrylates are produced by suspension polymerization[16]. After separation from the liquid, a powder is obtained which can be molded at higher temperatures of 150–200° C. The polymeric esters

are insoluble in water, alcohols and aliphatic hydrocarbons. They swell and dissolve in aromatic hydrocarbons, chlorinated hydrocarbons, ketones and esters. They are very resistant to dilute acids.

Copolymers with polymethacrylates are manufactured by means of emulsion polymerization, the dispersions being used chiefly for leather finishing and coatings. For this purpose butyl methacrylate is taken as the chief starting material as it gives more flexible films than the methyl or ethyl ester. Vinyl acetate and some acrylic esters are used as other monomers, since they produce highly adhesive polymers. For example, 100 grams of butyl methacrylate was mixed with 60 grams of vinyl acetate and 40 grams of ethyl acrylate; the mixture was emulsified in 600 ml water using 30 grams of sulfonated castor oil as an emulsifier, and small quantities of hydrogen peroxide, ferrous salt, pyrophosphate, and other additives as catalysts and modifiers. The emulsion was stirred and warmed up to 70° C, at which temperature the polymerization started, as evidenced by a sudden rise in temperature. After several hours most of the monomer is converted into the copolymer. The mixture is liberated from the unreacted monomers by distilling them (and a part of the water) under reduced pressure. The thick, creamy dispersion is convenient for coating. Flexible, transparent films remain on surfaces after evaporation of the water. The process of emulsion polymerization is illustrated in Fig. 97.

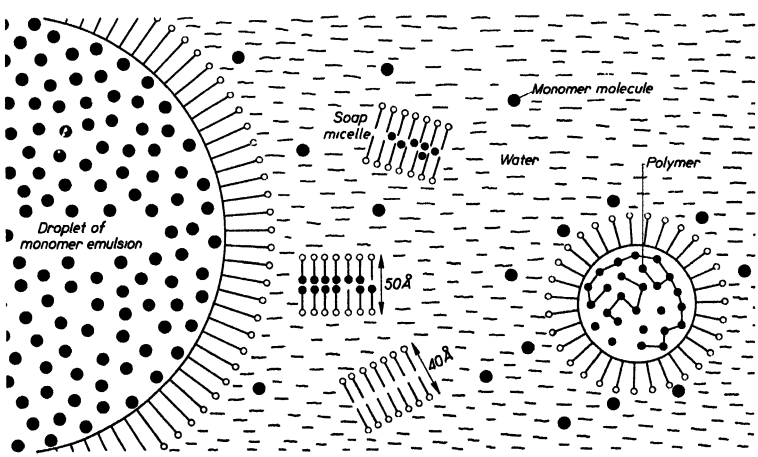

Fig. 97. Mechanism of the emulsion polymerization, according to Houwink.

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The amorphous, glassy state of the esters of polymethacrylic acid is due to the flexibility of the chain and bulkiness of the side residues. Due to the latter property, the chains cannot be oriented, i.e. aligned into highly parallel structures. Plexiglas is composed of kinked, interwoven chains of the polymethacrylate. The relatively high tensile strength is due to intermolecular attraction between the polar groups. The reason for the influence of the methyl group in elevating the softening temperatures of the methacrylates (in comparison to acrylates) has not been clarified as yet. It is, however, understandable that the butyl derivative has a lower melting point than the methyl ester (see Table 52). The bulkiness of butyl side groups hanging on the chains is greater than in the case of the methyl ester. The non-polar butyl residues interfere with the intermolecular attraction of the polar groups more than the methyl groups. The adhesive properties of the polymer are enhanced with increased branching.

The molecular weights of various preparations of polymethyl methacrylates were found to be very high, mostly between several hundred thousand and a million. As the viscosimetric method is one of the most convenient ones, many authors have tried to find reliable, quantitative correlations between the intrinsic viscosity and the various molecular weight averages. The light scattering method has been used recently as a standard method for obtaining the weight average molecular weights. According to Bischoff and Desreux[17], the following equations are valid:

$$\text{in methyl ethyl ketone} \quad |\eta| = 6.8 \cdot 10^{-5} M_w^{0.72}$$
$$\text{in chloroform} \quad |\eta| = 4.8 \cdot 10^{-5} M_w^{0.80}.$$

Chinai *et al.*[18] have found nearly the same expressions, namely:

$$\text{in methyl ethyl ketone} \quad |\eta| = 7.1 \cdot 10^{-5} M_w^{0.72}$$
$$\text{in chloroform} \quad |\eta| = 3.4 \cdot 10^{-5} M_w^{0.83}$$
$$\text{in toluene} \quad |\eta| = 7.1 \cdot 10^{-5} M_w^{0.73}$$

In a rather poor solvent, such as a mixture of 55 vol. methyl ethyl ketone and 45 vol. *iso*propanol, the power at M is only 0.55, hence, the molecules are highly coiled. In the work of Chinai and associates the molecular weight range of the polymers was 410,000–3,260,000.

6. Orientation Polymerization in the Presence of Polymethacrylate

Melville has noticed the interesting fact that pure methyl methacrylate polymerizes in the presence of its polymer[19]. It was ob-

served that the chains of polymethyl methacrylate prepared photochemically from the monomer possess the unusual property of continued growth in the dark. Since no catalysts were present in the monomer, it was difficult to understand why the polymerization should occur in the absence of light. It was found later that the polymerization of the monomer can be initiated by the polymer itself, prepared by any method. The possibility of contamination with catalysts was carefully checked, and the reaction could be observed even with the most thoroughly purified materials. Furthermore, it was found that no other polymer except the polymethyl methacrylate can promote the polymerization of methyl methacrylate, and that this effect is independent of the molecular weight of the polymer. Several hypotheses were proposed, e.g. that the polymer contains certain specific active groups, but none of these explanations could be substantiated by facts. It is also interesting to note that the molecular weight of the polymer formed in this type of reaction increased with increased temperature.

Szwarc calls this kind of reaction *'replica polymerization'*[20]. He has pointed out that the reason for this polymerization in the presence of the polymer is the formation of a regular pattern of the monomer molecules along the chains of the polymer. (Similar ideas have been put forward in the field of proteins by other authors.) According to Szwarc, the polymer facilitates the arraying of the monomer, and the polymerization then takes place in a kind of 'zipping up' of the arrayed units. Considerations regarding the energy changes also seem to support this explanation. The explanation is substantiated also by the observations that supercooled acetaldehyde does not polymerize as long as it is liquid; however, the molecules are rapidly zipped up as soon as crystallization starts. Hence, the polymerization is initiated by the crystalline surface on which the molecules are adsorbed.

The 'orientation' or 'replica polymerization' is a very interesting phenomenon, and its clarification may be of benefit for the solution of biological polymerizations. There are certain points which are not clarified as yet. For example, it is difficult to understand that adsorption in oriented arrays could be significant at 60–90° C, at which temperatures the methacrylate polymerizes in the presence of the macromolecules. The conditions would be more suitable for such arraying upon the crystallization of the supercooled acetaldehyde; however, the free energy liberated in the process of crystallization may have some activating effect on the monomer in this case.

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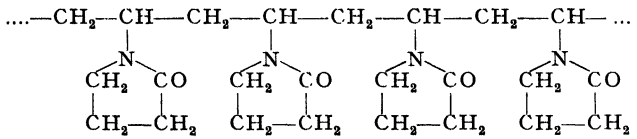
7. Polyvinylpyrrolidone

Polyvinylpyrrolidone (abbrev. PVP) is another interesting and practically important polymer in the series. The monomer, vinylpyrrolidone, is synthesized from acetylene using the high pressure method of Reppe[21]. Polymerization is accomplished easily, and the resulting PVP has linear molecules of the following constitution:



Polyvinylpyrrolidone is a colorless solid, soluble in water, alcohol, acetone, chloroform, and many other hydrophilic and lipophilic solvents. As a highly hydrophilic, non-toxic colloid it was used extensively during the last world war in the German army as a blood plasma substitute. As a linear polymer containing both polar and non-polar groups it has a considerable ability to bind (adsorb) many toxic substances, and it is also occasionally introduced into the blood stream as a vehicle. After a certain period of time it is excreted by the kidneys in the urine.

The ability of PVP to stabilize certain lyophobic colloids is remarkable. It was mentioned in Chap. 14 that PVP is able to stabilize colloidal silver against flocculation by means of salts. This stabilizing ability, however, appears to be selective, since ferric hydroxide or arsenous sulfide sols are not appreciably stabilized by the polymer. Nevertheless, the polymer is now widely used as a thickening agent and stabilizer, as well as an additive to adhesives and surface coatings. One of its most important applications is in hair sprays. Aqueous solutions of the polymer are more or less viscous, depending on the concentration and molecular weight of the PVP. The solutions are nearly neutral, and are compatible with a great variety of other substances.

PVP has certain unique solubility properties. It is soluble in water and also in dry acetone, but is insoluble in aqueous acetone. PVP is usually fractionated by gradual precipitation of aqueous solutions with acetone. It can be precipitated from its solutions by ammonium sulfate, which acts according to the same rules as any liquid precipitants, i.e. the larger the molecules and the more concentrated the polymer the less precipitant is required in order

to cause turbidity (see Chap. 13). However, PVP cannot be precipitated from its aqueous solutions by sodium chloride, calcium chloride or similar salts.

The viscosity of the solutions is also of considerable interest. The viscosity increases linearly with concentration, and the slope of the straight lines (reduced viscosity versus concentration) rises with the molecular weight[22]. In water-propanol mixtures there is a viscosity maximum at about 20 vol.-% propanol, and a flat minimum at 40–80 vol.-% propanol[22]. The viscosity of aqueous solutions is decreased by low and medium concentrations of salts, probably due to dehydration caused by the salts, and resulting tighter coiling of the macromolecules.

The molecular size and shape of the macromolecules of PVP has been thoroughly investigated by Scholtan, Hengstenberg, and others[23]. The ordinary PVP is of medium molecular weight, and fractions of the average molecular weight between 5000 and 150,000 are obtained. However, molecular weights greater than one million have also been reported. The equations proposed by different authors for the correlation of the molecular weight with the intrinsic viscosity of this polymer differ considerably. Even for fractionated specimens in aqueous solutions, and at nearly the same temperatures, different equations have been found valid. For the dependence of the intrinsic viscosity on the weight average molecular weight, the following equations have been proposed recently (ref. 23–25, respectively):

$$|\eta| = 1.4 \cdot 10^{-2} M^{0.70}$$
$$|\eta| = 4.1 \cdot 10^{-3} M^{0.85}$$
$$|\eta| = 6\,75 \cdot 10^{-2} M^{0.55}.$$

All three equations have been claimed valid for aqueous solutions. The reason for the discrepancies is not clarified, although the inhomogeneity of the specimens was pointed out as an important factor. Even the most carefully refractionated samples appear to be very inhomogenous with respect to molecular weight distribution. This has been substantiated by extensive studies on the sedimentation of PVP in the ultracentrifuge[24].

The molecules of the polymer are flexible, and are kinked in solutions to various degrees, depending on the solvent and other factors. The degree of kinking is greater the less the solvation, i.e. interaction with the solvent. This is reflected in the viscosity of the solutions as presented in Table 54 (*loc. cit.* ref. 22). According to

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TABLE 54

REDUCED VISCOSITY η_{sp}/c OF POLYVINYLPYRROLIDONE (aver. mol. wt. 50,000) IN VARIOUS SOLVENTS
T = 27.7° C (*loc. cit.* ref. 22)

Solvent	*Concentration of the polymer* c *grams in 100 cc*	η_{sp}/c
n-Propyl alcohol	4.0	0.497
	2.0	0.437
	1.0	0.416
	0.5	0.400
	0.25	0.385
	0.125	0.380
Methyl alcohol	4.0	0.445
	2.0	0.376
	1.0	0.329
	0.5	0.308
	0 25	0.302
	0.125	0.304
Water	4.0	0.385
	2.0	0.335
	1.0	0.302
	0.5	0.282
	0.25	0.256
	0.125	0.240
Aqu. ammonium sulfate 0.5 *M*/l	0.50	0.184
Aqu. ammonium sulfate 0.1 *M*/l	0.50	0.246
Aqu. KCl, 1.0 *M*/l	0.50	0.246
Aqu. $MgCl_2$, 0.25 *M*/l	0.50	0.172
Aqu. $MgCl_2$, 0.10 *M*/l	0.50	0.257

these data, the macromolecules of PVP in water are more kinked (less solvated and less extended) than in propanol. The viscosity of the aqueous solutions is decreased by ammonium sulfate which is a precipitant for the polymer, as indicated previously. At a certain "critical" salt concentration the interaction with the solvent is lessened to a degree at which the strongly coiled macromolecules begin to aggregate, i.e. they are pushed out of the solution.

It is interesting to note that PVP has some constitutional similarity to gelatin, since the latter protein contains many pyrrol-

idone rings, and is a linear colloid, like PVP. Gelatin, however, is insoluble in alcohols and similar solvents in which PVP is soluble. This is due to the more polar nature of gelatin, since its molecules contain many ionogenic groups which are not present in PVP. Noteworthy also is the precipitation of PVP with tannic acid. The latter reagent, when added in small amounts, reduces the viscosity of the polymer. Turbidity and flocculation occur upon addition of more tannic acid to the solutions. With this property PVP is like the proteins. However, urea and guanidine hydrochloride produce a totally different effect on the PVP than on globular proteins. These reagents denature globular proteins (Chap. 26), whereby the viscosity of the solutions increases. The viscosity of PVP solutions, on the contrary, is decreased by urea or guanidine salts. These highly polar substances apparently interact with the proteins but not with PVP. The reduction of viscosity in the case of PVP may be due to a dehydration effect of these reagents on the PVP coils, resulting in a still tighter coiling instead of expansion.

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CHAPTER 19

Linear Condensation Polymers

All of the synthetic polymers mentioned in the previous two chapters are produced by means of polymerization of specific monomers. The activated molecules of the monomer line up in long chains which are occasionally branched or are cross-linked to some extent. No side products are formed in these polymerizations. The end groups of all of these polymers are indefinite; they depend on the conditions of the polymerization, chiefly on the activators and modifiers added to the monomer. The properties of the polymer depend on the chemical composition, chain length, branching, and cross-linking, but they depend very little on the end groups, as the latter comprise a very small part of the total.

In this chapter some important examples of linear condensation polymers will be described. These polycondensation reactions usually are less spontaneous than the polymerization of the unsaturated monomers. Water is a common by-product in the polycondensations. The end groups are usually definite, and their amount can be determined by ordinary chemical procedures, if the molecules are not extremely large. The *polyesters* and *polyamides* are the most important representatives of this class of polymers. The type of polymers and their properties depend on several factors, chiefly on the number of functional groups in the monomers, and on the degree of polycondensation[1,2]. The polyesters and polyamides are both highly polar compounds. Bifunctional monomers, i.e. those reacting with two active groups, produce long chains. Tri- and polyfunctional monomers yield insoluble space polymers. In this chapter only the soluble linear products of polycondensation will be mentioned.

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1. Linear Polyesters

Linear polyesters can be synthesized by two methods: (1) by polycondensing hydroxy acids, and (2) by polycondensing glycols with dicarboxylic acids. Some examples of such polycondensations have already been mentioned in Chap. 4. It was indicated that the formation of macromolecular polyesters does not occur under all conditions, since lactone rings may also be formed in these reactions. It was shown by Carothers, Flory, and others[1,2] that cyclization instead of polycondensation occurs in the cases where stable five or six-membered rings can be formed. When the monomers have a structure such that these rings cannot be formed, two and more molecules react 'end to end', and linear chains will be produced instead of small rings, generally as follows:

(A) Hydroxy acid only:

$$\mathrm{HO{-}R{-}COOH + HO{-}R{-}COOH = HO{-}R{-}COOR{-}COOH + H_2O;}$$

$$\mathrm{HO{-}R{-}COO{-}R{-}COOH + HO{-}R{-}COOH =}$$
$$\mathrm{= HO{-}R{-}COO{-}R{-}COO{-}R{-}COOH + H_2O;}$$

also

$$\mathrm{HO{-}R{-}COO{-}R{-}COOH + HO{-}R{-}COO{-}R{-}COOH =}$$
$$\mathrm{= HO{-}R{-}COO{-}R{-}COO{-}R{-}COO{-}R{-}COOH + H_2O,}$$

and so forth;

(B) Glycol and dicarboxylic acid:

$$\mathrm{HO{-}A{-}OH + HOOC{-}B{-}COOH =}$$
$$\mathrm{= HO{-}A{-}OOC{-}B{-}COOH + H_2O}$$
$$\mathrm{HO{-}A{-}OOC{-}B{-}COOH + HO{-}A{-}OOC{-}B{-}COOH =}$$
$$\mathrm{= HO{-}A{-}OOC{-}B{-}COO{-}A{-}OOC{-}B{-}COOH + H_2O,}$$

and so forth.

There is a great difference between the addition polymerization of vinyl monomers and these polycondensations in the mechanism of the linear growth of the molecules. In addition polymerization the chains grow at a large number of nuclei similar to crystal growth. During the whole process of polymerization there are growing chains and a monomer. Reactions between free radicals occur at one end of the chain only. In the polycondensation reactions presented above, however, all of the monomer reacts at once. Dimers, trimers, tetramers etc., are formed at once, and the chains grow at both ends. This does not necessarily imply that the polycondensations

are faster and more spontaneous than addition polymerizations, since the reaction rate and completeness of conversion depend on other factors, chiefly on the energy changes involved.

Of the two types of polycondensations mentioned above, type B is of more practical importance than type A. Only the polycondensation of glycols and dicarboxylic acids will be discussed.

The following example shows how a polycondensation can be performed[1]. Succinic acid HOOC—CH_2CH_2—COOH is mixed with a slight excess of ethylene glycol, HO—CH_2CH_2—OH, and the mixture is heated in a distilling flask. At about 160–175° rapid esterification begins, accompanied by the liberation of water. When no more water is evolved the temperature is raised to 220–250° C and the excess glycol is distilled off under reduced pressure. The nonvolatile polyester remains in the flask in the form of a very viscous liquid, which solidifies on cooling to a hard, brittle mass. This can be dissolved in chloroform and precipitated as a granular powder by the addition of ether. The esterification proceeded according to scheme B, and the radicals A and B in this case are the same namely —CH_2CH_2—. The molecular weight of such polyesters is rather low, the solutions are semi-colloidal.

It is noteworthy in the above polycondensation that the reaction temperature was high, and that no catalysts were needed. Under the above conditions, esterification begins with virtually *all* molecules of the monomers participating. In the first stage of esterification practically all the monomer is converted into micromolecular esters composed of a small number (say 4–8) of mers. In a later stage the size of the molecules will increase but their number will decrease. The degree of polycondensation depends chiefly on the time of interaction.

Flory[2] has shown that the reactivity of the functional groups, namely —OH and —COOH, is the same in various stages of polycondensation. The viscosity of the system, of course, increases strongly with progressive growth of the chains. At a sufficiently high temperature, however, the mobility of the molecules is not affected appreciably by the relatively high consistency. Moreover, although the displacement of the whole macromolecule in a viscous medium is hampered, the motion of the segments of the chains will be affected relatively little. Even in a melt containing long polyester chains, the —OH and —COOH end groups will have plenty of chance to meet each other and react.

All esterifications are catalyzed by acids, and the yield of the

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ester depends on the removal of the water formed as a by-product. Since the reactions are reversible, the ester will be decomposed (hydrolyzed) if the water remains in the system, and all four components finally will be in equilibrium, as

$$R_1 \cdot OH + R_2 \cdot COOH \rightleftarrows R_1 \cdot OOC \cdot R_2 + H_2O.$$

In the example of polycondensing glycol and succinic acid mentioned earlier, water evaporates due to the high temperature. If the polycondensations are carried out at lower temperatures, water is usually removed by operation under reduced pressure. It is obvious that the course of the reaction will depend also on the relative amounts of the reacting components. Moreover, the reaction can be modified by adding monofunctional compounds. The end groups of the polymers depend on the relative amounts of the monomers. For example, if succinic acid were in excess in the described polycondensation, the polyester will have carboxyl groups on both ends of the chains. Added modifiers also will change the end groups. Lauric acid is sometimes used to modify the polycondensation. This monocarboxylic acid will react with all alcoholic OH groups and produce ester bonds, thereby terminating the growth of the chains:

$$\underset{\text{Lauric acid}}{CH_3(CH_2)_{10}COOH} + HO{-}A{-}O(-OC{-}B{-}COO{-}A{-}O)_n{-}OC{-}B{-}COOH =$$

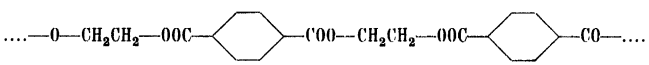
$$\underset{\text{Polyester}}{\qquad\qquad}$$

$$\underset{\text{Terminated polyester}}{CH_3(CH_2)_{10}COO{-}A{-}O(-OC{-}B{-}COO{-}A{-}O)_n{-}OC{-}B{-}COOH} + H_2O.$$

The standard method for the determination of the molecular weight (chain length) of the linear polyesters is by titration of the end groups. For example, the reaction product terminated by lauric acid is a monobasic acid. For a chemical molecular weight determination, the material is purified, weighed, dissolved, and titrated with a base of known normality. For a monomeric compound ($n = 0$) the molecular weight would be 344, when $A = B = C_2H_4$, according to the chemical formula. Hence one gram-equivalent of any base will be needed for the neutralization of 344 grams of such substance. The radical weight of each n is 144, hence, if $n = 10$, the molecular weight will be $344 + 1440 = 1784$. The amount of base needed for titration to the equivalence point will now be one gram-equivalent for 1784 grams. The greater the molecular weight of the polymer the more of it is required for the same amount of base. Generally, if g grams of the substance require eq equivalents

of base, the molecular weight $M = g/eq$. When, for example, in our case $n = 100$, then for an amount of $g = 1.47$ grams 0.1 milliequivalents or 1.0 ml of a 0.1 N base will be needed to reach the equivalence point.

The physical properties of the relatively low-molecular polyesters limit their practical applications. For this reason methods were developed in order to synthesize larger molecules. The molecular size and the chemical composition and structure are important points in the usefulness of any linear polymers, as indicated before. The *'terylene fiber'* (Dacron) is one example of linear polyester possessing excellent mechanical properties. It is prepared by polycondensing terephthalic acid with glycol. The chains are linear, and their structure is the following:



The polymer in the fiber is in a highly crystalline state, and the dimensions of the unit cell are known[3]. The high degree of crystallinity is due to the *smoothness* of the chains. As the material is drawn into fibers, the chains become *oriented*, and the strong intermolecular forces come into full play. Not only the polar ester bonds interact, but the flat benzene rings of the neighboring chains contribute significantly to the intermolecular bonding and strengthening of the whole fiber.

Extensive studies on the poleysters have been made in Staudinger's laboratory during the last ten years. Batzer and his colleagues[4] synthesized and studied a large number of various polyesters of wide molecular weight limits. Emphasis was placed on correlation of the constitution and chain length with the ability of the polymer to form films and fibers. The importance of the smoothness of the chain has already been emphasized (see also Chap. 16). When the chains contain any hydrocarbon residues attached to them, the ability to form fibers is lessened. For smooth chains, the ability to produce films and fibers is greater for the polymers which contain an even number of CH_2 groups in the mers than for those with an uneven number. This is due to steric effects, as ascertained also by X-ray analysis. Furthermore, the number of ester groups is more important than the number of CH_2 groups in the chain forming residues. In all cases films can be formed with lower molecular weight polymeric esters than fibers (Table 55).

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TABLE 55

MINIMUM MOLECULAR WEIGHTS AND NUMBER OF ESTER GROUPS (PER CHAIN) NEEDED FOR THE FORMATION OF FILMS AND FIBERS
(according to Batzer[4])

Polymer from	*Melt. temp. °C*	*Films formed at M*	*Number of ester groups*	*Fibers formed at M*	*Number of ester groups*
$HOOCCOOH + HO(CH_2)_6OH$	75	10,000	120	14,000	160
$HOOCCOOH + HO(CH_2)_7OH$	33	18,000	200	20,000	220
$HOOCCH_2CH_2COOH + HO(CH_2)_6OH$	55	14,000	140	18,000	180
$HOOC(CH_2)_3COOH + HO(CH_2)_6OH$	38	22,000	205	27,000	250
$HOOC(CH_2)_4COOH + HO(CH_2)_6OH$	57	16,000	140	20,000	175
$HOOC(CH_2)_5COOH + HO(CH_2)_6OH$	53	23,000	190	28,000	230
$HOOC(CH_2)_8COOH + HO(CH_2)_6OH$	64	20,000	140	24,000	170

According to the data of Table 55, the minimum number of ester bonds (per chain) needed for film formation is 120-140 for the monomers with an even number of methylene groups per mer. Fibers, however, can be formed only if at least 160-180 ester groups are present. These numbers are even larger for mers with an uneven number of carbon atoms.

The importance of stereochemical factors on the properties of the polymers is further elucidated by the findings on polyesters synthesized from 1,6-hexanediol and fumaric or maleic acid. The latter are unsaturated acids, HOOC—CH=CH—COOH, fumaric acid being the *trans*-isomer, and maleic acid the *cis*-isomer. The rotation in the molecules around the CH=CH double bond is restricted, contrary to the previously mentioned cases of succinic and other saturated acids. Hence, it could be expected that the chains with fumaric acid would be very stiff, the polyesters would crystallize readily, and that they will have a high melting point. The skewed molecules of the maleic acid, on the contrary, should not be able to form esters with smooth chains, they should not have any ability to crystallize and become oriented into fibers. The predictions were confirmed by the experiments of Batzer and colleagues[5]. The properties of the polyesters obtained from hexanediol and fumaric, maleic and succinic acids are compiled in Table 56.

TABLE 56

THE DEPENDENCE OF PROPERTIES OF POLYESTERS ON GEOMETRICAL ISOMERISM OF THE MONOMER (after Batzer and Mohr[5])

Hexanediol with fumaric or maleic acid (succinic acid for comparison).

Average M	*Succinic acid*		*Fumaric acid*		*Maleic acid*
	Melt. Temp.	*Appearance*	*Melt. Temp.*	*Appearance*	*Appearance*
6000	53–54	powder	100°C	powder	oil
15,000	54–55	film forming plastic	103–104	forms films and fibers	viscous oil
25,000	54–55	good for fibers	104	fibrous material	viscous oil
35,000	55–56	fibrous	104	strong fibers	viscous grease
50,000	56–58	strong fibers	105–106	strong fibers	soft, plastic

According to these findings, the polyesters from maleic acid are liquid even when the molecular weight is about 25,000. The only possible explanation for this behavior is the impossibility of the crooked chains to achieve a certain degree of orientation. This is due to the low degree of flexibility of the chain stiffened by the double bonds. The molecules cannot allineate into sheaves and other fixed arrangements, as the intermolecular forces are restricted by spatial conditions. The chains with fumaric acid (*trans*-isomer), however, can become oriented more easily than the flexible chains with succinic acid, because the fumaric polyester chains are stiff. Many other polyesters from a great variety of monomers have been prepared and studied. Regarding the softening (melting) point, the fumaric acid polyesters are between the saturated aliphatic polyesters on the one side and the very high melting terephthalic esters on the other side. The polyesters synthesized from glycols and the *trans*-isomers of the hexahydroterephthalic acid[6] also have high melting points of 110–160° C. Aromatic-aliphatic copolyesters, e.g. of terephthalic acid and sebacic acid, and many others have also been investigated[7]. The melting point of pure polyester of terephthalic acid and ethylene glycol is about 260° C, which is somewhat high for convenient shaping of the polymer. The co-polyesters with glycol, terephthalic and sebacic acid have lower melting points, e.g. 152° C (60 parts terephthalate and 40 parts sebacate). At a certain proportion of the monomeric dicarboxylic acids, the softening point of the co-polyester is even lower than of the sebacate (without terephthalate). Similar "eutectic minima" of melting points have been described in other series of copolymers.

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In contrast to the stiff molecules of the terephthalic and fumaric polyesters, the ordinary saturated aliphatic polyesters have macromolecules of moderate flexibility. The polymers are soluble in acetone, chloroform, benzene, and in many other solvents. The viscosity of the solutions increases with increasing chain length, and it depends also on the solvent. In a good solvent, such as chloroform, the viscosity of the same polyester specimen is higher than in benzene. The intrinsic viscosity dependence on the molecular weight was studied by Batzer on a great variety of polyesters, and it was found that the exponent a in the expression $|\eta| = KM^a$ changes between 0.5 and 1.0, depending on the chain length and the solvent[8]. Hence the macromolecules behave as any other moderately flexible polymers, in close agreement with the theoretical predictions made by Kuhn[9], Debye[10], Kirkwood[11], Stuart[12] and others (see Chap. 9). The values found experimentally for the exponent a are given in Table 57.

TABLE 57

THE MOLECULAR WEIGHT EXPONENT a FOR POLYESTERS OF VARIOUS CHAIN LENGTH (according to Batzer[8])

Number of chain-constituting groups	*The exponent* a *solutions in benzene*	*The exponent* a *chloroform solutions*
32	0.99	1.00
100	0.82	0.87
320	0.75	0.78
1000	0.68	0.72
3200	0.66	0.69
10,000	0.60	0.64
32,000	0.52	0.61

These data indicate that the short chains are almost straight ($a = 1$), and that the degree of kinking increases with increasing chain length. In a poor solvent (benzene) the very long macromolecules may even coil up, assuming a globular shape ($a = 0.5$).

2. Linear Polyamides

The bifunctional amino acids, like the hydroxy acids, may polycondense at sufficiently high temperatures, if the formation of stable

lactam rings does not occur. The ε-aminocaproic acid is the most suitable monomer for this kind of polycondensation. Gabriel and Maass, as early as 1899, found that this amino acid is converted into tough insoluble substances on heating. The study of these and similar polycondensations was undertaken by Carothers. The polycondensation proceeds as follows:

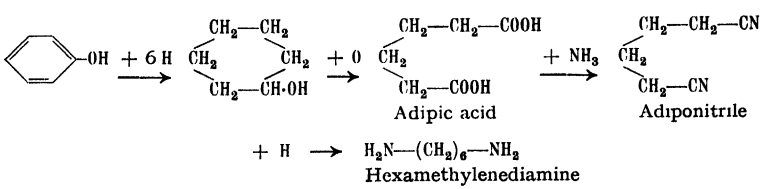
$$\mathrm{HOOC{-}(CH_2)_5{-}NH_2 + HOOC{-}(CH_2)_5{-}NH_2 =}$$
$$\mathrm{= HOOC{-}(CH_2)_5{-}NH{-}CO{-}(CH_2)_5{-}NH_2 + H_2O;}$$

$$\mathrm{HOOC{-}(CH_2)_5{-}NH{-}CO{-}(CH_2)_5{-}NH_2 + HOOC{-}(CH_2)_5{-}NH{-}CO{-}(CH_2)_5{-}NH_2 =}$$
$$\mathrm{HOOC{-}(CH_2)_5{-}NH{-}CO{-}(CH_2)_5{-}NH{-}CO{-}(CH_2)_5{-}NH{-}CO{-}(CH_2)_5{-}NH_2 + H_2O,}$$

and so forth.

The polycondensation continues in random interactions between the active end groups of the di-, tri-, tetra- . .. amides, and water is liberated as the by-product.

Even more important commercially are the polyamides which are manufactured by polycondensing dicarboxylic acids with diamines. The methods were elaborated by Carothers in the Du Pont laboratories, and they were applied later on a large scale[13]. The famous nylon and perlon fibers and fabrics are manufactured from polyamides, and the production output is rising steadily. Nylon was the first completely synthetic fiber which could compete with natural silk and wool. Phenol is the major starting material in the synthesis. It is converted into cyclohexanol by means of catalytic hydrogenation. The cyclohexanol then is dehydrogenated and oxidized, whereby the ring is opened and adipic acid is formed. This is one of the starting materials for the polycondensation. The other component, hexamethylenediamine, is manufactured from adipic acid, by converting it first into the dinitrile (by catalytic combination with ammonia); by further catalytic hydrogenation the nitrile is converted into hexamethylenediamine.



When adipic acid is mixed with hexamethylenediamine, a salt is formed, called "nylon salt", which for the sake of ease of handling,

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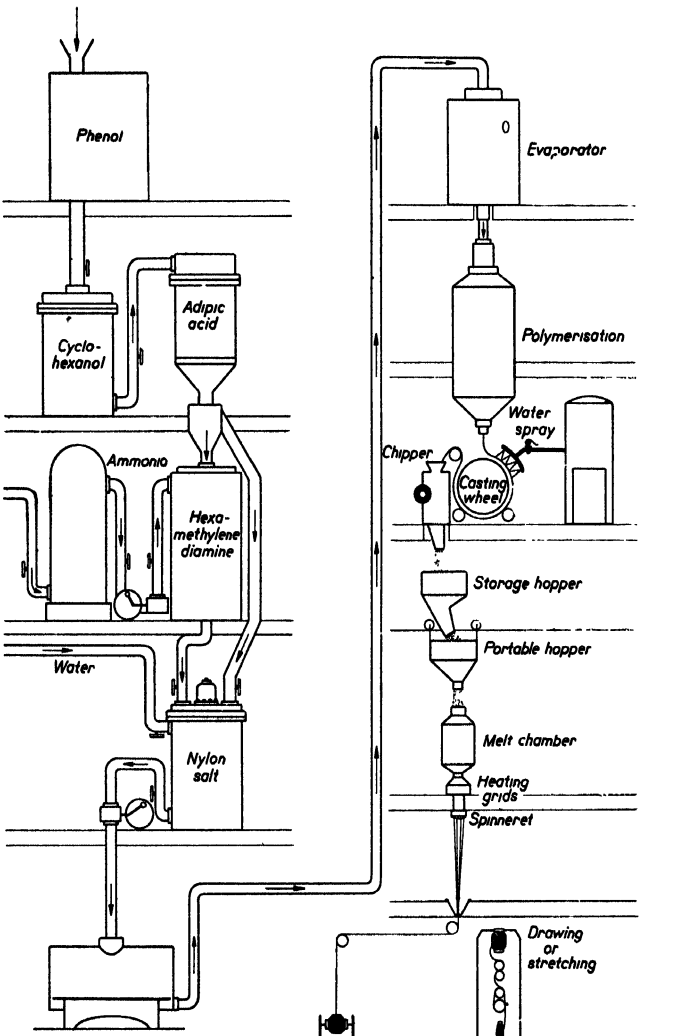

Fig. 98. Diagrammatic sketch showing the manufacturing of nylon fibers.

is dissolved in water. The polycondensation is accomplished by heating, the greatest part of water being removed before that by evaporation. The polyamide is formed according to the following reaction:

$$n\mathrm{H_2N(CH_2)_6NH_2} + n\mathrm{HOOC(CH_2)_4COOH} =$$
$$\mathrm{H{-}[HN(CH_2)_6{-}NHOC{-}(CH_2)_4CO]_n{-}OH} + (2n{-}1)\mathrm{H_2O}\,.$$

The nylon is removed from the reactor, cooled, and cut up into small chips which then are used as the raw material for nylon fibers or for molding. Fibers are formed by melting the polyamide and pressing the melt through fine orifices. The soft filaments solidify on cooling in the air, and smooth, round nylon fiber is obtained. The fibers are then gathered together and stretched by running them over a system of rollers. In this process of stretching out or drawing further orientation of the linear macromolecules occurs, and the tensile strength and crystallinity of the yarn increases considerably. The various steps are shown schematically in Fig. 98.

The changes in tensile strength on orienting the polyamide chains are remarkable. The tensile strength of molded nylon is only about 700 kg/cm², whereas that of cold drawn fibers is 3000–7000. By stretching at 120° C even higher tenacity up to 9300 kg/cm² is claimed. This increase is due solely to the *orientation* and thereby increased crystallinity. Hydrogen bonds between the NH . . .OC groups of neighboring chains can be formed all along the whole chain, if the chains lie parallel. (The same pertains to the cohesion between non-polar groups.) The intermolecular interaction between the macromolecules in a disordered state is weaker than in the oriented state, as was pointed out before. Calculations of the bond energy show that the force needed for the rupture of fibers is much smaller than the force needed for the cleavage of primary bonds in the linear macromolecules. Hence, the macromolecules are not torn to pieces by tearing a yarn, but they slip along each other under stress. This is shown in Fig. 99. According to this concept, the intermolecular forces are of paramount importance in determining the tensile strength of fibers. The length of the chains, of course, must also be considered, since in the case of long chains there are more points of contact between the chains than in the case of short macromolecules. In Table 58 are presented comparative data on the tensile strength of some of the most common fibers in dry and wet states.

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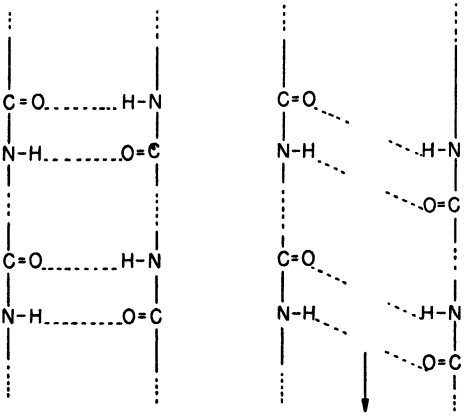

Fig. 99. Intermolecular forces and slippage of the chains on rupture of a nylon fiber.

TABLE 58

TENSILE STRENGTH (kg/cm²) OF DRY AND WET NYLON AND OTHER FIBERS
(according to L. F. Salisbury[13])

Fiber	*Dry*	*Wet*
Nylon	6700	5700
Silk, natural	6700	5000
Linen yarn	4600	5100
Cotton warp yarn	2400	2300
Viscose rayon	2700	1400
Acetate rayon	1900	1300

Nylon yarn shows an outstanding elastic recovery. Although not very elastic, nylon yarn recovers its length completely when stretched, whereas the elastic recovery of rayon or silk is incomplete. The most important applications for nylon are: fabrics, hosiery, tire cord, brush bristles, sewing thread, fishing lines and cords. Polyamide films are flexible and tough, and they can be made transparent. Films and fibers can be obtained not only by working the polyamide melts but also from solutions, i.e. by spinning into another liquid which causes coagulation of the dissolved polyamide.

The properties of the various polyamides are modified by taking different starting materials, such as cyclic amides, or by using

diamines and dicarboxylic acids with different number of CH_2 groups in the radicals. Thousands of various combinations have been tested in industrial research laboratories, in order to vary the properties of the polymer in various ways[14]. For example, it would be desirable to decrease the melting point of the material to be extruded from melts. The softening point of nylon is high (about 250° C), and the workability would be more convenient and inexpensive if the melting temperature could be lowered. This is possible indeed by using mixtures of diamines and dicarboxylic acids, e.g. adding to the ordinary nylon salt a certain amount of decamethylenediammonium sebacate. The latter alone gives a polyamide with the softening point of 200°, but the co-polycondensation products of both salts have lower melting points. A mixture of 40% nylon salt with 60% decamethylenediammonium sebacate softens as low as at 136°. Similar observations were made on other series of co-polycondensation products (*loc. cit.* ref. 7). The reason for the lowering of the melting points is the lessening of the smoothness and homogeneity of the linear macromolecules in copolycondensation. Because of this inhomogeneity, the intermolecular forces cannot display as completely as in very regularly built chains.

The structure of nylon fiber was established by means of X-ray analysis by Brill[15], Baker and Fuller[16]. Various modifications of the nylons have been investigated by other authors, and more detailed information was presented recently, especially by Bunn and associates[17]. The unit cell in the crystallites contains eight chain residues, and the arrangement is monoclinic. The arrangement of the CH_2 groups is planar, and the amide groups are tilted 7° from the plane. An important point in the structure is that alternate chains in the plane are oppositely directed, whereby all hydrogen bonds between the chains can be made perfectly. Depending on the type of the oriented polyamide, various identity periods have been found for the crystallites. The low melting specimens show less orderly structures with large identity periods. Interesting studies on the orientation of nylon and terylene have been made by Keller[18].

Ordinary nylon is resistant to solvents. The unoriented polymer can be dissolved in phenols and in formic acid. Polyamides which are composed of uneven chains (co-polycondensates), or those containing bulky hydrocarbon residues at the chains, may be soluble also in chloroform, esters, ketones, and alcohols. The molecular weight of various nylons is within the range 7,000–20,000. This was

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somewhat surprising, since all the other fiber-forming macromolecules are known to be larger. This fact substantiated the concept that the intermolecular attractive forces are even more important for the tensile strength than the chain length. The molecular weights of soluble polyamides made from sebacic acid and diisobutyl-hexamethylenediamine were determined by Staudinger and Jorder in solutions of cresol[19]. Values of 12,000–13,000 were found.

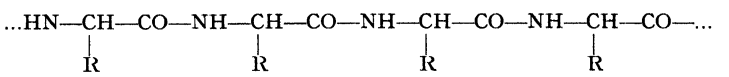
The minimum degree of polymerization needed for securing good fiber, according to Carothers[13], can be estimated best by measuring the viscosity. The polymer is dissolved in cresol, and the minimum reduced viscosity of a 0.5 % solution must be 0.4. Intrinsic viscosities of 0.8–1.0, however, will secure a better fiber. The intrinsic viscosity-molecular weight relationships have been studied by several authors[20]. The conclusion has been drawn that the linear macromolecules of nylon are kinked in solutions to a moderate degree.

The resistance of nylon to solvents is due to the same causes as the resistance to heating, namely the intermolecular attraction. The affinity of the solvent for the macromolecules must be greater than the intermolecular affinity between the chains. Consequently, whenever the macromolecules are split off the bulk and pass into solution, they must be solvated to a high degree. As only polar solvents are able to dissolve the polyamides, the polar amide groups and the end groups must be the loci where the solvation occurs. The amide bonds in the polyamides are remarkably stable toward hydrolytic cleavage by strong acids. The polymer can be dissolved in 90–95 % formic acid and even in cold 90 % sulfuric acid without being decomposed. The CO—NH bonds are hydrolyzed on heating with acids or alkalies. Depolymerization of the molten polyamides occurs at temperatures over 300° C. The original diamines and dicarboxylic acids can be partly recovered from the depolymerized material.

3. Polycondensation Products Related to Polyamides

In the macromolecules of nylon and similar polyamides the CO—NH groups are separated by a certain number of methylene groups. Natural silk resembles nylon in the linear shape of the macromolecules, as well as in the presence of the grouping CO—NH along the chain. However, the chains of natural silk are less uniform than nylon chains. Moreover, the peptide or amide bonds of the

same chain in silk are closer than in nylon. The chains of silk have the constitution:



The synthetic polyamino acids have a similar constitution, as indicated before (Chap. 4), and as will be shown later (Chap. 25). The polyamino acids synthesized by Smets (*loc cit.* ref. 15, Chap. 18), however, have a quite different constitution.

The linear polymers of the urethanes and urea derivatives are constitutionally related to the polyamides. For example, the reaction of 1,4-butanediol and 1,6-hexamethylene diisocyanate results in polyurethanes (Igamid U) as follows:

$$x\text{HO}-(\text{CH}_2)_4-\text{OH} + y\text{OCN}-(\text{CH}_2)_6-\text{NCO} =$$

$$\ldots-\text{O}-(\text{CH}_2)_4-\text{OCO}-\text{NH}-(\text{CH}_2)_6-\text{NH}-\text{OCO}-(\text{CH}_2)_4-\text{OCO}-\text{NH}-$$

$$-(\text{CH}_2)_6-\text{NH}-\text{OCO}-(\text{CH}_2)_4-\text{OCO}-\text{NH}-(\text{CH}_2)_6-\text{NH}-\text{CO}-\ldots$$

This reaction is not a true polycondensation, as no by-products are formed. However, the polyurethanes obtained in such reactions resemble the polyamides. The polyurethanes have nearly the same molecular weight as the polyamides, they can be drawn into high quality fibers and molded into films and other shapes. These polymers are even more resistant to solvents and other chemicals than the nylons are.

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CHAPTER 20

Cross-linked Space Polymers

The polyesters and polyamides described in the previous chapter are composed of linear macromolecules. It was shown that the possibility of fiber and film formation depends largely on the constitution, composition and size of the linear macromolecules. Smooth, linear chains composed of groups capable of intermolecular interaction proved to be the best for high quality fibers. When the molecular chains contain bulky side groups the intermolecular attraction and orientation of the chains is greatly hampered, preventing the formation of fibers. The less oriented and crystallized are the chains, the greater is the solubility. Most of these and other properties of the polymers can be explained, as we have seen, by simple geometrical reasons.

The presence of bulky side groups and branches at the chains results in increased solubility, low softening point, amorphous state, and adhesivity, provided that the side groups of adjacent chains do not interact mutually. If intermolecular linking of the chains occurs, the properties of the polymer are changed profoundly. The cross-linked space polymers formed in such reactions are insoluble. Their properties depend on the relative number of the cross-links: if the polymer is only slightly cross-linked it is more or less elastic, it swells in solvents but does not dissolve. A greatly cross-linked polymer is hard, non-elastic, infusible and insoluble. It is obvious that such properties may be desired. However, it is also clear that such polymers will be more difficult to shape than the highly plastic linear polymers. This difficulty can be overcome if the material is formed before the process of cross-linking is completed. The most important of such space polymers will be described in this chapter.

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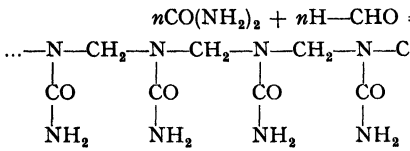
1. The Aminoplasts[1]

The aminoplasts are condensation polymers synthesized from urea and formaldehyde. The mechanism of polycondensation is only partly elucidated. It is probable that the mode of interaction is different under various conditions. The temperature, relative amounts of the reacting components, and the acidity of the mixture are important factors which regulate these polycondensations. It is very difficult to follow the course of reactions in the system after the cross-linking has started and the material becomes insoluble.

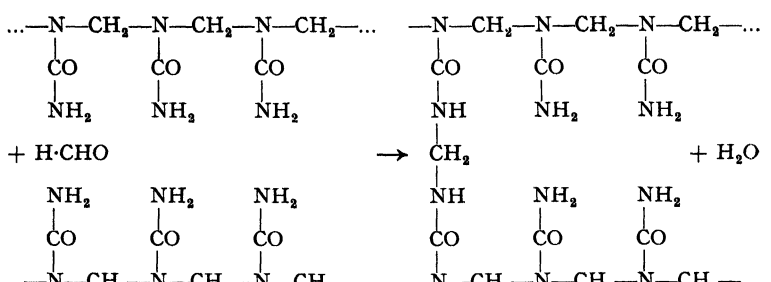
Disregarding the various possibilities in the reaction between urea $CO(NH_2)_2$ and formaldehyde H—CHO in the first phases of reaction, we shall consider only two major stages: the stage of linear polycondensation, and the cross-linking. The linear polycondensation can be formulated as follows:

$$n\mathrm{CO(NH_2)_2} + n\mathrm{H{-}CHO} =$$



$$+ x\mathrm{H_2O}.$$

The cross links are formed in further reactions between the NH_2 groups of the linear polymer and formaldehyde, whereby neighboring chains are interlinked:



The important feature of these polycondensation reactions is that the polymer is more or less completely soluble and fusible when the reaction has not proceeded too far. This is extremely important for the workability of these materials. The common technique is to

fill preheated forms with the incompletely polycondensed material, which is still fusible, and the polycondensation is completed at a certain high temperature during the shaping and cooling off. Materials prepared this way are called *thermosetting* materials. After complete polycondensation, a very tough product is obtained which is insoluble and cannot be melted without decomposition. This change is explained by the *increase in the number of cross-links* during the last phase of polycondensation. Various convenient techniques have been elaborated for industrial application of these processes. For example, a partly polycondensed material of low or moderate molecular weight may be prepared first. The macromolecular chains in such material are short, and they are very little cross-linked. Such products have the appearance of brittle masses which can be ground to powders. In order to shape tough plastics the powder is mixed with a filler, hexamethylenetetramine, and other additives, filled in steel forms, and heated. The chief purpose of the filler is to reduce the price; the mechanical properties do not suffer from addition of the filler, and sometimes may even be improved. Cellulose is used as filler for the best materials, and wood flour for the cheapest products. The added hexamethylenetetramine releases formaldehyde upon heating in the form, whereby further polycondensation and cross-linking is secured. Of the other additives colored matter often is added. The hardening temperature is between 135–180°, and the properties of the obtained plastic depend on this temperature and time of hardening.

The aminoplasts are produced in large amounts. A great variety of things are manufactured from the thermosetting aminoplasts, for example, plastic cups and spoons, toys, furniture parts, and automobile interiors. The plastic is light, durable, and available in a variety of bright colors. It also has sufficient resistance to heat and chemicals. Pure aminoplasts (without fillers) are transparent, glassy masses, although the material is not suitable as organic glass because it does not remain transparent for long periods of time. Incompletely polycondensed products have other uses, such as for glueing together plywood sheets, and for laminating cardboard and paper. Furthermore, the soluble polymer is used for the treatment of textiles, especially of cotton, rayon and linen. After impregnating the textiles with the polymer, heating, and removing the excessive chemicals by washing, the cellulose fabrics are less prone to crease. This anti-crease treatment reduces shrinkage in washing also, and even increases the wet strength of rayon. Another interesting

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application of the incompletely polycondensed urea-formaldehyde resin is its use as an additive to paper pulp in order to improve the wet-strength of paper and reduce the flow of ink. In all of these cases the final hardening, i.e., further polycondensation and cross-linking is the chief process responsible for the various effects. For example, in the treatment of paper the fibrous mesh of cellulose is simply bound together by the overall cross-linked resin. The channels between the fibers are filled, and the paper attains more of the compactness of leather.

It must be pointed out that the amount of cross-linking, even in the hardened resins, must be only a moderate one, although various degrees of cross-linking are present in different cases. The harder the plastic the more cross-linked it is. The resin on cellulose textiles is less cross-linked and more flexible than the material which was thermoset at the 150° treatment. It is impossible to apply in these cases the ordinary criteria of molecular size, as the sizes are indefinite, and a piece of cross-linked resin can be considered as a giant molecule.

A large number of modifications have been proposed regarding the starting materials and further treatment. Mixtures of thiourea $SC(NH_2)_2$ and urea are used for some purposes. Another interesting starting material is melamine (cyanuric triamide) which can be synthesized conveniently from calcium cyanamide. Melamine is polycondensed with formaldehyde like urea, and cross-linked space polymers are formed. Melamine-formaldehyde resins with a low degree of cross-linking are used, for example, in treating wool fabrics. This treatment aids in preventing shrinkage and felting, and improves the washability. After impregnating the textile with the soluble resin the fabric is hardened in order to produce the desired cross-linked resin. Sponge materials are produced by blowing air through partly polycondensed resin, and hardening. This spongy material is very light, and is used for heat insulation. Melamine resins are superior to urea resins for such insulating plates, and are superior also for improving paper.

In Table 59 are shown some properties of urea and melamine resins.

The chief disadvantage of these plastics is their relatively great affinity for water. Although the hardened resins are insoluble in water, they absorb relatively large amounts of moisture. This is due to the highly polar nature of the constituting groups of this resin. For the same reason, pure resin (without filler) is unsuitable

TABLE 59

PROPERTIES OF AMINOPLASTS (WITH CELLULOSE FILLER), after Widmer and Frey, *loc. cit.* ref. 1

	Urea + H·CHO	*Urea + thiourea + H·CHO*	*Melamine + H·CHO*
Molding temperatue °C	135–165	140–155	120–190
Tensile strength kg/cm²	422–914		562
Flexural strength kg/cm²	774		914
Hardness (Rockwell M scale)	120		120
Heat distortion °C	132		196
Water absorption at 20° C (in 25 hours in %)	0.75–2.0	0.7–1.0	0.3–0.6
Boiling water test (10 minutes % weight increase)	3.4	2.3	0.4
Dielectric constant	6.3–7.5		7

as organic nonbreakable glass, since the decrease in transparency is caused chiefly by the adsorption of water, with the resulting slowly occurring structural changes. It may be mentioned that this ability to absorb water indicates that the solid framework of the resin is not extremely compact, i.e., that there is only a limited cross-linking. This is substantiated by the low density (1.4–1.5) of the materials. Finally, it should be mentioned that the urea and melamine resins are amorphous. Hence, the chains are interlinked at random, and are not oriented in any way.

2. The Phenoplasts[2]

The phenoplasts are polycondensation products manufactured from phenols and formaldehyde. They were introduced by Baekeland in 1909, and are sometimes called "bakelites" (although the latter name has been assigned also to other plastics). They belong to the most important of the synthetic polymers, their consumption being about two times greater than that of the urea formaldehyde resins or polystyrene. The phenoplasts, like the aminoplasts, are thermosetting space polymers. The polycondensation occurs according to the following scheme[3]:

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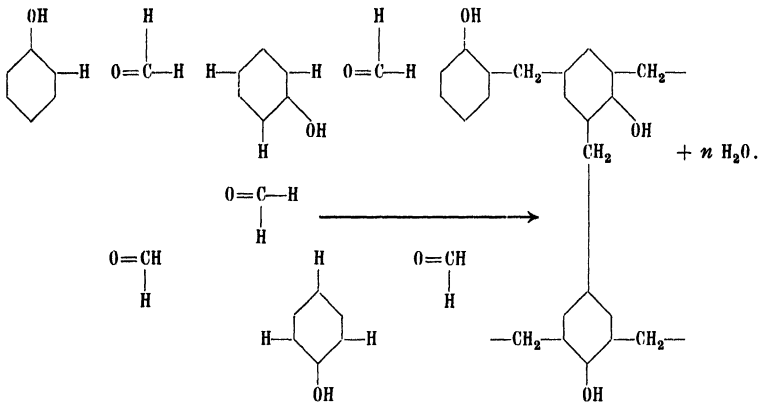
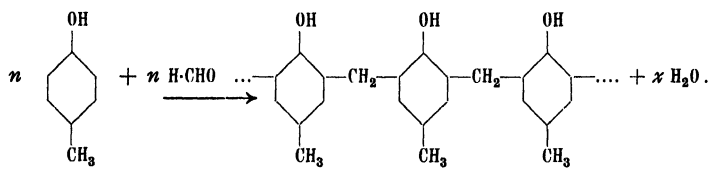


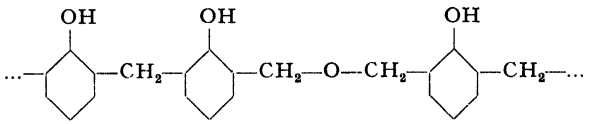
Phenol reacts in this polycondensation as a *trifunctional* monomer. The two *ortho*-hydrogens and the *para*-hydrogen of the benzene rings react with the formaldehyde. The rings thereby are linked through the methylene groups into a three-dimensional network, and water is formed as a by-product. The phenolic hydroxy groups do not participate in this linking directly[3]. It is interesting to consider the reaction of *ortho*- and *para*-cresols with formaldehyde. The *o*- and *p*-cresols contain only two active hydrogens in their molecules and therefore it may be expected that only linear polymers will be formed with such starting material, e.g., with *p*-cresol the reaction is expected to proceed as a linear polycondensation:



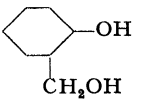
These polymers are indeed linear, soluble substances, although at higher temperatures partially cross-linked materials also can be formed.

Polycondensation with the three-functional phenol and formaldehyde can be altered depending on the acidity and other conditions, such as the relative amounts of the components. According to Houwink[4], the polycondensation is predominantly linear if the

reaction is carried out with an acidic mixture. The products obtained under such conditions are fusible and soluble, and are called *novolacs*. The constitution of these macromolecules differs from the previously mentioned structures, and seems to be as follows:



It can be assumed that in the first stage of the reaction formaldehyde combines with the phenol (in *o*- or *p*- position), and alcoholic compounds, such as



are formed. When one molecule of water is eliminated from two $—CH_2OH$ groups, the ether bridge $—CH_2—O—CH_2—$ between two rings is established[5]. It is obvious that the presence of the alcoholic groups in the polymer increases its polarity and renders it more soluble in water. The hydrophilic nature of the colloid can be reduced by introducing lipophilic residues in the polymer.

The chief starting material for the commercial production of these resins is rather impure phenol which contains certain amounts of the various cresols. For this reason, the reactions occurring during the condensation and hardening of these thermosetting resins are very complex. In the first stages of the reaction more or less soluble and fusible products are obtained, even in an alkaline polycondensation. Materials possessing various degrees of polycondensation and cross-linking can be obtained by interrupting the reaction at a desired stage. The resins and plastics are usually dark colored due to the highly colored reaction products of phenols. However, practically clear, colorless resins and plastics can also be obtained. For this purpose pure phenol and other pure starting materials are used; traces of iron and other metals must be avoided. The polycondensation is carried out in aqueous alkaline solution, formaldehyde being added in excess in order to avoid the presence of some unreacted phenol in the final product. As the polycondensa-

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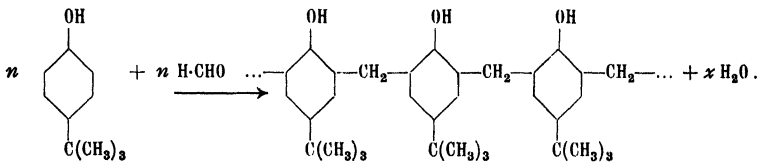
tion proceeds, the solution becomes turbid, and the resin precipitates. The properties of the resin depend greatly on the method of this polycondensation. In further processing, the resin may be either separated, stored or shipped, or the dispersion can be used directly for either molding, for laminating plywood and paper, or for varnishes, etc. The degree of polycondensation and cross-linking can be regulated by changing the degree of acidity. Fillers, dyes and softeners are added to the mixture. The excess of water is removed by evaporation, more formaldehyde is added (in the form of solid hexamethylenetetramine), and the mixture is poured into molds. The hardening is carried out in ovens at moderate temperatures of 150° C. The polycondensation now proceeds still farther, the material becomes more cross-linked, and more water is formed in the reaction. The removal of water from the resin can be facilitated by working under reduced pressure. This is especially important when clear plastics are desired, since small vapor bubbles remaining in the material will cause opacity.

The phenoplasts are used generally for the same purposes as the aminoplasts. The phenoplasts are more resistant to water than the aminoplasts; the tensile strength and resistance to heat is about the same for both. Cotton fibers, paper pulp, wood flour, asbestos and many other materials are used as fillers. Complicated objects, e.g., radio cabinets and housings for telephone apparatus are molded under high pressure. The use of these resins for laminating plywood and cardboard is important. Further polycondensation and cross-linking thereby occurs, and the phenoplast glues together the cellulose fibers and other ingredients into a tough material. The tensile strength of phenoplast-laminated paper can be as high as 890 kg/cm^2. Even more tough and also resistant to high temperatures (up to 215° C) are laminated products of glass cloth which are hardened with the phenoplast resins.

Very interesting is the utility of the phenolic resins for varnishes, paints and laquers. The greatest obstacle in the application of the incompletely polycondensed resin for varnishes was the insolubility in drying oils and hydrocarbons. This difficulty has now been overcome in several ways. One of the oldest methods was to combine the phenol-formaldehyde resin with rosin. The abietic acid and similar compounds of the rosin react with the phenol-formaldehyde resin, acting as a solubilizing agent, and the compound with rosin is soluble in tung oil. Another way of producing oil-soluble phenol-formaldehyde resin was to use as starting materials substituted

bifunctional phenols. If, for example, the para-position is substituted with the lipophilic tertiary butyl group $(CH_3)_3C$—, a linear polycondensation product of the following constitution can be obtained:



This polymer is more compatible with oils than the ordinary phenolic resins. Another advantage is its stability to discoloration by light. A third method of obtaining oil-soluble resins is to combine the phenol-formaldehyde resins with unsaturated fatty acids. The combining is accomplished by adding the fatty acid at an early stage of polycondensation when little cross-linking has occurred. The compounds then are soluble in drying oils due to the increased lipophilic character produced by the fatty acid 'tails'.

The aminoplasts are amorphous. No orientation can be observed by means of X-ray analysis, either on further hardening or deformation. The incompletely reacted materials are somewhat elastic, but on further cross-linking they become hard. The thermoset plastics are insoluble, but they swell somewhat in certain solvents. According to De Boer[6] and Houwink[7], even the thermoset phenoplasts are not cross-linked to the greatest possible degree. This conclusion was based on a consideration of the tensile strength, elasticity, specific gravity and other properties of the plastic. The exact amount of cross-linking, however, is unknown.

3. The Anilinoplasts

Formaldehyde reacts with aniline in the presence of acid catalysts. Depending on the conditions, linear and soluble or cross-linked space polymers can be formed:

Linear polycondensation:

$$n C_6H_5NH_2 + n H{\cdot}CHO =$$

$$...—NH—C_6H_4—CH_2—NH—C_6H_4—CH_2—NH—C_6H_4—CH_2—... + x H_2O.$$

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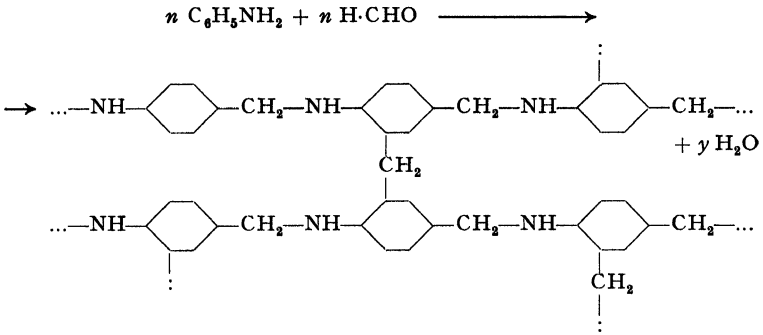
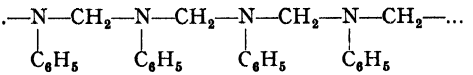
Polycondensation with cross-linking:



The NH_2-group, like the phenolic OH, activates the *o*- and *p*-hydrogens of the ring, and formaldehyde attacks the rings at these positions, as well as reacting with the amino-groups[8]. Other structures have also been proposed for these polymers, e.g., the chains



which can then be interlinked through the *p*- or *o*-loci of the benzene rings with other chains.

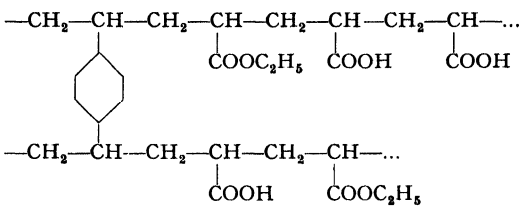
The polymers are thermoplastic, and their properties depend largely on the relative amounts of the reacting components. The heat resistance of these plastics is greater than that of the phenoplasts and aminoplasts.

4. Space Polymers as Ion Exchangers[9]

The ion exchange resins were discovered by Adams and Holmes[10], and in a short time these important products became indispensible for various practical purposes. The ion exchange resins are space polymers containing ionogenic groups as constituents of the framework. For example, if the basic phenylenediamine is added to aniline in the polycondensation with formaldehyde, a cross-linked space polymer with the basic —NH_2 groups attached at the carbon skeleton is obtained. In aqueous medium, ionization takes place at these basic groups: $R—NH_2 + H_2O = R{\cdot}NH_3^+ + OH^-$. (The degree of ionization may be increased by treating the resin with

methyl iodide.) The cations of the resin are able to bind anions, releasing an equivalent number of hydroxyl ions into the solution, as mentioned previously. Similarly, the cation exchange resins contain the anionic sulfate, sulfonate or carboxyl groups. Ion exchange resins of cross-linked sulfonated polystyrene, cross-linked polyacrylic acid, or sulfonated phenol-formaldehyde space polymers are of importance. For example, when phenolsulfonic acid $C_6H_4(OH)SO_3H$ is polycondensed with formaldehyde, an acidic resin is obtained which binds cations, and releases equivalent numbers of hydrogen ions into the solution. The affinity of the heavy metal ions for the macro-anion of the resin is usually greater than the affinity of sodium or potassium. Efforts have been made to produce ion exchange resins with a high degree of selectivity, e.g., in exchanging sodium for potassium.

Deuel and associates[11] have thoroughly studied the cation exchangers which were obtained by cross-linking polyacrylic acid with divinylbenzene. The acid was partially esterified, and thus the charge density was altered. The space polymer studied had the following structure:



It could be shown that the selectivity of the ion exchanger depended on the degree of esterification, i.e., the charge density, as only the unesterified carboxyl groups are capable of dissociation and ion exchange. The polyacrylic resins bind lithium and sodium ions more weakly than ammonium ions, while hydrogen, calcium and barium ions are bound more strongly. The selectivity was found to be greater the greater the charge density of the resin.

The *degree of cross-linking* was also found to be an important factor in determining the selectivity of ion exchange[12]. First, the porosity of the space polymer depends largely on the cross-linking. Excessive cross-linking will result in highly compact solids which are ineffective as exchange resins, since ions will be unable to penetrate into the solid, and the exchange will occur only at the

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surface. The less the cross-linking, the larger will be the channels in the space polymer. Hence, depending on the average size of the pores, ions of various sizes may be permitted to enter the exchanger and participate in the exchange reactions. It may be concluded from this that only slightly cross-linked, loosely built space polymers may be the best. This, however, is not true for all purposes. For example, if a solution containing a variety of solutes must be liberated from only some of the ions, it would be desirable that only these particular ions enter the space polymer. If the other ingredients are also bound to the resin, the attempt to separate the substances will fail. For example, such problems may arise when semicolloidal peptides of predominantly basic (cationic) nature must be liberated from some inorganic cations by this method. If the pores are too large, the material to be purified may itself be bound in the resin.

5. The Alkyd Resins

The alkyd resins or glyptals are space polymers obtained by polycondensing the trifunctional glycerol with dibasic acids, chiefly phthalic acid. The two hydroxyls of the glycerol react with the two carboxyls of the phthalic acid, producing linear polyesters. The chains then are linked by the third function of the glycerol reacting with more acid.

The resin is applied before the polycondensation is completed, as the completely cross-linked material is a hard solid. The incompletely cross-linked hot melt is very adhesive, but is less stable to moisture than the previously mentioned aminoplasts and phenoplasts. The properties of these resins can be modified by adding unsaturated fatty acids to the monomers. This has two effects: first, it reduces the molecular weight and the cross-linking, since the monofunctional fatty acid reacts with the alcoholic OH-groups of glycerol and prevents the growth of the chains; and, second, the product becomes more lipophilic and less hydrophilic than the ordinary alkyd resin. Resins of this type are used for varnishes. Rosin, benzoic acid, and many other ingredients are used in modifying these resins for various purposes. Pentaerythritol $(CH_2OH)_2C(CH_2OH)_2$ is sometimes used instead of glycerol, since this alcohol with its four functional groups offers more chances for cross-linking chains than the trifunctional glycerin.

6. Space Polymers in Drying Oils

Linseed oil and other drying oils are composed of triglycerides of unsaturated fatty acids, such as oleic, linoleic, and linolenic acids. The double bonds of the acid radicals react with the oxygen of air slowly, whereby the oil becomes more viscous. Thin layers may solidify in rather short time. 'Drying' in this case is not solidifying due to evaporation but occurs due to reactions which lead to the formation of large molecules. The interlinking of the triglyceride molecules probably occurs by opening some of the double bonds in the radicals of the unsaturated acids, and by forming oxygen bridges. Migration of double bonds was revealed by means of spectroscopic analysis of the ultraviolet part of the spectrum. When the oils are heated, a conjugated system of double bonds is formed in the unsaturated acids which have two or more double bonds in the molecules. Cross-linked solids are also obtained by treating these 'drying oils' with vulcanizing agents such as sulfur monochloride S_2Cl_2. The formation of space polymers is secured by the fact that three acid residues are present in each triglyceride molecule; moreover several double bonds are available in each residue[13]. The starting material is polyfunctional. The 'drying' or hardening is promoted by light, and also by traces of metal oxides and other catalysts. Linoleum is manufactured by oxidation of linseed oil which is mixed with sawdust, cork dust, and other fillers.

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CHAPTER 21

Cellulose and Similar Polysaccharides

1. Cellulose and Its Accompanying Macromolecular Substances[1]

The chief solid constituent of our flora is cellulose. Owing to its fibrous constitution, cellulose is the most efficient material for the building of cell walls and other structural tissues. The magnificent slender trunks of our sequoias and pines, and the stalks of all grasses are built of the long, thin macromolecules of cellulose.

Cellulose of the woody tissues of trees and shrubs, or of the stalks and hulls, is not pure, but is mixed and combined with a large number of other organic and inorganic substances. The most important (quantitatively) organic substances which accompany cellulose in plant tissues are the *pentosans* and *lignin*. The amounts of these ingredients in various plant tissues are mentioned in Table 60.

The pentosans are also polysaccharides, but they are constitutionally non-related to cellulose. The pentosans are polycondensation products of the pentoses, chiefly of xylose and arabinose. Lignin, which is even less chemically related to cellulose than the pentosans, is a benzene derivative. Contrary to cellulose and the pentosans, it is a non-fibrous polymer in which the fibrous macromolecules of the polysaccharides are embedded. Its structure will be discussed in the following section.

Not all plant materials have compositions similar to those shown in Table 60. For example, seeds are very rich in other polysaccharides, such as starch. Proteins and fats are also found in seeds in relatively large amounts. Sugar cane and sugar beet contain large amounts of saccharose. Acidic polysaccharides, such as agar-agar, are found in abundant quantities in sea weeds. Nevertheless, cellulose

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TABLE 60

PARTIAL ANALYSIS OF SOME WOODY TISSUES

(after Whistler and Smart[1])

Tissue	*Cellulose*, %	*Pentosan*, %	*Lignin*, %
Pine (Western Yellow and White)	58	7	27
White spruce	53	12	28
Yellow Cedar	54	8	31
Redwood	48	8	34
Sugar Maple	58	22	24
Flax shives	45	25	29
Wheat straw	48	27	18
Corn stalk	40	26	16
Soybean stalk	50	25	19
Oat hulls	34	30	14

is the major building material of the bodies of plants. An electron micrograph of a section of spruce fiber is presented in Fig. 100.

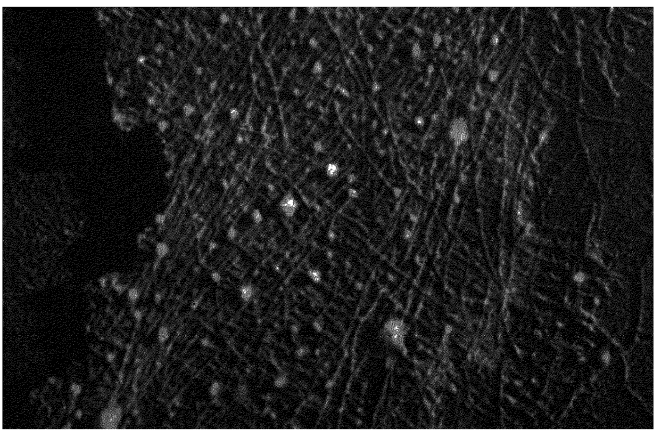


Fig. 100 Longitudinal section of a fragment of spruce fiber. Magnification 29,000 ×. (From E. Ribi, *Expil. Cell Research*, 5 (1953) 161.)

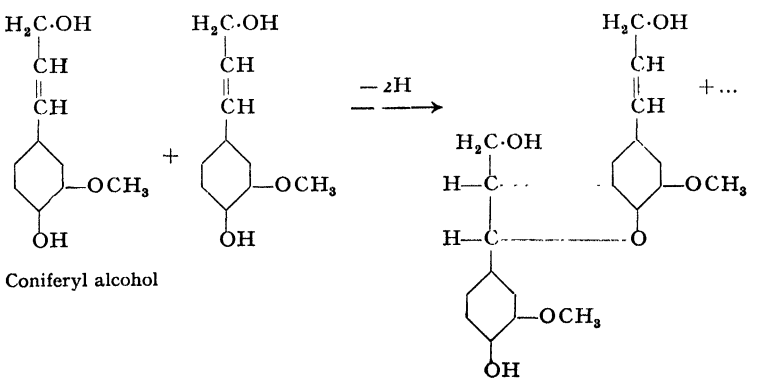
The origin and formation of cellulose in cells is unknown. The macromolecules are synthesized in the cells in order to produce a suitable building material for the body of the plant. From our human point of view, cellulose is a suitable material for making clothing in order to protect our bodies. Cotton is still the most important textile, and cotton is a relatively pure cellulose. Linen

and rayon are other types of cellulose fibers and fabrics. Cellulose is used also as the starting material for numerous industrial products, such as paper, plastics, glues, gun-powder, and many other items.

2. Lignin — a Polymer Accompanying Cellulose[2]

The problem of lignin was one of the hardest in the field of natural organic substances. One reason for this is the simple fact that lignin is located in plant tissues between the long, tightly-linked fibers of cellulose and other fibrous polysaccharides. Lignin itself has always been a very undesirable material, the only objective being to liberate the desired cellulose from it. However, this appeared to be impossible without drastic treatment of the material. There are no methods available for separating lignin from cellulose without degrading both of them. For example, chopped wood or straw in paper mills is boiled with calcium hydrosulfite $Ca(HSO_3)_2$ in order to liberate the cellulose from lignin. Cellulose thereby remains in a coarse dispersion (pulp) and lignin is dissolved. According to the currently prevailing views, cellulose is slightly degraded during this treatment, and lignin is cleaved to a considerable degree.

According to the extensive studies of Freudenberg and his colleagues, lignin is a polycondensation product of coniferyl alcohol, sinapinic alcohol, and similar carbocyclic, partially methylated phenols which have unsaturated side chains containing alcoholic groups. Coniferyl alcohol could be converted into lignin by a fungal enzyme. It was shown that dehydrogenation occurs in this reaction, and that the polymer obtained gives all the reactions of lignin. The first step is believed to occur as follows:



Coniferyl alcohol

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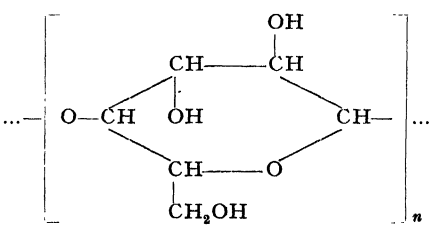
Further monomeric molecules of the coniferyl alcohol can be added in the reaction of the unsaturated side chain with another ring, two hydrogen atoms being subtracted at each step. It is not known how large the molecules of native lignin are, as the substance can not be obtained in a native state. Freudenberg, however, could prove that coniferin reacts with the enzymes (redoxases) of plants in vivo during lignification. By introducing radioactive coniferin into spruce it was confirmed that spruce lignin is a copolymerizate of dehydrogenated coniferyl and sinapinic alcohols[3].

The mode of interlinking lignin and cellulose is unknown. Even if there is not any chemical interlinking, the fibrous nature of the components, especially the cellulose, secures ample secondary bonding and strong cohesive forces. Lignin is amorphous, and the highly crystalline patterns of natural fibers are due solely to the crystalline cellulose.

Among the various fission products of the lignins from various sources vanillin, $C_6H_3(OH)(OCH_3)CHO$, is of interest. It has been isolated in high yields by oxidative degradation of lignin with nitrobenzene[4]. Many other aromatic substances have been isolated from the decomposition products. The kind and amount of these degradation products depend upon the method of degradation and the source of the lignin.

3. The Macromolecules of Cellulose

Cellulose is one of the polysaccharides which can be designated by the formula $(C_6H_{10}O_5)_n$. The mer in the macromolecules is anhydroglucose. Glucose is obtained on hydrolytic cleavage of cellulose, this process being accomplished by boiling with strong acids or by enzyme action at ordinary temperatures. Contrary to the starches and glycogen, the macromolecules of cellulose are not branched. Cellulose is a perfect linear colloid, as established by both chemical and physical methods. The details of constitution have been studied extensively, and the following structure is now accepted:



Hence, the linear macromolecules are composed of pyranoid six-rings which are linked by oxygen atoms. Each glucose residue or ring contains three free alcoholic hydroxyl groups which give all the ordinary reactions of alcohols. For example, these hydroxyls can be etherified or esterified. One of the end groups of the chains should contain a reducing group, and at the other end should be four alcoholic hydroxyls in the anhydroglucose residue. Hence, if cellulose is completely methylated and then hydrolysed, some tetramethyl glucose should be found as well as the trimethyl glucose. It was found, indeed, that both the four hydroxyls and the aldehyde end group can be detected. It was also found that the relative amount of these end groups is greater the lower the molecular weight of the original cellulose. However, only in the partially degraded specimens of average molecular weight 5000–20,000 could the end groups be detected by chemical methods. In native cellulose, which was prepared with particular care, no end groups could be demonstrated, probably due to the fact that the end groups then comprize a vanishingly small part of the total.

The mode of linking of the glucose residues in the chain was established by extensive chemical studies, due chiefly to Freudenberg, Haworth, Willstaetter and Zechmeister[5]. It could be shown that the molecules contain 1,4-β-D-glucosidic linkages. Not only acetylation products of the disaccharide β-cellobiose, but also other intermediate degradation products composed of 3, 4, 8, etc., mers have been isolated.

Further evidence concerning the constitution of cellulose was obtained by physical-chemical studies of the optical rotation, X-ray diffraction, and kinetics of hydrolytic decomposition. According to Freudenberg and Kuhn, the optical activity of the methylated and acetylated decomposition products of cellulose (e.g., acetates of cellobiose, triose, and tetrose) changes regularly with the increase of chain length or number of mers. A straight line relationship could be postulated, and cellulose was shown to fit on these straight lines as if it were a long chain β-glucosidic compound of the same constitution as β-cellobiose[6]. The kinetics of hydrolysis by acids also indicates that the structure throughout the chain is uniform, although oxidative degradation has shown that there may be a few weaker links. The latter seem to appear regularly at intervals of approximately one per 500 glucose units[7]. Some authors claim to have found other reactive groups, such as carboxyls, but these findings could not be confirmed by very careful examination of

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pure specimens of native cellulose. Cotton wool is one of the purest kinds of native cellulose, and it shows a negative charge on electrophoresis. It is, however, very likely that this charge is due to some acidic polysaccharides which accompany cellulose as impurities.

A very important contribution in the elucidation of the structure of cellulose was rendered by X-ray analyses[8], as indicated earlier (Chap. 11). The unit cell is composed of four glucose residues, and it has the dimensions $a = 8.3$ A, b (fiber period) $= 10.3$ A, and $c = 7.9$ A. The completely dry fiber has a density of 1.57, which is very close to the density calculated from X-ray data (1.60 ± 0.05). From this it may be concluded that the chains are packed throughout the fiber as tightly as they are in the crystalline portions of the fiber. Truly amorphous regions and branched chains should result in a lower density. Consequently, even the regions which are not perfectly crystalline are highly oriented. The molecular chains are twisted, but they are all largely parallel in native fiber. The successive links of the β-glucose residues are rotated through 180°, hence the cellulose chains have a diagonal screw axis as a symmetry element. In regenerated cellulose the density and degree of orientation, of course, is lower.

The microstructure of cellulose fibers is revealed by microscopy, especially by electron microscopy. Cellulose appears under the electron microscope as composed of long fibers which can be seen as bundles of smaller fibrils (Fig. 101). The latter, however, are not the macromolecules but sheaves of the long and thin linear macromolecules. The diameter of the original native cotton fibers, which can be discerned by the naked eye, is 0.012–0.042 mm. The fine fibrilla discernable in the electron microscope have a diameter of 0.00002 mm or 200 A. The diameter of a single macromolecule of cellulose is only about 6 A. The best fibers of flax and jute are thicker than cotton fibers, but the macromolecular structure in all cases is practically the same[9].

The molecular weight of cellulose is very high, although it is known with accuracy only for the specimens of cellulose which are probably more or less degraded in the process of isolation. The milder the methods of isolation, the larger the figures which have been obtained for the molecular weights. It must be admitted that the problem is very difficult, and is far from being solved as concerning native cellulose in wood and fibers. The fibrous structure and polarity of the linear molecules make it almost impossible to liberate the original fibers from the bundles without damaging them.

Because of the uniformity of structure and high degree of crystallinity, the macromolecules cling together very tightly. This produces a mechanically very strong structure. It is a difficult task to bring cellulose into solution, i.e., to split the fibrillar sheaves into single macromolecules.

Fig. 101. Electron micrograph of cellulose in developing oat coleoptile cell wall. Magnification 22,500 ×. (Courtesy of Dr K. Muehlethaler, *Biochim. Biophys. Acta*, 5 (1950) 1.)

The purest cellulose is prepared by the following method. Cotton fiber is first extracted with alcohol and ether in order to liberate the material of any lipids and waxes. The fibers then are treated with boiling 1% sodium hydroxide in order to split off the traces of lignins and other organic substances usually found in natural fibers. The fibers then are washed with dilute acetic acid and distilled water.

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For the molecular weight determination, cellulose must be dissolved. If the treatment for purifying and dissolving the macromolecules is too drastic, they will be split. With incomplete purification and dispersion the entities brought into solution may not be macromolecules but aggregates, e.g., fragments of the microfibrils. Cuprammonium hydroxide $[Cu(NH_3)_4](OH)_2$ (Schweizer's reagent) is the only important solvent which is able to dissolve cellulose, although even then oxygen must be carefully avoided, as oxidative cleavage of the chains occurs easily. According to Staudinger, only one milligram of oxygen is needed in order to split 7.5 grams of cellulose with an average degree of polymerization of 3000 down to 1500[10]. When this was overlooked, falsely low values for cellulose were often measured. Molecular weights of many millions have been measured by some workers, but there is some doubt whether the entities present in these cases have been single and not aggregated macromolecules. The molecular weights of regenerated cellulose have been measured by various methods, such as osmotic pressure, sedimentation in the ultracentrifuge, and viscosity. The molecular weight range commonly found for these macromolecules is about 50,000–200,000. Some figures for native cellulose, obtained by means of sedimentation measurement in the ultracentrifuge, are presented in Table 61.

TABLE 61

MOLECULAR WEIGHTS OF CELLULOSE IN CUPRAMMONIUM HYDROXIDE[11]

Cellulose	*Average degree of polymerization*	*Molecular weight*
Unbleached cotton linters	9,200	1,500,000
Raw Georgia cotton	6,200	1,000,000
Nettle fiber cellulose	10,800	1,760,000
Ramie	11,300	1,840,000
Sulfite wood pulp	2,900	460,000

The results obtained by the viscosity method give lower results, for example 492,000 for native ramie[7]. The very long and thin macromolecules of cellulose seem to be linked to a certain degree in solutions.

Schulz and Marx[12] in a recent extensive study isolated cellulose from various sources under mild conditions, and determined the

molecular weights by viscosity and sedimentation methods. The largest values for the degree of polymerization (P) were found for flax cellulose — 8000, whereas cotton and ramie cellulose specimens had P values of 6200–7800. The degree of polymerization of wood cellulose was found to be much lower, namely 1150–3300. It could be shown that the more mild the method of isolation the higher the molecular weights. Relatively low molecular weights were obtained also for the cellulose isolated from sprouts and root ends, as well as from microbial material.

High quality commercial cellulose is prepared by boiling cotton linters with dilute sodium hydroxide. The material may be bleached by hypochlorite, and is then washed with acetic acid and water. This cellulose is used for the preparation of high grade paper, rayon, cellulose acetate and similar products. Ordinary commercial cellulose is manufactured from wood. Lignin and other ingredients (resins, tannic acid, gums) are removed either by boiling with an acid solution of calcium hydrosulfite, or with an alkaline mixture of sodium sulfide and sodium hydroxide. The liberated cellulose fibers, after being washed, still contain about 1–5 % lignin and 4–8 % pentosans and similar polysaccharides. Most of this pulp is used for making paper.

4. The Derivatives of Cellulose

Cellulose is a highly polar and hydrophilic compound, i.e., it adsorbs water, the amount adsorbed being determined by the texture of the specimen, temperature, and other factors. Pure cellulose under ordinary conditions usually contains about 6–10 % water. Cellulose, however, is insoluble in water and most aqueous solutions. This is due, as indicated previously (see chiefly Chap. 12), to strong intermolecular forces. These intermolecular forces are able to show their strength for the simple geometrical reason that the macromolecules are linear and oriented.

Cellulose swells in alkaline solutions, and this swelling is called mercerization (after John Mercer, who discovered and studied this process about one hundred years ago). Complete dissolution occurs in the alkaline cuprammonium hydroxide, in complex copper-ethylenediamine solution[13], and in some quaternary ammonium hydroxides. Complex compounds in which both copper and the nitrogen compounds participate are thereby formed in the solutions. It is very interesting that the optical activity of the complex

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with copper and ammonia is very high, with specific rotation values as great as −1200° being obtained with blue light[14].

Cellulose can be regenerated from the solutions by precipitating with acid or salt solutions. The regenerated material is amorphous but it can be oriented on stretching or compression. Artificial cellulose fibers are spun from the cuprammonium solutions (cuprammonium rayon). The tensile strength of such fibers, however, is less than that of natural cotton or linen.

Cellulose is partially soluble in concentrated solutions of sodium hydroxide and other strong alkalies. The soluble components are called β- and γ-celluloses, the fraction insoluble in 17.5% sodium hydroxide is α-cellulose. The former usually contain various impurities and low molecular degradation products, while the latter resemble the native cellulose. It is assumed that some of the alcoholic hydroxyls are somewhat more acidic than others, and that alkoxides or alcoholates (R—O—Na) are formed. Of great technological importance is the reaction of the alkaline cellulose with carbon disulfide. Soluble cellulose xanthates thereby are formed, according to the reaction:

$$\mathrm{R{-}O{-}Na + CS_2 \longrightarrow R{-}O{-}\overset{\overset{\mathrm{S}}{\|}}{\mathrm{C}}{-}S{-}Na.}$$

Viscose rayon is spun from solutions of these cellulose xanthates. Cellulose pulp is treated first with sodium hydroxide, then with carbon disulfide, and the yellow xanthate is "ripened" for a certain time. It appears that only one xanthate group is introduced into each two or three glucose residues of the macromolecules. The xanthate is dissolved in an alkaline solution, and this solution is pressed through fine orifices into an acidified bath. The xanthate groups thereby are decomposed, and the cellulose is regenerated, precipitating as a fibrous product. The decomposition of the xanthate occurs as follows:

$$\mathrm{R{-}O{-}\overset{\overset{\mathrm{S}}{\|}}{\mathrm{C}}{-}S{-}Na + H_2SO_4 \longrightarrow R{-}O{-}H + CS_2 + NaHSO_4.}$$

Huge quantities of rayon are spun according to these reactions. Cellophane is another material made of cellulose xanthate by regenerating cellulose from it. Cellulose is amorphous in the transparent foils of cellophane, the macromolecules being woven into

a random meshwork. Moreover, the macromolecules in cellophane are shorter than in native cellulose fibers.

The manufacture of viscose rayon and cellophane is controlled by measuring the viscosity of the solutions. Oxidative decomposition of the macromolecules is unavoidable, in fact, it is even desirable for the sake of material which is more easily handled. Extensive degradation, however, would result in mechanically unsatisfactory products.

Staudinger proved that the degree of polymerization does not change in these polymer-analogous reactions of cellulose if oxidative degradation is avoided. In Table 62 are compiled data for the intrinsic viscosity and degree of polymerization of cellulose xanthates and cellulose regenerated from them. The xanthates were dissolved in 2 *N* sodium hydroxide, the regenerated cellulose specimens in cuprammonium hydroxide. The xanthates contained 18.0–21.5% sulfur and 10.0–13.1% sodium.

TABLE 62

THE INTRINSIC VISCOSITY OF CELLULOSE XANTHATES AND CELLULOSE REGENERATED FROM THEM (according to Staudinger[15])

$\lvert\eta\rvert$ *of xanthate* ($\lvert\eta\rvert_x$)	$\lvert\eta\rvert$ *of regenerated cellulose* ($\lvert\eta\rvert_c$)	$\frac{\lvert\eta\rvert_x}{\lvert\eta\rvert_c}$	*Average degree of polymeriz. of xanthate*	*Aver. degree of polymeriz. of cellulose*
0.970	1.71	0 57	330	340
1.19	2.12	0.56	400	425
2.01	3.35	0.60	610	670
2.54	4.30	0.59	820	860
2 75	5.41	0.51	1080	1020
3.38	6.24	0.54	1170	1250
3.43	6.64	0.52	1270	1330

It is obvious that the intrinsic viscosity is very high, and that it differs considerably, depending on the specimen. The viscosity depends not only on the degree of polymerization (chain length), but also on the solvent and on the degree of xanthogenation, i.e., the number of xanthate residues introduced. In the 2 *N* sodium hydroxide the macromolecular chains of the xanthate seem to be coiled to a higher degree than the molecules in cuprammonium hydroxide. Moreover, the complex formation in the latter contributes to an increase in viscosity, since the macromolecules thereby become highly solvated. Hence, the quantitative relationships be-

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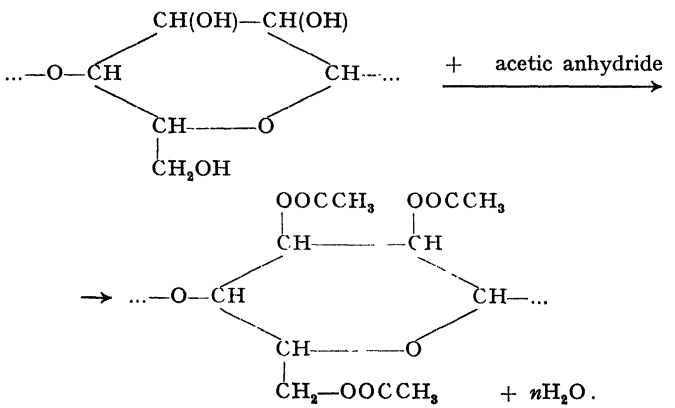
tween the degree of polymerization and viscosity are different for the xanthate and cellulose in the different solvents. Despite these complications the ratio of the intrinsic viscosities (third column in Table 62) is remarkably constant. According to Staudinger, this is the best evidence that the degree of polymerization has not changed upon regeneration of the cellulose from the xanthate. Hence, it is very unlikely that the colloidal particles in the solutions of these substances might be aggregates of smaller molecules (micelles) as some authors believed.

Cellulose acetate (acetyl cellulose) is another commercially important derivative of cellulose. It is manufactured as follows. Cotton linters or high quality wood pulp is pretreated with acetic acid in order to produce swelling, and the swollen cellulose is acetylated with a mixture of acetic acid, acetic anhydride and sulfuric acid. Two to 2.6 acetyl groups are introduced into each glucose residue. Temperature must be controlled in order to avoid excessive degradation. The acetate is dissolved in acetone, and acetate rayon is spun by forcing the solution through spinnerets into warm air. For molding various plastics the acetate is compounded with plasticizers (tricresyl phosphate, dibutyl phthalate).

Cellulose esters with butyric acid instead of acetic acid are prepared in a similar way, and mixtures of cellulose acetate and butyrate are very suitable for molding.

Pure cellulose triacetate is prepared by acetylating pure cellulose with acetic anhydride in the presence of pyridine. In the complete acetylation reaction all three hydroxyls of the glucopyranose residues react with the acetylating agent, as follows:



It is obvious from the formula that the chains become more bulky on acetylation. If butyl residues are introduced instead of acetyl, the side groups become even more voluminous. A cellulose acetate plastic is amorphous, with partial orientation in the fibers. Butyl cellulose is more difficult to orientate than acetyl cellulose due to the bulkiness of the side groups.

Cellulose acetate is soluble in glacial acetic acid, *m*-cresol, and other solvents. The viscosity of the solutions is very high, an indication of the presence of a linear colloid. According to Staudinger, the degree of polymerization does not change when the triacetates are saponified in the absence of oxygen, and the molecular weight of the regenerated cellulose is determined. The data are shown in Table 63[16]. The specimens with low viscosity originally have been more degraded than those of high viscosity, the degradation occurring during the purification and acetylation of the cellulose.

TABLE 63

THE INTRINSIC VISCOSITY OF CELLULOSE ACETATES AND CELLULOSE REGENERATED FROM THEM (Staudinger[16])

Intrinsic viscosity of acetate in m-*cresol*	*Average* P	*Intrinsic viscosity of cellulose in cuprammon. hydrox.*	*Average* P	*Viscosity ratio*
1.80	285	1.53	305	1.17
2.14	340	1.76	350	1.22
3.18	505	2.50	500	1.27
5.04	800	3.91	780	1.29
5.43	870	4.24	850	1.28
6.04	960	4.79	955	1.26
7.44	1180	5.83	1165	1.27
10.45	1660	8.40	1680	1.24

In this instance the intrinsic viscosities of the cellulose regenerated from the acetates is lower than the viscosity of the acetates. The viscosity ratio (last column), however, is remarkably constant, and it indicates that the chain length has not changed in the regeneration. The extremely high viscosity of the acetate solutions in cresol seems to be due to a high degree of stretching of the macromolecules. According to Svedberg and Gralen[11], the macromolecules of cellulose in cuprammonium hydroxide are kinked. The acetyl cellulose chains in cresol may be less kinked, possibly due to the bulkiness of the side groups and very high degree of solvation.

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This is illustrated in Fig. 102. Observations on similar derivatives of cellulose (nitrocellulose) support this conclusion. Hence, the chains of cellulose derivatives in good solvents are very extended; the macromolecules of cellulose in cuprammonium hydroxide are moderately kinked, and those of the xanthates in concentrated sodium hydroxide probably are more coiled than the former.

Cellulose nitrate is another important ester of cellulose[17]. It is prepared industrially by treating cotton or wood pulp with a mixture of nitric and sulfuric acids. The material obtained is highly explosive, and it is used as an ingredient in various explosives. For films and plastics nitrocellulose is compounded with camphor. The plastic, the so-called celluloid, is not explosive but is highly inflammable.

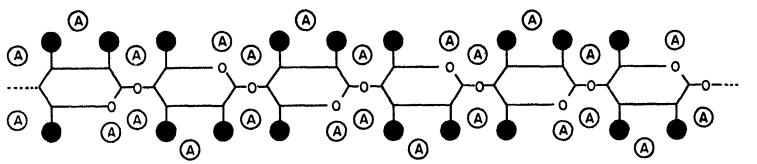

Fig. 102. The solvated macromolecular chain of acetyl cellulose.
A – solvent molecules bound to the chain.

It is interesting that the macromolecules of cellulose are very little degraded during this treatment with concentrated acids. The most suitable method for nitration without excessive degradation is by the use of nitric and phosphoric acid mixtures. The nitrocellulose obtained has about the same chain length as the original cellulose. This can be explained by the fact that the nitration occurs rapidly, and that the nitrates formed are stable toward hydrolytic cleavage by the acids. Staudinger has shown that the intrinsic viscosity of cellulose nitrate in acetone is even greater than the intrinsic viscosity of cellulose in cuprammonium hydroxide. The macromolecules of nitrocellulose are little kinked in acetone solution (see also p. 155). There is strong evidence that the colloidal particles present in these acetone solutions of nitrocellulose are single, not aggregated macromolecules. Hence, the very high viscosity of these solutions cannot be explained by linear aggregation or excessive solvation. The most likely cause is a high degree of stretching of the long, thread-like macromolecules. Viscosity data are presented in Table 64 (*loc. cit.* ref. 16).

TABLE 64

VISCOSITY OF CELLULOSE (IN CUPRAMMONIUM HYDROXIDE) AND NITROCELLULOSE (IN ACETONE) PREPARED FROM THESE CELLULOSE SPECIMENS
(according to Staudinger[16])

$\lvert\eta\rvert$ *of cellulose*	*Average* P	$\lvert\eta\rvert$ *of nitrocellulose*	*Average* P
0.76	152	1.50	135
1.05	210	2.00	180
1.68	336	3.40	310
2 25	450	4.80	440
3.58	716	7.40	670
7 70	1540	15.0	1350
10.0	2000	20.0	1800
16.8	3400	39.0	3540

Nitrocellulose specimens with even longer molecular chains have been prepared, for example, with a degree of polymerization of 6000 or even 8600 (number average mol. weight 2,400,000)[18].

Gralen[19] has studied a series of nitrocellulose specimens by means of sedimentation and diffusion, and weight average molecular weights between 230,000 and 780,000 were found for various specimens. The frictional ratios and dimensions of the molecules also were calculated. An ellipsoidal model was assumed, and the calculated length and diameter of the highly asymmetric units are presented in Table 65. The greatest molecular weight and also the highest asymmetry were found for the nitrocellulose prepared from

TABLE 65

MOLECULAR WEIGHTS, FRICTIONAL RATIOS, AND MOLECULAR DIMENSIONS OF NITROCELLULOSES (IN ACETONE SOLUTIONS) according to Gralen[19]

Source of cellulose	*Mol. wt.*	*Frictional ratio* f/f_0	*Length in A*	*Diameter in A*
American cotton linters, unbleached	780,000	12.2	9800	11.3
American cotton linters, bleached	400,000	10.6	6600	9.8
Sulfite cellulose	430,000	9.6	5900	10.8
Cellulose from spruce	340,000	6.3	3200	13.1
Rayon pulp	240,000	6.0	2700	12.0

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unbleached American cotton linters. The molecular weight and asymmetry are reduced upon bleaching the starting material. The cellulose of rayon pulp appeared to be even more degraded, and this material gave nitrocellulose of even lower molecular weight.

The axial ratios, according to these data, are between 220 and 870, i.e., the molecules are very long and thin. The molecular shapes, however, cannot be considered definite or permanent, as the string-like macromolecules possess some flexibility, and the unit is in Brownian motion. Not only the whole macromolecule is displaced in solution but the configuration of the chain also changes. Contrary to the very flexible vinyl polymers, the cellulose derivatives with their bulky and highly polar groups are more rigid (see Fig. 102); in solutions they are probably shaped in loops and spirals instead of tight coils.

Of the many recent studies concerning the dependence of the intrinsic viscosity on the molecular weight of nitrocellulose, the excellent work of Doty and coworkers will be mentioned[20]. The polymer was carefully fractionated by precipitating the fractions from acetone solutions with pure hexane. Weight average molecular weights were determined by means of light scattering. The viscosity was measured with solutions of various concentrations, and also at various rates of shear. Osmotic pressure measurements showed that the weight average molecular weight obtained by light scattering measurements was about twice as great as the number average weight calculated from osmotic pressure data. The following relation was found to be valid between the intrinsic viscosity (corrected also for shear gradient) and weight average degree of polymerization P_w:

$$|\eta| = 5.0 \cdot 10^{-3} P_w .$$

This relationship holds for a wide range of P (between P 250 and 9000). This work confirms fully the classical equation of Staudinger, in which 10^{-4} is used in place of 10^{-3} and the coefficient 5.0 of Doty's equation is replaced by the K_m constant, which for nitrocellulose is close to unity. The replacement of the coefficient 5.0 by 10 is justified by the fact that the weight average degree of polymerization P_w is twice as large as the number average degree of polymerization P_n (i.e., $P_w = 2P_n$), and that the P_n values obtained by osmotic measurement were used by Staudinger and others for the estimation of the K_m constant.

Cellulose ethers can be prepared by treating cellulose, swollen

in alkali, with an alkyl halide or sulfate. For example, the commercially important ethyl cellulose is manufactured by the reaction of ethyl chloride with alkali cellulose. The polymer ether is used for lacquers, coatings, as a paper size, etc. The water soluble hydroxyethyl cellulose is made by the action of ethylene oxide on alkali cellulose. Carboxymethyl cellulose is another interesting derivative of this type[21]. It is prepared by treating alkali cellulose with chloroacetic acid:

$$R\cdot ONa + Cl\cdot CH_2COOH \longrightarrow R\cdot O\cdot CH_2COOH + NaCl.$$

(R=cellulose residue)

The resulting product is an acid. The sodium salt of it is used widely as an ingredient of washing powders for laundering. This water soluble cellulose derivative has high adsorptive capacities. It is adsorbed on cotton and other cellulose fibers strongly, and prevents the redeposition of dirt. This is due chiefly to the negative charge of the carboxyl. The particles of dirt are usually negatively charged, hence, they are repelled from the negative surface[22].

The degree of substitution of the hydroxyl hydrogens in these etherification reactions is generally very low. Usually only one hydroxyl group of two or three glucose residues becomes etherified in the technological procedures of making these derivatives. Hence, all the ether derivatives must be considered as polyhydroxy compounds.

The diethylaminoethyl (DEAE) cellulose is another important derivative of cellulose. This polymer, contrary to the carboxymethyl cellulose, is positively charged, and it is used now widely as an anion exchanger, especially for the fractionation of proteins[23a].

5. The Hemicelluloses

The term 'hemicellulose' includes a variety of polysaccharides which accompany cellulose in plants. The hemicelluloses are soluble in sodium hydroxide, and are generally of lower molecular weight than native cellulose. Contrary to cellulose, which is a chemically definite macromolecular substance, the hemicelluloses are mixtures of many components. The amount and nature of these hemicelluloses vary depending on the source, e.g., wood being richer in these substances than flax stalk. The mixture of fibrous polysaccharides which is obtained after removal of lignin is called holocellulose. Hemicellulose is removed from the holocellulose by treating it with

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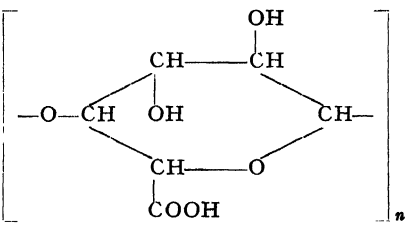
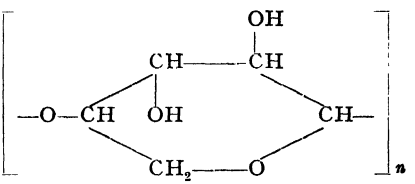
4% sodium hydroxide. This alkaline extract can be fractionated further, for instance, by neutralizing it and thereby precipitating a fraction. Another fraction can be obtained by the addition of alcohol to the supernatant. None of the fractions, however, are definite chemical compounds, but they are mixtures. Polyglucuronic acid, xylan, and mannan are the chief ingredients, all of them being mixtures of polymer-homologs of various molecular weights. The structures of polyglucuronic acid and xylan are shown below.



Polyglucuronic acid



Xylan

Mannan is a stereoisomer of cellulose, mannopyranose residues being linked in chains, similar to the macromolecules of cellulose.

In addition to these polysaccharides, many other acidic and neutral macromolecular compounds have been found in plants, e.g. araban, yielding arabinose on hydrolysis, and the galactans. Moreover, the hemicelluloses contain some lignin. In technological processing of wood pulp the hemicelluloses are not removed, since they are beneficial for the quality of paper. The cellulose fibers felt more easily when the hemicellulose is present than when it is absent.

Xylan is levorotatory, the specific rotation values of alkaline solutions varying between –80 and –109° (sodium light). The specific rotation of the oligosaccharides xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose increases in that order. Xylobiose has a value of –26°, the triose –46°, the tetraose –60°, the pentaose

–66°; hence, the levorotation increases with increasing chain length. However, the increase becomes smaller when the molecule has grown large.

The molecular weights of the various hemicelluloses have been found to vary between 10,000 and 300,000, the lower values of 10,000–30,000 being the most common ones. Husemann has shown that these polysaccharides are macromolecular substances, not micellar colloids, since the degree of polymerization did not change on conversion to chemical derivatives[24]. The molecular weights were determined by osmotic measurements. Data are presented in Table 66.

TABLE 66

POLYMER-HOMOLOGOUS CONVERSIONS OF HEMICELLULOSES
(after Husemann[24])

Substance	*Average* P	*Derivative*	*Average* P
Xylan from straw	119	Methyl-xylan	113
Xylan from beech wood	150	Benzoyl-acetyl xylan	147
Mannan from spruce wood	160	Benzoyl-acetyl mannan	147
Arabo-galactan from larch I	220	Arabo-galactan acetate	210
Arabo-galactan from larch II	267	Arabo-galactan acetate	287

The molecular weights of the derivatives mentioned in Table 66, of course, differ from the molecular weights of the original hemicelluloses. This is due to the fact that the mers of the derivatives are heavier than the mers of the hemicelluloses.

More data on the hemicelluloses can be found in special literature[25]. The chief technological use for the pentosans is conversion into furfural. Hemicelluloses improve the quality of paper pulp. The higher molecular derivatives of xylan and mannan can be used as sizes, and they form reasonably tough films comparable with cellophane and similar cellulose products.

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CHAPTER 22

Starch and other Branched Chain Polysaccharides

The polysaccharides mentioned in the preceding chapter are linear colloids. Cellulose and its derivatives are composed of unbranched macromolecules. In this chapter the polysaccharides possessing branched macromolecules will be discussed. Starch is the most important representative of this group. Due to the characteristics of its molecular shape and structure, starch is not found in structural tissues but among reserve substances in seeds, etc. The classification into strictly linear and branched structures, however, has its limitations. It appears that starch is not a homogeneous substance but, like many other natural polymers, it is composed of several components, some of which possess linear macromolecules.

1. Starch[1]

As an important reserve substance, starch is stored by the plant chiefly in the seeds, where its content is usually between 40–70%. Tubers, fruits, roots and stem pith contain a smaller amount, usually about 5–30%. In contrast to cellulose, starch is digestible, and hence it is one of the most important ingredients of human and animal food. A variety of refined food products is manufactured by a highly developed food product industry, with corn, wheat, potato, and rice being the most important starting materials. Moreover, large quantities of starch are used in laundering, adhesives, etc. The annual production of corn starch in U.S.A. is about 4,000,000,000 pounds.

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Starch occurs in plants in the form of discrete particles or granules which have a size of 5–50 microns (0.005–0.05 mm). The granules swell in water, and dissolve at a specific temperature, whereby a colloidal solution or a gel is formed. The size and also the shape and other microscopic features are characteristic for starches of various sources. The granules are optically anisotropic, which indicates a high degree of orientation of the macromolecules in the grains. More definite data on the crystalline structure are provided by X-ray spectral analysis. The cereal starches render a somewhat different type of spectrum (A type) from that of the tuber starches (B type).

Swelling and dissolution are facilitated by increase in temperature, as well as by alkalies, acids, and some salts, such as iodides and thiocyanates, but not sulfates (Hofmeister series!). Prolonged heating with strong acids, however, will cause degradation. The same occurs in the digestion of starch in the presence of enzymes. Semicolloidal dextrins appear as intermediates in this degradation, maltose and glucose being the final products.

Cereal starch is manufactured as follows: Shelled grain (e.g. corn) is cleaned by processing through a grain mill, and softened at 50° C with a dilute solution of sulfurous acid. (The acid facilitates swelling, prevents growth of undesired microbes, and acts as a bleaching agent). The softened grain then is milled, and the slurry liberated from coarse fragments of the grains by filtration. The remaining filtrate contains the partly swollen starch grains suspended in a solution of proteins and the micromolecular ingredients of the grain. The starch is separated from this dispersion by centrifugation. The manufacture of potato starch is simpler. The cleaned tubers are ground in the presence of sulfurous acid, the slurry is screened, and starch is separated from the dispersion by allowing the grains to settle, or by centrifuging.

Starch is composed of two types of macromolecules: *amylose* and *amylopectin*[2]. The former is a linear polysaccharide, the latter is a branched chain polymer, chemically similar to amylose. Both are mixtures of polymer-homologs, differing in the average degree of polymerization, and also in the presence of small amounts of extraneous material. Some trace materials, for example, phosphoric acid, may be chemically bound to the carbohydrate. Small amounts of lipids also are found in starch. The branched amylopectin is the chief ingredient of most starches, the usual proportion of the two components being 4 : 1 or 5 : 1.

Data on grain size, gelation temperature, and amylose content of starch from various sources are presented in Table 67.

TABLE 67

STARCHES FROM VARIOUS SOURCES
(after Whistler and Smart, *loc. cit.* ref. 1)

Source	*Size of the granules (in microns)*	*Gelation temperature, °C*	*Amylose content in %*
Corn	4–26	64–71	26
White potato	15–100	62–68	23
Sweet potato	15–55	82–83	20
Tapioca	5–36	69–70	18
Wheat	2–38	53–64	25
Rice	3–9	65–73	
Arrowroot	7–75	75–78	21

Separation of amylose and amylopectin can be achieved in various ways, for example by precipitating the amylose with butanol[3]. Finely ground starch is first extracted with methanol in order to remove lipids. A dilute dispersion of the starch grains then is heated under pressure, whereby a colloidal solution of the starch is obtained. Coarse granular fragments are removed by centrifugation, *n*-butanol is added to the hot solution in an amount somewhat more than is required for the saturation of an equal volume of water at room temperature, the mixture is stirred, and allowed to cool slowly. Amylose precipitates slowly as a microcrystalline compound. The amylopectin remains in the colloidal solution.

The chemical structure of amylose and amylopectin is disclosed by studying the degradation products of the acetylated, methylated and otherwise modified carbohydrates. Both polysaccharides appear to be composed of α-D-glucopyranose residues linked through carbons 1 and 4, as in cellulose. Hydrolysis of completely methylated amylopectin yields about 4% tetramethyl glucose together with the trimethyl glucose. Only about 0.3% tetramethyl glucose is obtained from amylose treated in the same way. Since only the end groups can give tetramethyl glucose, amylopectin contains more end groups than amylose. The simplest explanation would be that, in the case of amylopectin and amylose of similar molecular weight, the former possesses branched molecules, whereas the latter

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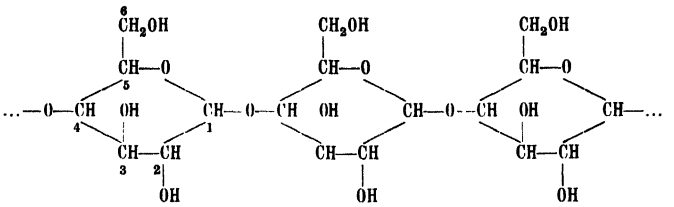
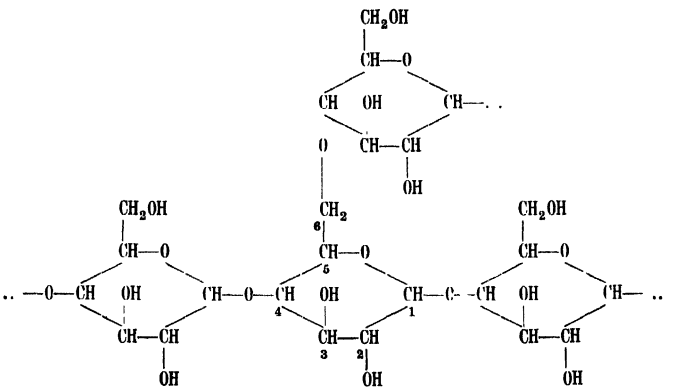
is a straight chain linear colloid. The constitution of amylose and amylopectin is shown in the following formulas and Fig. 103.



Amylose chain



A section of the branched amylopectin chain.

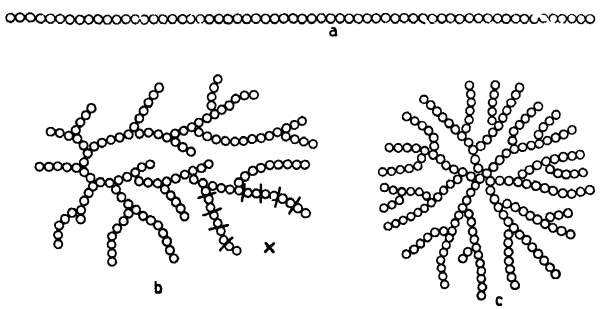


Fig. 103. Molecular shapes of starch molecules.
(a) Expanded amylose chain; (b) amylopectin; (c) glycogen.

Both amylose and amylopectin are polymolecular colloids, and the molecular weights found for various preparations depend on the method of isolation and fractionation, as well as on the method by which the molecular weights are determined (see Chap. 8). The low molecular weights formerly found for unfractionated starch were calculated, for example, on the basis of chemical end group analyses. As the chains are branched, the end groups are present in a relatively large number, and the molecular weight cannot be determined in this way without knowing the exact amount of branching. The true molecular weight for native, undegraded amylopectin is very high. Values between 1,000,000, and 6,000,000 have been found for various amylopectins by osmotic pressure measurements[4]. Molecular weights between 7 and 73 million were found for potato amylopectin by means of light scattering[5]. In spite of these large molecular weights, no fibers can be drawn of amylopectin, and films of it are brittle. Streaming birefringence is not produced by solutions of amylopectin. All this indicates that the macromolecules of this polysaccharide are not linear but branched. The large molecule of amylopectin can be called a *microgel*, as the highly hydrated unit carries a large amount of liquid with it. Amylopectin is the ingredient of starch which is responsible for the adhesive properties and formation of pastes. Amylopectin forms red-violet complexes with iodine.

The macromolecules of the amyloses are linear, as the solutions show streaming birefringence, they are highly viscous, and films and fibers can be obtained. The molecular weight of amylose is lower than that of amylopectin. Values of 70,000–300,000 have been reported for various preparations of amyloses, chiefly on the basis of osmotic pressure measurements[6]. In contrast to amylopectin, the linear macromolecules of the amylose are easily oriented into crystallites. Amylose forms a blue complex with iodine. Amylose acetate films and fibers are similar to cellulose acetate films and fibers. The degree of crystallinity of these materials increases with the degree of deformation (stretching). Amylose is hydrolyzed completely to maltose by the enzyme β-amylase, whereas amylopectin is incompletely degraded by this enzyme to limit-dextrins.

Starch grains contain 15–20% water, part of which is bound hydrate water. Starch gels and pastes contain about 90–95% water. The coherent network of the gels is formed by interlinking some parts of the macromolecules through hydrogen bonds. Intermolecular swelling of the macromolecules of amylopectin must be

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assumed, and large quantities of water are immobilized inside and in between the branched structures (intra- and intermolecular immobilization).

In contrast to the action of β-amylase, α-amylase is able to hydrolyze amylopectin to glucose, the low molecular dextrins and maltose being intermediate products. The so called diastase of malt, which contains α-amylase, hydrolyzes about 90% of the starch to fermentable sugars. This is a very important procedure in the fermentation industries, e.g. in brewing. Large amounts of starch are converted into glucose by acid hydrolysis, according to the equation:

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6.$$

The acid is neutralized by sodium carbonate or calcium hydroxide, and the salts formed are removed by ion exchange resins. The solution of glucose is concentrated by evaporation. The resulting syrup is used for manufacturing candy and other food products; glucose can be crystallized from the syrup by seeding with glucose crystals. The course of enzymatic or acid degradation is checked by the reaction with iodine, by viscosity measurements, and by chemical glucose determinations. The course of enzymatic degradation is different with various enzymes, and it has been studied in detail. The enzymes of *Bacillus macerans* convert starch into the so called Schardinger dextrins, which seem to be cyclic degradation products. These dextrins are crystalline and of relatively low molecular weight[7]. The hypothesis on their cyclic structure is supported by the fact that no tetramethyl glucose could be found among the degradation products of these methylated dextrins. Only trimethyl glucose was found, as if cyclic chains were present.

Hanes, Freudenberg, Rundle[8], and other authors have tried to explain the formation of such cyclic dextrins by assuming a helical structure for the chains in the macromolecules of starch. According to this model, about six glucopyranose residues are present per turn of the helix. This would explain, first, the formation of cyclic dextrins composed of six glucose residues. Another set of facts which support this model is furnished by the study of the starch-iodine complex. The helix has room for the iodine molecules inside it. According to Rundle and Baldwin[8], starch-iodine complex exhibits dichroism of flow; light with its electric vector parallel to the flow lines is more strongly absorbed than light with its electric vector normal to the stream lines. This requires that the iodine

molecules must be parallel to the long axis of the starch-iodine complex, i.e. the iodine molecules form linear arrays (Fig. 104). The dichroism is especially pronounced in solutions of crystalline amylose which gives the blue complex with iodine, and no definite dichroism could be observed with amylopectin-iodine complex. The fact was also pointed out that only degradation products composed of at least six glucose residues give a color with iodine. The structural specificity, however, is not important for color formation, since iodine gives blue complex compounds also with polyvinyl alcohol, and other substances. The arraying of the iodine molecules within helices or along linear macromolecules seems to be essential.

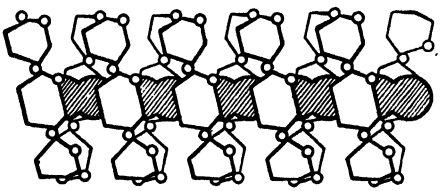

Fig. 104. Schematic sketch of the iodine-filled amylose helix, according to Rundle, Foster, and Baldwin.

Another important colloid-chemical property of starch is *'retrogradation'* (meaning—denaturation, gradual loss of valuable properties). Aqueous starch solutions become opalescent and turbid in time, and gradual precipitation occurs. Recent light-scattering studies on retrogradation of amylose have been published by Foster and Sterman[9]. The viscosity decreases at the same time, and the resistance to enzymes increases. In terms of modern colloid chemistry, this is a process of ageing. This is a slow, spontaneous aggregation, due to Brownian movement. Occasional aggregation occurs upon collision, and the aggregates gradually grow bigger. The aggregates can be more or less oriented (crystalline) depending on the components: amylose aggregates more easily, and forms better oriented particles than amylopectin. Retrogradation of amylose depends also on the molecular weight (chain length) of the macromolecules. There is a maximum of retrogradation at a medium chain length[10]. This is due to the fact that extremely long macromolecules may be randomly kinked, and therefore will have difficulty in bundle formation; very short chains, on the contrary, will be in too intense Brownian motion, and there will be less chance of displaying intermolecular attraction than in the case of

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chains of medium length. Starch gels also exhibit retrogradation, which in this instance may be called syneresis (see Chap. 15). Although the particles in the gel are more or less fixed, some chains possess a limited mobility, and they tend to aggregate, as macromolecules do in solutions. The meshwork of the gel in time becomes coarser, and part of the immobilized water is pressed out of it. It is obvious that the adhesive capacities of aged (retrograded) starch, dextrin, and similar pastes will deteriorate with ageing, as the compact aggregates have less active groups (smaller surface) than completely dissolved macromolecules in a fresh paste. If, however, a fresh paste is applied to a surface (e.g. wall paper and wall), only a limited retrogradation can occur in time, since the macromolecules are fixed to solid surfaces. As chemically similar substances, starch and cellulose exhibit mutual intermolecular attraction, due to hydrogen bonding. For this reason, starch is also used as an additive to cellulose in the manufacturing of paper.

Among the trace ingredients of starch, phosphoric acid and fatty acids are important. Phosphoric acid is found in the amylopectin of potato starch, and in starch of the pith of sago palm, whereas most cereal starches are free of phosphorus. The amount of phosphorus found is about 0.08%, and it is chemically bound as phosphoric acid ester to the primary alcoholic hydroxyls (position 6 in the glucopyranose ring). The fatty acids are present to about 0.5%, palmitic, oleic and linoleic acids being detected. The presence of phosphoric acid renders a negative charge to the macromolecules, as only one function of the tri-basic ortho-phosphoric acid is bound to the starch. Consequently, the amylopectin of potato starch migrates in electrophoresis experiments. Adsorbed fatty acids also may be partly responsible for the charge. The charge of the amylopectin, however, seems to be unimportant for the adhesion of starch to cellulose, as amylose adheres to cellulose even better than amylopectin.

Since the glucopyranose residues in the macromolecules of starch contain three free hydroxyl groups, the starches react with acids, whereby esters are formed. The polymer-analogous conversions with some relatively low molecular fractions of starch have been mentioned in Chap. 11. It is interesting to note that similar conversions have been performed with the very large molecules of amylopectin[5]. Amylopectin of molecular weight nearly 14 million was acetylated, and the molecular weight of the acetate was determined in various solvents by means of light scattering. The ester then was saponified

by means of 0.2 *N* sodium hydroxide, and the molecular weight of the recovered amylopectin was determined in aqueous solution. Only a slightly lower value, namely 9.4 million was found for the recovered amylopectin. This indicates that in these polymer-analogous reactions only an insignificant cleavage of the giant molecules has occurred. It is also significant that the molecular weight of the same acetate in various solvents was found to be practically the same. The results are presented in Table 68. The molecular weights appear to be remarkably constant, and they are independent of the solvent. This is the best kind of evidence that no significant association has occurred, and that the entities in the solutions of the acetates are single large molecules.

TABLE 68

MOLECULAR WEIGHT OF AMYLOPECTIN ACETATE FROM LIGHT SCATTERING MEASUREMENTS IN ORGANIC SOLVENTS (according to Witnauer, Senti and Stern[5])

Solvent	M *of amylopectin acetate*	M *of the corresponding amylopectin*
Acetone	$18.0 \cdot 10^6$	$10.8 \cdot 10^6$
Chloroform	15.9	9.5
Dioxane	15.5	9.5
Nitromethane	15.4	9.5
Acetonitrile	15.6	9.7
Acetone II	17.4	10.4

Solutions of starch are dextrorotatory, an indication that α-glucosidic linkages prevail. Specific rotation values of about 200° have been measured. According to Staudinger and Husemann[11], the specific rotation of the larger molecules is higher than of the smaller ones. It was also confirmed that the starch recovered from its triacetate possessed the same specific rotation as that of the original starch[11].

Concluding this brief account on starch, the important studies of Haworth, Hirst, Jones, and their associates on the chemical constitution of starch should be mentioned[12]. Extensive studies on the degradation products, especially of completely methylated starch, supported the branched chain formula as presented on p. 376. The end groups produce 2, 3, 4, 6-tetramethyl glucose, the straight chain residues yield 2,3,6-trimethyl glucose (which is the major

product), and the residues of amylopectin at which branching occurs, form 2, 3-dimethyl glucose. The amount of the 2, 3-dimethyl glucose was found to be equivalent to that of the tetramethyl glucose, hence there is one end group per one branching point. According to the studies of end group determination by means of periodate oxidation, amylose macromolecules have one end group per 250 or more glucopyranose residues, whereas amylopectin has one end group for each 25–27 units[13].

Interesting is the fact that starch can be dissolved without degradation in liquid ammonia or formamide at low temperatures. Corn starch can be made water soluble by means of a pretreatment with liquid ammonia[14]. If such pretreated starch is stirred with water, a very adhesive paste results. This procedure is advantageous practically, since autoclaving thus can be omitted. Moreover, the disorganizing effect of liquid ammonia is beneficial for the study of the chemistry of the macromolecules: all active groups become exposed and are available for reagents. Trimethyl and triethyl ethers of amylose and amylopectin of high intrinsic viscosity were prepared by the aid of the liquid ammonia method[14]. A smooth acetylation of starch could be achieved in formamide at −15° C[15].

Synthetic amylose has been prepared some time ago by means of enzymatic synthesis[16]. Glucose-1-phosphate has been used as the starting material. Although the synthetic amyloses possessed smaller molecular weights than the natural products, the achievement is noteworthy.

2. Glycogen and Similar Polysaccharides

Glycogen solutions have been mentioned before in several chapters (e.g. Chap. 9). According to Staudinger, glycogen is a typical spherocolloid[17]. The viscosity of its solutions is low, the reduced osmotic pressure is constant over a wide concentration range, and single macromolecules as round dots were observed even in the electron microscope. Chemically glycogen is very similar to starch. Both are made up of D-glucose residues, both are highly dextrorotatory and both possess highly branched macromolecules. Completely methylated glycogen yields more tetramethyl glucose on hydrolysis than completely methylated starch. This indicates that glycogen is more branched than starch. The low viscosity of glycogen solutions also indicates that the molecules are more compact and spherical than those of starch. In fact, there is no sharp border-

line between the starches and glycogens, as polysaccharides have been found which could be classified in either group. For example, the polysaccharide of sweet corn, because of the source, could be expected to be a starch. However, it is easily soluble in water, it has more end groups than amylopectin has, and it gives a red-brown color with iodine instead of blue or violet[18]. Some authors prefer to call all similar polysaccharides which are isolated from animal tissues glycogens, and all those isolated from plants starches.

Liver is one of the richest sources of glycogen. The polysaccharide is extracted from the ground tissue by either hot water or alkali. The proteins are removed by precipitation with trichloroacetic acid. Coarse impurities are centrifuged off, the solution is dialyzed, and the glycogen can be precipitated either by alcohol or concentrated acetic acid. Pure glycogen is a white powder, easily soluble in water without noticeable swelling, and the solutions do not set. Glycogen is the macromolecular reserve carbohydrate. The potential chemical energy is stored in it, and the energy is released whenever necessary in the complicated reactions in muscular tissues and elsewhere. Because of these functions it must be easily transportable, and the compact, spherical shape of the macromolecules satisfies this condition. However, as already mentioned, macromolecules of similar constitution have also been found in plants. Yeasts and some bacteria also contain polysaccharides which could be called glycogens.

It has been established that the chemical structure of glycogen is essentially the same as that of amylopectin. The D-glucopyranose residues are linked in the 1,4 positions in chains which have many branching points, most probably through the carbon-6 of the rings. The whole macromolecule can be imagined as an irregular lattice (Fig. 103), as the substance appears amorphous on X-ray examination. The "holes" in this lattice are much smaller than those in starch, hence the interior of the macromolecule must be less accessible for the molecules of various reagents than the macromolecules of starch. A complete methylation or acetylation of glycogen, indeed, is more difficult to achieve than with starch. The analysis of degradation products indicates that about 10–20 glucose residues are in the unit chains[19]. The enzyme β-amylase digests only about half of the macromolecule. The core of the macromolecule seems to be even more compact and more branched, as tetramethyl glucose could be isolated from the degradation products

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of methylated material in 18% yield[20]. According to Meyer, this would indicate chain units of only 3–7 glucose residues. This was found in the case of glycogen from mollusks, and other glycogens may have a somewhat different structure. This is ascertained also by the variety of colors produced on addition of iodine to the glycogens from various sources. Light yellow-brown, red, and violet colors have been observed. Although the color depends on the concentration of the components and the temperature[21], the origin of the specimen is most important. These observations support the idea that the unit chains (unbranched straight segments) may have different lengths in different glycogens. It is known (see sect. 1) that complex compounds of blue color are formed only with long straight chains. When one branch is present for approximately each 20 glucopyranose units, the color with iodine is red or red-brown, and it becomes lighter when the chains are even more ramified.

All preparations of glycogen have been found to be polymolecular. Staudinger and Husemann[22] prepared glycogen fractions which had molecular weights between 66,000 and 900,000. Polymer-analogous conversions into acetates were also performed, and the molecular weights of the acetates were determined. The acetates finally were saponified, the glycogens were recovered, and the molecular weight and other properties were studied. It was found that the original macromolecules can indeed be recovered. Husemann also showed that the molecular weight of glycogen can be determined by means of precipitation titration[23]. Ordinarily this method is considered only as a semiquantitative method (see Chap. 7). However, in the case of fractionated glycogens the method gave results which agreed well with the osmotically determined molecular weights.

TABLE 69

MOLECULAR WEIGHTS OF GLYCOGEN SPECIMENS
(according to Bell, Gutfreund, Cecil and Ogston[24])

Specimen source	*Solvent*	*Mol. wt. from sedimentation and diffusion*	*Mol. wt. from osmotic pressure*
Rabbit liver glycogen	water		500,000
ditto	NaCl, 1 *M*	4,400,000	2,000,000
Rabbit liver glycogen V	NaCl, 1 *M*	4,300,000	
Ascaris lumbricoides glycogen	water	700,000	100,000
ditto			500,000

The molecular weights, determined by means of sedimentation in the ultracentrifuge and diffusion measurements, appear to be higher than the osmotically determined values. This is due to the fact that the osmotic method gives the number average molecular weight, which is smaller than the ultracentrifuge-diffusion-method average for polymolecular colloids. In Table 69 are presented some data obtained by these methods[24].

3. Dextrans

The dextrans occupy an exceptional place among the many types of polysaccharides, because of their importance as blood plasma substitutes (volume expanders). Dextrans are produced in the United States and other countries by utilizing the ability of certain microorganisms, especially strains of *Leuconostoc mesenteroides*, to convert sucrose to dextrans. The very high molecular dextrans then are partially hydrolyzed, in order to obtain fractions suitable for medical applications. Hydrolysis is accomplished by heating with dilute hydrochloric acid, and fractionation is performed by precipitating with ethanol.

Dextrans, as the name indicates, are dextrorotatory, and the specific rotation is nearly the same as that of starch and glycogen. The low molecular products of hydrolysis rotate the plane of polarized light somewhat less than the larger molecules. Complete hydrolysis yields D-glucose. These data indicate that the dextrans are polycondensation products of α-D-glucose. Chemical studies have shown that the anhydroglucose residues are linked through α-1,6-glucosidic linkages[25]. The chains are branched, and on the average, one branch unit occurs for every 20 glucose residues. In spite of this branching, the substance should be classified as a linear colloid, since its solutions are viscous, and they exhibit double refraction of flow. X-ray analysis indicates that the macromolecules are oriented to a high degree. Filaments have appeared also in electron micrographs[26]. A section of the macromolecule of dextran is illustrated on p. 386.

The molecular weights of various dextran specimens vary widely, depending on the fractionation, i.e. whether the first or last precipitated fractions are examined. As even the most carefully separated fractions are polymolecular, the average values of molecular weights depend also on the method applied. The variance then permits conclusions about the degree of heterogeneity, i.e. size distribution.

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Some examples of such determinations of molecular weight by various methods are presented in Tables 70 and 71.

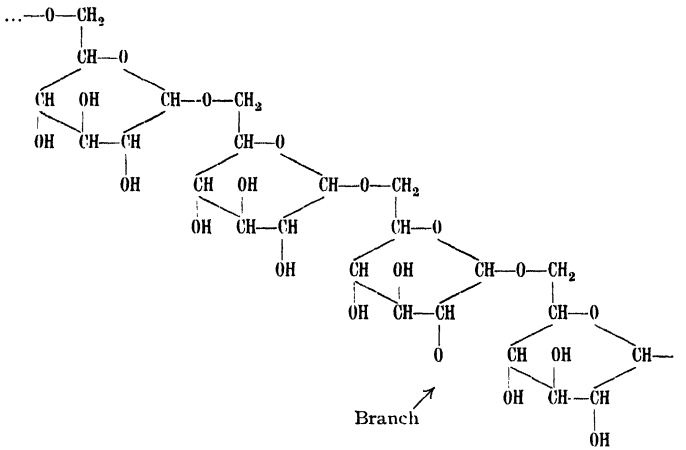

TABLE 70

MOLECULAR WEIGHTS OF DEXTRAN FRACTIONS DETERMINED BY VARIOUS METHODS (after Wales *et al.*[27])

Designation of the fraction	*Number average mol. wt.* M_n	*Weight average mol. wt.* M_w	*Z-average* M_z
C–5	41,000	47,000	57,000
C–15	38,000	50,000	57,000
NRRL–3136–54–1	64,000	76,000	106,000
N–158(0–45),Subf. 3	95,000	170,000	230,000
NRRL–3136–71–1	240,000	540,000	700,000

The differences in molecular weights obtained for the same samples with different methods are obvious. The variation of the Huggins viscosity-concentration slope constant k (see Chap. 9) for the various fractions (Table 71) is remarkable. This variation is difficult to explain, especially the fact that the constant attains a minimum value of about 0.40 when the dextran has the molecular weight of several hundred thousand. According to Senti and his colleagues[28], the dependence of the intrinsic viscosity on weight

TABLE 71

MOLECULAR WEIGHT, INTRINSIC VISCOSITY, AND HUGGINS CONSTANTS FOR VARIOUS DEXTRAN FRACTIONS (after Senti *et al.*[28])

Fraction	M_n	M_w	*Intrinsic viscosity*	*Huggins constant*, k
1	14,500	17,700	0.129	0.54
4	25,400	37,700	0.197	0.57
7	57,600	65,600	0.252	0.48
9	71,300	92,600	0.284	0.50
12		185,000	0.378	0.41
15		422,000	0.458	0.63
18		975,000	0.633	0.84
22		6,940,000	0.850	1.16

average molecular weight (determined by light scattering) can be expressed by the equation:

$$[\eta] = 9.78 \cdot 10^{-4} M_w^{0.50}.$$

This equation is valid for dextran fractions of molecular weights below 100,000, such as those used for blood plasma substitutes.

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CHAPTER 23

Pectic Substances and Mucilages of Plants

The variety of polysaccharides found in plants is very great, although cellulose, the starches, pentosans, and other substances already mentioned in previous chapters dominate in quantity. Most of these macromolecular compounds are built of anhydro-D-glucose residues linked in straight or branched chains. However, many other monosaccharide units besides glucose are found in other polysaccharides. Anhydroxylose, anhydrogalactose and anhydromannose units have been found. Inulin is a polysaccharide built of anhydrofructose. This semicolloidal carbohydrate is found in the roots of many plants, e.g. in chicory. Detailed information on these substances can be found in recent reviews[1].

All of the major polysaccharides mentioned so far have been unsubstituted polycondensation products of certain simple sugars, the hydroxyls (and the aldehydic end groups) being the functional groups of the macromolecules. In this chapter, certain other polysaccharides of practical importance will be mentioned which contain other functional groups beside the hydroxyls. Such substances are found in various plant gums and mucilages. Esterified or free carboxyl groups are the most common ones found in these macromolecules. The pectins are the most important representatives of these substances[2].

1. Pectins

Pectins are polymeric acids the solutions of which set easily (pectos in Greek means rigid, frozen). They are found in various plant

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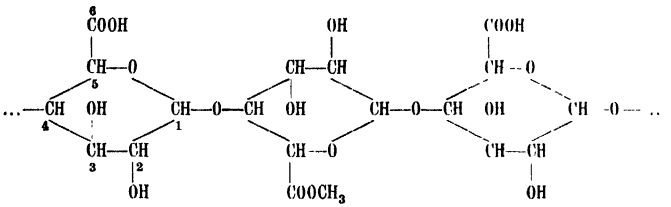
tissues in both soluble and insoluble forms. Fruits, stalks, and other parts of plants contain pectins in different amounts depending on the species, age of the plant and other factors. The macromolecules are fibrous, hence the solutions are highly viscous, and this property determines the functional significance of these substances in the life of the plant. Pectins are useful in the food product industry, as stiff jellies can be obtained with as little as 0.2% pectin. Citrus waste (peelings), apple pomace, and sugar beet pulp are the chief sources of pectins. The pectins are extracted from this material by either dilute acids or water. The former cause partial degradation of the acid polysaccharide, whereas water extracts only part of the pectin. The methods employing a mild acid treatment seem to be the most useful, the pH being kept in the limits of 2.0–2.5, or even 3.5. Alkaline extraction is used when the non-jellying sodium salts of pectins are desired; they are employed as thickening agents, and for coatings. Acidic extracts of pectin can be purified by precipitation with ethanol, and washing the precipitate of pectin with dilute alcohol. Ash can be removed by dialysis or electrodialysis, or by ion exchange resins.

The pectin substances isolated in this way appear to be composed of several chemically different carbohydrates. The chief ingredient is partly esterified polygalacturonic acid, galactan and araban being the other components. It is possible that the latter two are chemically bound (at least partly) to the chief components at the carboxyl groups. The relative amounts of the components vary depending on the source[3]. The components themselves also differ in some degree. Most important is the polygalacturonic acid which determines the ability to form jellies. The macromolecules of this acid may have different sizes, and the carboxyl groups may be esterified to a greater or lesser extent. Drastic methods of isolation with hot acids cause a certain degradation of the chains, and the products obtained by such methods are more heterogeneous than those isolated by mild treatment.

D-Galacturonic acid is the major compound obtained from the products of total hydrolysis of pectins. Methyl alcohol is another product of cleavage. Galactose and arabinose can be isolated in smaller amounts, occasionally also rhamnose and acetic acid. Methyl alcohol comes from the methyl ester, acetic acid from acetyl groups. Sugar beet pectin contains 4% of acetyl groups, but other pectins contain only traces of it[4]. The basic chemical data on the components of pectin was furnished by Ehrlich and associates

(1926–29), although the concept of Ehrlich that pectin is a micellar colloid proved to be wrong. Henglein and Schneider determined the molecular weights of pectin nitrate, and found high values of 41,000 and 82,000 for two preparations. Polymer-analogous conversions into acetates yielded derivatives which showed molecular weights of 40,200 and 79,100 respectively[5]. These findings proved that pectins are not micellar but molecular colloids. The high viscosity[6], double refraction of flow[7], and the formation of films and oriented fibers[8] indicate that the macromolecules are linear. The constitution of the chain is generally accepted to be as follows:



As the materials are highly dextrorotatory (+210 to +290° with sodium light), the residues of galacturonic acid are joined by α-1,4-glucosidic linkages. About 20–60% of the carboxyl groups present in the macromolecular acid are esterified, depending on the source of the pectin. The fibrous material appears to be paracrystalline, the degree of crystallinity depending on the stress applied to the solid. Fiber periods of about 13–14 A have been measured by means of X-ray analysis. This is somewhat less than the period of the fiber axis of cellulose, indicating a puckered chain in pectin[9].

The molecular weight of pectins has been determined by osmotic pressure measurements, sedimentation in the ultracentrifuge[10], and by the viscosimetric method[11]. Depending on the specimen used, as well as on the method of measurement, values between 20,000 and 360,000 have been obtained. Hence, with regard to molecular size and shape, the pectins resemble starch amylose. The molecules in solution, like other linear polysaccharides, are less kinked than the very flexible macromolecules of vinyl derivatives. As the pectins are polyelectrolytes, the degree of kinking must depend on the dissociation of ionizable groups (see Chap. 10). The stretching of the chains must depend also on the presence of micromolecular electrolytes, since they influence the electrokinetic potential and

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charge. Moreover, the stretching should depend on the degree of esterification. Maximum stretching can be expected of fully ionized, unesterified macromolecules in the absence of other electrolytes, since negatively charged segments then will strongly repel each other. Experimental observations support these expectations. For example, the intrinsic viscosity decreases as the degree of esterification increases[12]. The double refraction of flow also increases with increasing viscosity. Axial ratios of the stretched macromolecules of 53 : 1 up to 165 : 1 have been calculated (Owens *et al.*, *loc. cit.* ref. 11)

Pectin jellies can be formed by cooling hot fruit juices. The formation of strong jellies is facilitated by maintaining the acidity at a specific level of pH 2.5–3.5, which often is the acidity of the natural juices and saps. Moreover, a high concentration of sugar facilitates gelation. However, sugar is not the most important ingredient, as pectin gels can be prepared without sugar. This can be accomplished by adding calcium or similar ions to the pectin solutions. An important condition for the formation of such calcium gels is a low degree of esterification of the polygalacturonic acid.

According to the views outlined in Chap. 13, gelation is an unsuccessful attempt of precipitation. The tendency to precipitate or crystallize may be caused by a number of reasons, though two factors are of paramount importance: the presence of agents competing for the solvent, and the temperature. Sugar is such an agent competing with the pectin for the solvent (water), as both are strongly hydrophilic substances. The solubility of pectin decreases with a reduction in the temperature, hence the solutions tend to set on cooling. A gel is formed instead of a precipitate because of the electrical charge on the macromolecules, especially in the case of sugar-containing jellies. The linear macromolecules cannot aggregate into compact particles, but they become partially linked by the secondary bonds of the various polar groups present in the macromolecules. In the pectin-calcium gels parts of the coherent meshwork of the gel may be linked also through the ionic R—COO—Ca—OOC—R bonds between several chains. In this instance the degree of esterification is important, and gels are not formed when too many of the carboxyls are esterified, since such groups cannot react with the calcium ions. Another important factor which determines the ability of certain pectins to set is the molecular weight of the pectin. The longer the macromolecular chains, the less of the substance is needed in order to produce firm jellies. Another way to approach this question is to measure the rigidity of jellies

prepared from various pectin specimens (Chap. 15). Special testing devices have been used for this purpose[13].

Data illustrating the high viscosity of the pectins are presented in Table 72. The number average molecular weights were calculated from osmotic pressure movements, the weight-average M_w from viscosity data.

TABLE 72

INTRINSIC VISCOSITY AND OTHER DATA OF PECTIN SPECIMENS

(according to Owens *et al*, *loc. cit*, ref. 11)

Pectinic acid	*Intrinsic viscosity at 25° C*	M_n	M_w
10.7.No 1	4.2	31,000	67,000
10.7.No 2	8.7	39,000	115,000
10.7.No 3	1.2	18,000	27,000
10.9	5.7	27,000	86,000
6. 2B	3.3	23,000	56,000

The intrinsic viscosity was obtained by plotting the reduced viscosity values η_{sp}/c against concentration, the values of c being very low (in the range 0.05–0.3%). A 0.15 M sodium chloride solution was used as the solvent in order to reduce the charge effects. Most of the measurements presented in Table 72 were made in nearly neutral solutions (pH 6). The intrinsic viscosity decreases in strongly acidic solutions from pH 3.5 to pH 0.5. This is due to suppression of dissociation of the polyelectrolyte by the added hydrogen ions, as discharge leads to increased kinking of the linear macromolecules (see Chap. 10). According to Deuel[2], a solution of pectin possessed an intrinsic viscosity of 1.8 at pH 3.5, but only 0.95 at pH 2.0–2.5. Further diminution of the pH did not change the intrinsic viscosity appreciably.

2. Pectic Araban and Galactan

The partially esterified polygalacturonic acids, as mentioned in the previous section, are accompanied in plants by other polysaccharides. The commercial pectins usually contain certain amounts of araban and galactan. The aqueous or acid extracts of plants containing pectic substances may contain different amounts of

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araban and galactan. The latter can be more or less completely removed from the polygalacturonic acid by extraction with 70% ethanol. Galactan can be separated from araban by hydrolysing the mixture with 0.05 *N* sulfuric acid, which decomposes only the araban. One of the best sources for the preparation of araban is peanut meal[14]. After liberation of oil, the meal is extracted with 10% sodium chloride in order to remove the proteins. The pectins then are dissolved in very dilute alkali, precipitated, and purified by extraction with 70% alcohol. The alcoholic extracts containing araban are evaporated and the araban is eluted with pyridine. According to Hirst and Jones[14], araban is a semicolloidal polysaccharide. Only L-arabinose is obtained on complete hydrolysis. Hirst and Jones conclude that araban is a branched polymer composed of arabofuranose residues. The specific rotation (Na light) of araban is $-160°$.

Pectic galactan appears with araban in various amounts, e.g. in apple pectin the amounts of araban and galactan are about the same. Very little galactan was found in citrus pectins, and none in flax pectin[15]. The best source for the preparation of this colloidal polysaccharide is *Lupinus albus*[16]. Ground lupine seeds are treated in the same way as the peanut meal, i.e. fats and proteins are extracted first, and the pectic substances are eluted with dilute alkali. Further separation of the pectic substances is accomplished by means of fractional precipitation with methanol, and by removal of the polygalacturonic acid by precipitating it with calcium chloride. Separation from araban can be accomplished either by acid treatment, in which the araban is hydrolysed but galactan remains unchanged, or by taking advantage of the different solubility of the methylated products. Pectic galactan is composed of D-galactose residues, the pyranoid rings being linked in β-1,4 positions. About a hundred mers are linked in straight chains. The polysaccharide is slightly levorotatory. The presence of araban and galactan does not improve the gelation of pectin solutions.

More complex arabans and galactans have been isolated from wood. For example in larch (*Larix occidentalis*) up to 18% of such arabogalactans have been found. The polysaccharide is isolated by extracting wood flour with water, and (after removal of various impurities) precipitating with alcohol. On total hydrolysis, D-galactose and L-arabinose are found in the proportion of 6:1[17]. The polysaccharides probably are very heterogeneous. Two components have been revealed in sedimentation experiments in the ultracentrifuge,

the molecular weight of one being estimated as 16,000 and of the other 100,000[18]. The polysaccharide is suitable for various industrial applications, e.g. as adhesive, thickener for drilling muds, paper pulp size, etc.

3. Agar and other Seaweed Polysaccharides[19]

Agar is one of the acid polysaccharides found in seaweeds (algae). It is soluble in hot water, and the solution sets on cooling. The ability of agar to form gels is very pronounced, as 0.5% or even 0.2% solutions may set, depending on the agar specimen used. Solutions containing only 0.1% of the polysaccharide are highly viscous. The various uses of agar are based on these properties. Most important are the applications in the food product industry, for example in the preparation of canned meat, fish, ice cream and firm-jellied candies. Agar is superior to gelatin, since mixtures with the same amount of agar give firmer gels than with gelatin. Moreover, agar is resistant to most putrefying bacteria and acts therefore as a protective agent against spoilage of the food. The digestibility coefficient of agar is low, hence it may be incorporated in diets composed in order to reduce weight. In Japan, however, certain sorts of agar are important food products.

The manufacturing process for agar is simple: seaweeds are extracted with boiling water (or hot dilute acid or alkali). The hot solution is filtered, decolorized, eventually neutralized, and the agar is further purified by a freezing-thawing process. Most of the impurities are removed with the water leaving the gel on thawing. Further purification can be achieved by soaking agar in cool distilled water, and by electrodialysis. The presence of calcium ions appears to be very important for the gelation, as agar completely liberated from calcium does not set[20]. This indicates that gelation in the case of agar is similar to that of pectin in the presence of calcium salts.

According to Percival and Somerville[21], 2,4,6-trimethyl-D-galactose is obtained in the hydrolysis of methylated agar. Hence, the chains of this polysaccharide are built of D-galactopyranose residues which are linked at the carbons number 1 and 3. This was confirmed by later work of Percival and other investigators, although monosaccharides other than galactose have also been found among the products of hydrolysis. Also interesting is the fact that all specimens of the best purified agars contain between 0.3 and

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3.7% sulfur. This sulfur is responsible for the acidic nature of the polysaccharide. A 1% solution of highly purified agar has a pH of 2.0, the acidity being due to the half-ester of sulfuric acid bound to the macromolecules. No sulfate can be detected in solutions of pure unhydrolyzed agar, but the reaction for SO_4^{--} is positive after hydrolytic cleavage with hydrochloric acid. One —O—SO_3H group is present in about one of each 8–50 galactose units in the chains. It is possible that calcium ions participate in the gelation by interlinking the chains through bridges of the type

$$R\text{--}O\text{--}SO_3\text{--}Ca\text{--}O_3S\text{--}O\text{--}R.$$

Hydrogen bonding may also be important in the formation of the coherent network. Jellies containing 1–2% agar are very firm, and they are used as bacteriological culture media. The polysaccharide is not digested by most bacteria, the gels remain coherent at 37°, and they do not synerize, or synerize very little. The specific rotation (Na light) of the calcium salt of agar is -33°.

Agar is a linear polyelectrolyte, and the viscosity, flocculation and other colloid-chemical properties of the solutions have been thoroughly studied by Kruyt, Bungenberg de Jong and others[22]. The reduced viscosity η_{sp}/c of dilute solutions of agar varies with concentration in the same way as does that of polyacrylic acid and similar polyelectrolytes (Chap. 9). At very low concentrations of 0.1–0.5% the reduced viscosity decreases with increasing concentration, and then increases when the concentration of the colloid is increased (see Fig. 105). The abnormalities disappear when a small amount of salt is added to the solution. The viscosity is reduced on addition of salts, and the polyvalent cations have a much greater effect than monovalent cations (see Fig. 106). These measurements have been made with warm solutions (at 50° C) to avoid gelation. The effect exhibited by the salts is due to the reduction of the repulsive forces originating in the ionogenic groups R—O—SO_3^-. The highly stretched macro-ions coil up to a certain degree as the repelling force between the charged segments is reduced. The viscosity thereby decreases. The kinking is due to the intramolecular attraction between the segments. When the temperature is lowered sufficiently, intermolecular linking and aggregation also occur. However, due to the fact that the macromolecules are highly hydrated, the colloid does not flocculate but is converted into a gel.

Carrageenan is another polysaccharide found abundantly in marine plants. While agar is produced from seaweed of chiefly

tropical regions, carrageenan is produced from marine algae of the shallow waters of the northeastern coast of North America and the coasts of western Europe. The polysaccharide is extracted from the plants with hot water, and it can be purified by precipitation with propanol. Pure carrageenan is a white powder, fairly soluble in warm water, but firm gels can be obtained only with 5% of the polysaccharide. Chemical studies have shown that the colloid is a branched chain polysaccharide composed chiefly of D-galactose residues, part of the hydroxyls being esterified with sulfuric acid. The specific rotation (Na light) of carrageenan is +32 to +48°.

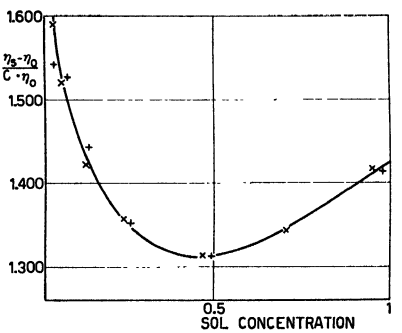

Fig 105.

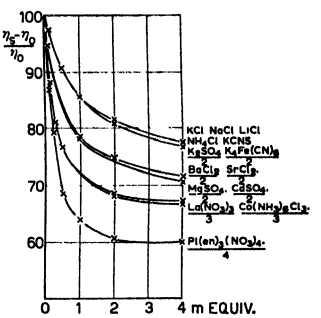

Fig. 106.

Fig. 105. The dependence of the reduced specific viscosity of agar on its concentration, according to Kruyt and Bungenberg de Jong.
Fig. 106. The viscosity of agar in the presence of various salts. On the abscissa is plotted the concentration of the salts in milliequivalents per liter, on the ordinate the specific viscosity. Concentration of the agar 0.14%. Temperature 50° C.

It has been shown that the viscosity of the solutions and the ability to set depends on the salts present[23]. Carrageenan, like agar, is used as a thickening agent, as a sizing agent for textiles and high grade paper, for stabilizing casein paints, as clarifying agent for liquors, and as an emulsifying agent. Its most important application, however, is as a stabilizer for the dispersion of chocolate in chocolate milk[24]. Carrageenan, like agar, is only partly digestible. The molecular weight of carrageenan is very high, and the macromolecules tend to associate in networks which are split into single chains in very dilute solutions[25].

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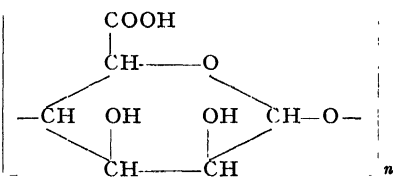
4. Alginic Acid

Alginic acid, as the name indicates, is another substance found in algae. Various species of the brown algae are rich in this polysaccharide, which has about the same functions in the algae as cellulose has in terrestrial plants. The acid occurs in the form of calcium, sodium, potassium and magnesium salts. The sodium salt, called algin, is the most important derivative of alginic acid. It is extracted from kelp, by means of sodium carbonate. The soluble sodium alginate can be purified in several ways, e.g. by precipitating with calcium chloride or hydrochloric acid. The latter precipitates a fibrous alginic acid. Chemical studies have shown that alginic acid is composed entirely of D-mannuronic acid residues joined by β-1,4-glycosidic linkages[26]. Each residue contains one free carboxyl group, and the macromolecule is not branched, hence the structure can be illustrated simply as follows:



The geometric pattern of alginic acid is almost the same as that of pectins and cellulose. Structural analysis by means of X-rays indicates that alginic acid is highly oriented, and the fibrous structure is similar to that of cellulose[27]. The molecular weight of alginic acid is lower than that of cellulose. Molecular weights of 50,000–75,000 have been found for the soluble salts by means of sedimentation and diffusion studies[28], and 48,000–186,000 by osmotic pressure measurements on other preparations[29]. The solutions are viscous, and the macromolecules seem to be highly extended. Alginate gels have been studied extensively by Thiele and associates[30].

The alginates are used for about the same purposes as carrageenan, e.g. as thickeners in creams, lotions and pastes. Fibers can be spun from alginic acid derivatives[31].

5. Gums and Mucilages

Plant gums and mucilages are polysaccharides resembling in properties and structure the previously mentioned colloids. Gums are

exudates on the bark or leaves. They are composed chiefly of salts of acidic polysaccharides, and they are soluble in water. Mucilages are found in various plant tissues; they are either neutral or acidic and are composed of galactose, mannose, and other units.

Gum arabic is one of the plant gums of most practical importance, and one of the oldest known adhesives and thickening agents. It is an exudate from several species of shrub-like leguminous trees of the genus *Acacia* which grow in various subtropical countries. The gum is soluble in water and can be purified by reprecipitation with alcohol. L-Arabinose, D-galactose, L-rhamnose, and D-glucuronic acid are the major hydrolysis products, and the macromolecular chains are branched[32]. The glucuronic acid residues confer acidic nature to the macromolecules, and the purified material is sometimes called arabic acid. The solutions are non-reducing, and the pH of moderately concentrated aqueous solutions is 2.2–2.7. The acid and its salts are levorotatory, specific rotations of $-20°$ to $-30°$ being measured with sodium light. The colloid-chemical properties of the solutions have been extensively studied by Kruyt, Bungenberg de Jong, and their associates[33]. The solutions are highly viscous, and the viscosity changes upon change of concentration or addition of electrolytes. Coacervation, flocculation, or gelation may occur on mixing the colloid with salts of high concentrations, alcohol, or gelatin. The effects produced depend on the pH of the solutions. Due to the presence of carboxyl groups in the macromolecules of arabic acid, the colloid is negatively charged, hence polyvalent cations influence the viscosity and stability much more than do salts with monovalent ions. The effects produced by the amphoteric gelatin and similar polyampholytes depend on the pH (see Chap. 14).

The many other gums of commercial importance, such as gum tragacanth, karaya, damson, cherry, and egg-plum gums are similar to gum arabic both in chemical constitution and colloid-chemical properties[1]. They are all mixtures of closely related polysaccharides of more or less acidic nature.

The term "mucilages" covers a variety of ill-defined polysaccharides, neutral or acidic, linear or branched macromolecules composed of various monosaccharide residues. The molecular varieties may be infinite, and they may differ from species to species, and also depend on the age of the plant, and other conditions. This is one example of the many fields open for further research. The information available at the present time on these substances is scanty, and reference is made to special literature[1].

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In Table 73, some data are compiled on the molecular weights of some plant gums and mucilages.

TABLE 73

MOLECULAR WEIGHTS AND FRICTIONAL RATIOS OF GUMS AND MUCILAGES (after Greenwood[34])

Polysaccharide	*Method*	*Mol. wt.*, M	*Frictional ratio* f/f_0
Gum arabic	Osmotic pressure	191,000–245,000	
Ditto	Sedimentation and diffusion	256,000–326,000	2.2
Gum tragacanth	Sedimentation and diffusion	840,000	
Karaya gum	Sedimentation and diffusion	9,500,000	2.4
Locust bean mucilage	Ditto	310,000	1.24

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CHAPTER 24

Animal Polysaccharides with Fibrous Macromolecules

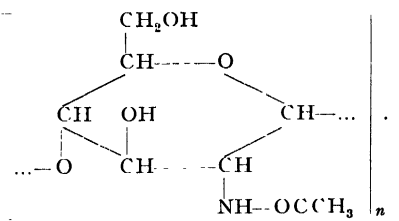
Although the chief source of polysaccharides is plant tissue, similar substances are found also in animal bodies. Thus far, only glycogen has been mentioned as an example of such an animal polysaccharide. It has also been pointed out that there is no strict borderline between starches and glycogens, since many types of amylopectins resemble the corpuscular glycogens to a great extent. The spherocolloid glycogen, however, is not the only polysaccharide found in animal tissues. Various linear polysaccharides play important roles as structural materials in the animal body. Chitin, chondroitin sulfuric acid, and hyaluronic acid are the most important representatives to be described briefly in this chapter.

1. Chitin[1]

Chitin is the organic skeletal substance of invertebrate animals. It is very widely distributed in crustaceans and insects. However, chitin is found also in lower plants, especially in the cell walls of fungi. The presence of chitin in lower plants is thought to be of significance in establishing phylogenetic relations between lower species.

The cuticle of insects, lenses of their eyes, wings, shells of crabs and similar materials contain about 20–80% chitin. Crab and lobster shells are the most common sources of this polymer. Cleaned shells are crushed, dried and ground. The powder then is treated with dilute hydrochloric acid, which removes calcium carbonate from the material. The residual chitin is washed with water and

sodium hydroxide in order to remove the remaining proteins. Bleaching and washing with alcohol and ether follow. The resulting chitin is a colorless solid, insoluble in water, alcohol, dilute acids, alkalies, and lipophilic solvents. It can be dispersed in hot concentrated solutions of certain salts. It can be dissolved also in concentrated hydrochloric acid, whereby gradual hydrolytic cleavage of the macromolecules occurs with a gradual change in specific rotation from $-14°$ to about $+56°$ (Na light)[2]. Complete hydrolytic degradation by means of hot, concentrated acid gives glucosamine and acetic acid in equivalent amounts and theoretical yields. This indicates that the macromolecules are built up of acetyl glucosamine residues. Extensive chemical studies have established that the repeating unit is N-acetyl-D-glucosamine linked by β-1,4 bonds[3]. Hence, the structure of this nitrogenous polysaccharide can be illustrated as follows:



The above formula was substantiated by X-ray structural analysis by Gonell, Meyer and Mark[4]. Fibrous chitin appeared to be highly crystalline, the X-ray diffraction patterns strongly resembling those of cellulose. The crystalline regions in the preparations appeared to be several hundred angstroms thick, and very long. According to Meyer, the macromolecular chains may be as long as cells. Since the material is highly insoluble, it is impossible to determine the molecular weight. The very high viscosity of fresh solutions in acids indicates that the molecules are of about the same size and shape as those of cellulose. According to X-ray structural analysis, the unit cell is rhombic and contains 8 acetylglucosamine residues. The length of the cell along the fiber axis is 10.46 A (Fig. 107). Such crystalline oriented chains appear in chitin specimens which are obviously fibrous (e.g., tendons of rock lobster), whereas other specimens may be much less oriented. For example, chitin from butterfly wings and fungal chitin yield powder dia-

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grams. However, the X-ray diagrams of crustacean and some specimens of fungal chitin are essentially the same[5].

The resemblance between chitin and cellulose is obvious, and the insolubility and stability toward enzymatic and acid hydrolysis are due to the smooth linear structure and strong intermolecular attraction. Whereas most of the previously mentioned polysaccharides have been inhomogeneous mixtures or macromolecules with several repeating units in the chains, chitin and cellulose are chemically homogeneous. The inhomogeneous pectins and galactomannans can be compared with the synthetic copolymers, the homogeneous chitin and cellulose are comparable to the uniform polyesters or to polyethylene. The geometrical uniformity and smoothness of the chains is the major prerequisite for orientation and crystallinity.

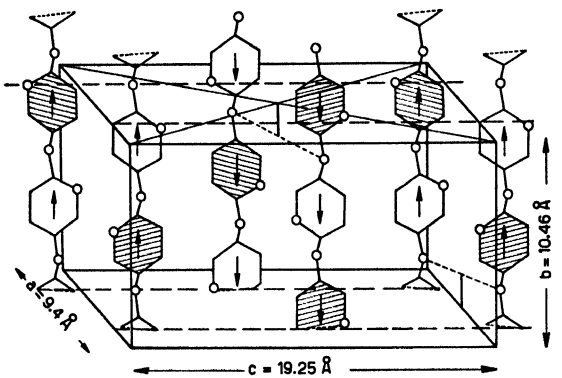

Fig. 107. Diagram of the unit cell of chitin, according to X-ray spectroscopy data of K. H. Meyer and G. W. Pankow. (From K. H. Meyer, *Natural and Synthetic High Polymers*, Interscience Publ., New York, 1950, p. 450.)

The second condition securing highly coherent and stable structures is intermolecular attraction. In chitin and cellulose there is ample opportunity for this, due to the high polarity of the groups constituting the chains. It is interesting that the heat of activation of acid hydrolysis of chitin and cellulose is about the same (approximately 30,000 cal. per mole of the glucosidic linkage)[6].

Noteworthy is the fact that chitin is very resistant to enzymatic hydrolysis, although some soil bacteria, as well as certain fungus-feeding beetles and snails, are able to hydrolyze chitin slowly. Intact chitin, however, can be found even in fossil remnants of

insects many million years old[7], a spectacular example of stability. The enzymatic hydrolysis of either animal or fungal chitin is essentially identical, and leads to the same products in each case[8]. The identity of animal and fungal chitin has been established also by other methods[9]. Fungal chitin, however, is tenaciously bound to a nitrogen-free polysaccharide, probably a hemicellulose, and it is difficult to obtain it in a pure state.

Chemical treatment of chitin with alkalies results in gradual deacetylation of the chitin. Partial degradation occurs at the same time, and the resulting chitosan is an ill defined, weakly basic mixture of polymerhomologs of polyanhydroglucosamine.

2. Chondroitin Sulfuric Acid

Chondroitin sulfuric acid is found in cartilage and other tissues of vertebrates chiefly as the calcium salt. Cartilage is the richest source, containing about 40% of this polysaccharide, collagen being the other major constituent. The separation and isolation of both these linear colloids is a difficult task, as they constitute a tenacious fibrous network of connective tissue in which intermolecular attraction is very strong. The intermolecular bonds between the polysaccharide and protein seem to be especially strong in skin and umbilical cord, since from these tissues chondroitin sulfuric acid can be extracted only with strong alkali, the latter probably causing some degradation of the macromolecules. From cartilage, however, chondroitin sulfuric acid can be extracted with neutral calcium chloride, hence no primary bonding to protein need be assumed. These calcium chloride extracts yield a rather impure polysaccharide containing some protein. The purest preparations of chondroitin sulfuric acid and its salts are obtained by the method of Einbinder and Schubert[10].

Wet cartilage is extracted repeatedly with a concentrated solution of potassium chloride. The extracts are purified by adsorbing the extraneous material on kaolin, and filtering off the solid. The filtrate is acidified with acetic acid buffered with potassium acetate, and treated repeatedly with kaolin. After filtering and evaporating the filtrate in a vacuum more potassium acetate is added, and the polysaccharide is precipitated by pouring the solution into a large volume of ethanol. The resulting product is potassium chondroitin sulfate, a white solid, soluble in water, and insoluble in alcohol and other lipophilic solvents. Further purification of the products by means of adsorption, and by adding moderate amounts of

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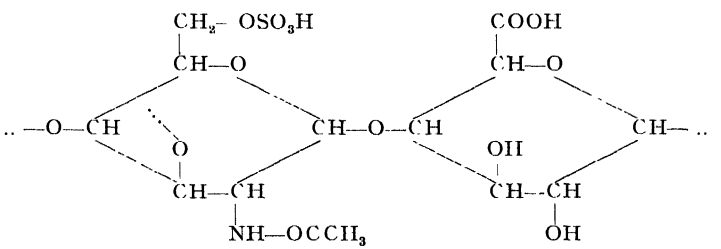
alcohol and calcium chloride yielded crystalline preparations of calcium chondroitin sulfate[11].

According to the analytical data, the macromolecules of chondroitin sulfuric acid are built of galactosamine, glucuronic acid, sulfuric acid and acetic acid. The amino group of the galactosamine residue is acetylated, and the OH at carbon-6 of the residue is esterified with sulfuric acid. The remaining acid function of the sulfuric acid and of the carboxyl groups confer acidic properties to the polymer. Its specific rotation (Na light) is about —30°.

According to Meyer and associates, the macromolecules of chondroitin sulfuric acid are linear, galactosamine and glucuronic acid residues being linked in long chains[12]. Although all the structural details have not yet been disclosed, the major structural element of the chains seems to be the following:



The molecular weight was evaluated by Meyer and associates[12] from viscosity and end group analysis, and values about 20,000–50,000 have been reported. Higher values of 260,000 were reported by Blix and Snellman[13] from streaming birefringence and viscosity data. Treatment with alkali causes degradation, and lower molecular weights of only 30,000 are then found.

Chondroitin sulfuric acid and its salts are linear polyelectrolytes possessing a high charge density on the macromolecules. The polymer appears homogeneous in electrophoresis experiments[14]. In animal tissues, the ionogenic sulfate and carboxyl groups may form salt bonds with the basic amino groups of proteins. Moreover, the polyelectrolyte acts in cartilage as an ion exchanger[15]. Experiments with veal costal cartilage showed that the tissue has equal binding capacities for sodium, calcium and barium. The binding capacity for these ions was correlated closely with the sulfate content of the tissue, indicating that chondroitin sulfate was responsible for the binding.

Mathews pointed out that variations in charge density, due to

variations in dissociation and binding of ions, may cause changes in configuration of the macromolecules of this polyelectrolyte[16]. As a typical linear polyelectrolyte, chondroitin sulfuric acid exhibits very high resistance to flow in distilled water, and the viscosity is strongly reduced on the addition of salts (Chap. 9 and 10). The reduced viscosity of pure chondroitin sulfuric acid in distilled water decreases with increasing concentration of the polymer, and becomes constant at a concentration of about 0.5 %[16]. In salt solutions the dependence of the reduced viscosity on concentration is normal, i.e., it increases linearly with concentration (Fig. 108). The intrinsic viscosity in salt solutions was found to be 0.51–1.10, depending on the preparation. Data on the potentiometric titration of chondroitin sulfuric acid[16] are in agreement with the theory of Katchalsky and Gillis[17] which considers the effect of ionization upon the degree of coiling. The macromolecules of the polyelectrolyte appear to be highly stretched in nearly neutral solutions. Removal of cations, i.e., their replacement with hydrogen ions, causes coiling. Configurational changes of these macromolecules, or their aggregates with linear proteins, in connective tissues and membranes may be significant for the permeability of membranes and similar physiological processes[18].

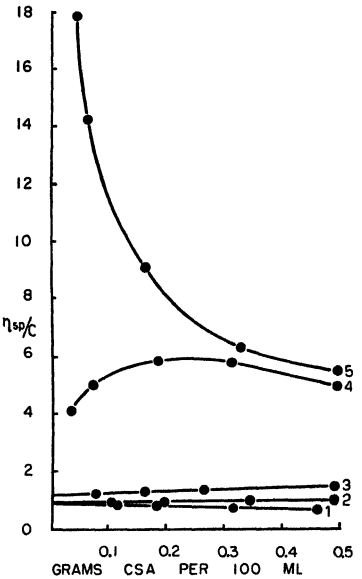


Fig. 108. Effect of electrolytes upon viscosity of chondroitin sulfuric acid, according to Mathews. On the abscissa is plotted the concentration of the chondroitin sulfuric acid, on the ordinate the reduced specific viscosity. Curve 1, 0.15 *M* sodium phosphate buffer pH 7.0 with 0.2 *M* NaCl; curve 2, 0.066 *M* sodium phosphate buffer pH 7.0, curve 3, 0.022 *M* sodium phosphate buffer pH 7.0, curve 4, 0.001 *M* sodium phosphate buffer pH 7.0; curve 5, distilled water.

3. Hyaluronic Acid

It was shown by Karl Meyer and his associates that several other acidic polysaccharides besides chondroitin sulfuric acid are present

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in animal tissues[19]. The most important one of these is hyaluronic acid. In the form of its salts, this polyelectrolyte is present in all connective tissues, where it serves as one of the components of the jelly-like matrix which holds the cells together. This colloidal matrix is the so-called "ground substance" which is important not only as a structural factor, but also as a medium in which the spreading (diffusion) takes place of the substances participating in the physiological processes. The spreading of foreign material, such as drugs, also depends largely on the submicroscopic structure of this ground substance. The spreading is greatly facilitated by introducing certain enzymes—hyaluronidases—which are able to cleave the fibrous macromolecules of the polyelectrolyte. Moreover, hyaluronates, the salts of hyaluronic acid, serve as lubricants and shock-absorbers in joints. Often appearing together with chondroitin sulfates in skin, cartilage, umbilical cord or vitreous humor, the hyaluronates differ from chondroitin sulfates in that the former do not contain any sulfate, and contain glucosamine in place of galactosamine. Hyaluronic acid is a linear polymer built of the residues of N-acetyl-D-glucosamine and D-glucuronic acid. The carboxyl groups of the latter confer a negative charge to the linear macromolecules.

Hyaluronic acid in tissues is firmly associated with proteins, and separation without degradation is a difficult task. Crude preparations can be obtained by extracting ground defatted tissue with trichloroacetic acid and subsequently precipitating with ethanol. Such preparations always contain protein. The latter can be removed more or less completely by shaking the solution with a mixture of chloroform-amyl alcohol. The polysaccharide-protein complex also can be liberated from the protein component by digesting the proteins with proteolytic enzymes, such as pepsin and trypsin[20]. Precipitation of the protein with ammonium sulfate, extraction of the hyaluronate with weakly alkaline solvents, and other methods have also been proposed for purification. Pure preparations of hyaluronic acid should not give a positive reaction for amino acids after hydrolysis. D-Glucosamine hydrochloride and D-glucuronic acid are the major products of hydrolysis of the polysaccharide with concentrated hydrochloric acid. If the macromolecules were composed of equivalent amounts of N-acetyl-D-glucosamine and D-glucuronic acid, the pure preparations should contain 3.5% nitrogen, 40.7% hexosamine, 44.8% glucuronic acid, and 10.9% acetyl. The values actually found are close to these figures, i.e.,

3.0–3.8% nitrogen, 36–50% glucuronic acid, 34–42% hexosamine, and 0.5–11.0% acetyl being found in different well-purified preparations. Sodium hyaluronate possesses a specific rotation of —70° (sodium light).

Solutions of the hyaluronates are very viscous, and they exhibit double refraction of flow[13]. The viscosity shows the usual anomalies of linear polyelectrolytes; the viscosity is extemely high in very dilute solutions, and the reduced specific viscosity decreases with concentration. These anomalies disappear on adding salts[21]. No reliable values for the molecular weights of undegraded material are available. Molecular weights of 200,000–400,000 were estimated by Blix and Snellman[13], but it is possible that the polysaccharide was somewhat degraded. According to these authors, the macromolecules are highly elongated and asymmetric. Ogston and Stanier[22] arrived at different conclusions. The material they used was certainly not degraded, as it was obtained from synovial fluid (of ox) simply by ultrafiltration and dialyzing the filtrate against a phosphate buffer of pH 7.3. The substance isolated in this way, however, was not a pure hyaluronate but a complex of the polysaccharide and protein. Physicochemical studies on this preparation indicated that the particles of the complex have a relative weight of approximately 8 million. The particles in solution seemed to behave as coiled fibers encompassing large quantities of liquid. As this complex contained about 30% protein, it is hard to deduce what the behavior of protein-free hyaluronate would be. According to Ogston and Stanier[22], the protein cannot be split off without partial degradation of the polysaccharide. It is possible that the double refraction of flow in this case is due to stretching of the coils under the influence of the flow gradient, although asymmetry of the original particles of the complex is not quite ruled out.

The hyaluronates are poly-anions, hence they will combine with the positively charged amino groups of adjacent protein molecules. Hydrogen bonding between the protein-hyaluronate and the polysaccharide molecules themselves probably also contributes to the formation of coherent gels in the tissues. When a hyaluronate is mixed with an acid protein solution, flocculation occurs, due to ionic interaction. The latter is weakened at a pH alkaline in relation to the isoelectric point of the protein, since then the protein molecules will carry an excess of negative charges (see Chap. 14).

It is interesting that enzymatic degradation of the macromolecular hyaluronates occurs in vivo. The particular enzymes able to

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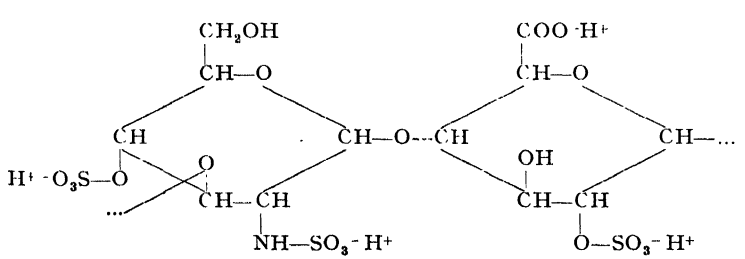
hydrolyze the hyaluronates are found, for example, in testicular extracts or spermatozoa, in leech heads, and in snake venoms. The biological significance of these facts is obvious. The enzymes break the gelatinous framework of the ground substance, and make the tissue more porous and easily accessible to the spreading of certain agents in the tissue. These important problems will be treated in more detail in Chapters 29 and 31.

4. Heparin

Heparin is an anticoagulant of blood and, in contrast to hyaluronic and chondroitin sulfuric acids, is found in various tissues in relatively small amounts. This polysaccharide is also bound to proteins. A crystallized acid barium heparinate has been isolated from beef liver and lung[23]. Minced tissue is extracted with a mixture of ammonium sulfate and alkali, and the crude heparin-protein complex is precipitated with acid at pH 2.5. The precipitate is suspended in alcohol, dissolved in dilute alkali, and the protein is removed by digestion with trypsin. The remaining heparin is precipitated with alcohol and acid. Purification is continued by redissolving and reprecipitating the polysaccharide with acid, acetone, and other reagents. Heparin liberated in this way from impurities can be crystallized by adding barium acetate and glacial acetic acid to the solutions[24]. Analysis of the purified material shows that the polysaccharide is composed of D-glucuronic acid and glucuronylglucosamine, the amino groups of the latter being esterified with sulfuric acid. The sulfur content is unusually high, and it must be assumed that more than one sulfate group is present in each residue, the others forming ester bonds with one or two of the remaining hydroxyls. Hence, this polysaccharide is unique because of the extremely high charge density on the macromolecules, having about two dissociable groups for each anhydrohexose, e.g.:



The molecular weight of heparin is lower than that of the previously mentioned polysaccharides of animal tissues, values of approximately 20,000 being mentioned[25]. The viscosity of the solutions is moderate in comparison with that of hyaluronic acid.

Heparin intervenes in the process of blood clotting by blocking some enzymes. Because of this ability to react as an anticoagulant, heparin has great therapeutic value as a prophylactic agent for the prevention of thrombosis.

5. Blood Group Polysaccharides[26]

Among the many other polysaccharides found in animal tissues, the blood group polysaccharides are of particular interest. Human blood can be separated into various types, the latter being determined by specific polysaccharides found in the red cells of the blood. The richest source of these substances, however, is not the red cells, but gastric juice or ovarian cysts. The A-group substances have been the most thoroughly investigated. They can be extracted, for example, from cyst fluid with phenol, and precipitated from the extract with ethanol. Further purification was accomplished by reprecipitation from ethylene glycol or formamide with ethanol. The polysaccharide appears to be composed of N-acetylhexosamine (46%), L-fucose (18%) and D-galactose (17%)[27]. In contrast to the other polysaccharides, the blood group substances also contain amino acids as integral constituents. However, the composition of the same specific group substance differs depending on the source. On mild acid hydrolysis the blood group substances lose their immunochemical activity.

The A-group specific polysaccharide was found to be homogeneous in electrophoresis, sedimentation, and solubility tests[27]. The molecular weight was found to be 260,000 to 278,000 according to sedimentation, diffusion and other measurements[28]. The macromolecules seem to be highly asymmetric. Higher molecular weights for the A substance were found by means of light scattering[29].

The B-group substances have also been isolated recently in a highly purified state, and their chemical and physical properties have been studied[30]. Morgan and his associates showed[30] that at least two B-group substances were present in the same source (ovarian cyst fluid). The acid hydrolysis products contained L-fucose, D-glucosamine and D-galactosamine, 18–20% fucose being found. Moreover, eleven amino acids could be identified in the

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products of hydrolysis. Although carbohydrate constitutes the major portion of the macromolecular compound, the amino acids amount to about 10% of the total. Physicochemical examination revealed that the B substances are homogeneous on electrophoresis at pH 8 and pH 4. The molecular weight calculated from sedimentation and diffusion data was found to be 460,000 for one of the B substances, and 1,800,000 for the other. Both preparations of B appeared somewhat polydisperse, and the macromolecules were found to be highly asymmetric in shape. The frictional ratio for one specimen was found to be 3.9, and for the other 5.7. These findings are of great significance, since they demonstrate that considerable physicochemical differences may exist between different substances belonging to the same group.

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CHAPTER 25

Poly-amino Acids and Semi-colloidal Proteins

The great and diverse domain of proteins and protein-like substances is the most challenging branch of organic colloid chemistry. In spite of the many achievements in the chemistry and physical chemistry of proteins, their diversity and complexity have only recently become fully apparent. Native proteins appear to be more complex than it seemed some twenty years ago. Moreover, a great variety of synthetic poly-amino acids appeared on the stage. The number of newly discovered micromolecular and semi-colloidal substances possessing protein-like structure also is growing steadily. Some of these compounds appear to be of paramount importance in the biological functions of living tissues.

The scope of this treatise does not permit a thorough discussion of the chemistry and physical chemistry of this field. Colloid-chemical aspects of molecular size and shape will be treated preferentially. However, as shown already in the case of other examples in the preceding chapters, the colloid-chemical properties depend on chemical composition and constitution. Hence, a brief account of the chemical structure must be given. Excellent comprehensive treatises on proteins are available for those who want to become more thoroughly acquainted with this field[1].

1. Colloid-chemical Aspects of Classification

Two colloid-chemical criteria are dominant in the classification of proteins and protein-like substances: (1) the *size*, and (2) the *shape* of the molecules. With regard to molecular size, the material may be:

(1) micromolecular
(2) semi-colloidal
(3) colloidal
(4) coarse.

Although micromolecular substances do not belong to the realm of colloids, some of them are of extreme importance in the understanding of protein structure. The amino acids and simple polypeptides belong to this group. Substances with semi-colloidal dimensions are the more complex polypeptides and the intermediates isolated in the degradation of proteins, as well as most of the synthetic poly-amino acids. All soluble proteins are colloidal substances. Coarse dispersions can be obtained of insoluble proteins.

With respect to molecular shape the substances under consideration can be classified into two large groups:

(1) globular (sphero-) proteins, and
(2) fibrous (linear-) proteins.

Examples of the former are: albumin, hemoglobin, pepsin. Examples of fibrous proteins are: fibrinogen, myosin, collagen.

In this chapter only the structural principles and properties of the micromolecular and semi-colloidal protein-related materials will be discussed.

2. Basic Structural Features

The *chemical criterion* relating a certain substance to the protein family is that the major products of hydrolytic degradation of the substance under question must be *α-amino acids*. Hydrolytic cleavage of proteins may sometimes occur in steps, and intermediates other than amino acids may be encountered. Amino acids, however, are the final products of acid hydrolysis or enzymatic degradation with proteolytic enzymes. Amino acids, on the other hand, are also the starting materials in the synthesis of more complex substances more or less resembling proteins. Highly complex polypeptides and poly-amino acids have been synthesized, but the synthesis of even the simplest true protein has not yet been achieved. The course of degradation and synthesis may occur in the following steps:

Degradation:

Protein ⟶ Sub-units of the protein ⟶ Polypeptides ⟶ Amino acids.

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Synthesis:

Amino acid ⟶ Dipeptide ⟶ Tripeptide ⟶ Polypeptide (stepwise synthesis),

or Amino acid ⟶ Poly-amino acid (polycondensation).

Chemically, amino acids, polypeptides, poly-amino acids, and proteins are all similar in that they all are *ampholytes*, the molecules in solutions carrying positive and negative charges at the same time. Due to intramolecular neutralization, the amino group becomes positively charged, and the carboxyl group acquires a negative charge:

$$H_2N{-}R_x{-}COOH \rightleftarrows \underset{\displaystyle O\text{---}CO}{\overset{\displaystyle NH_3}{\overset{|}{\underset{|}{}}}}{-}R_x \rightleftarrows {}^{+}H_3N{-}R_x{-}COO^{-}.$$

The residue R_x depends on the kind and number of amino acids. In the simplest case, the amino acid glycine (amino acetic acid), R_x is CH_2. In the case of large protein molecules R_x is very complex. In this instance it is composed of a large number of peptide bonds (—CO—NH—), asymmetric carbon atoms

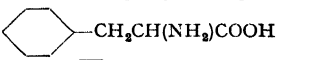
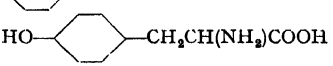
$$\begin{array}{l} \quad\ H \\ \quad\ | \\ C{-}C^{*}{-}NH, \\ \quad\ | \\ \quad CO \end{array}$$

and a certain number of other atomic groups. In contrast to synthetic polymers and polysaccharides in which only one, two or three (very seldom more than two or three) mers build up the chains, in the macromolecules of proteins about 20 different amino acid residues are found. These are listed in Table 74.

All of the amino acids, except glycine, possess one (or two) asymmetric carbon atoms in the molecules, and the amino acids obtained from natural sources, e.g., the hydrolysis products of proteins, are optically active. The specific rotation changes with the change of ionization of carboxyl and amino group, i.e., the specific rotation of an amino acid in water changes upon adding hydrochloric acid or sodium hydroxide to the aqueous solution. For example, the specific rotation of aspartic acid in water (Na light) is $+5°$. The specific rotation increases up to $+19.5°$ if one equivalent of HCl is added to one equivalent of aspartic acid. If, however, one equivalent of alkali, is added instead of acid, the specific rotation becomes negative, namely $-19°$. Natural amino

TABLE 74

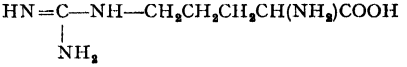
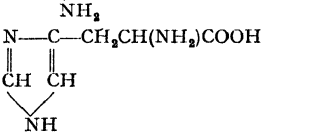
THE TWENTY IMPORTANT AMINO ACIDS FOUND IN MOST PROTEINS

Glycine	$CH_2(NH_2)COOH$
Alanine	$CH_3CH(NH_2)COOH$
Valine	$(CH_3)_2CHCH(NH_2)COOH$
Leucine	$(CH_3)_2CHCH_2CH(NH_2)COOH$
Isoleucine	$CH_3CH_2CH(CH_3)CH(NH_2)COOH$
Serine	$HO—CH_2CH(NH_2)COOH$
Threonine	$CH_3CH(OH)CH(NH_2)COOH$
Cysteine	$HS—CH_2CH(NH_2)COOH$
Cystine	$HOOC—CH(NH_2)CH_2S—SCH_2CH(NH_2)COOH$
Methionine	$CH_3—S—CH_2CH_2CH(NH_2)COOH$
Phenylalanine	
Tyrosine	

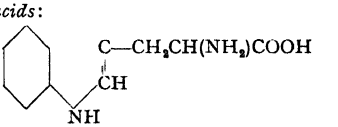
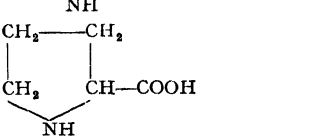
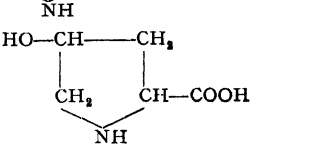
Dicarboxylic amino acids:

Aspartic acid	$HOOC—CH_2CH(NH_2)COOH$
Glutamic acid	$HOOC—CH_2CH_2CH(NH_2)COOH$

Diamino (basic) amino acids:

Lysine	$H_2N—CH_2CH_2CH_2CH_2CH(NH_2)COOH$
Arginine	
Histidine	

Neutral heterocyclic amino acids:

Tryptophan	
Proline	
Hydroxyproline	

In some proteins other amino acids are found in addition to those mentioned in this table.

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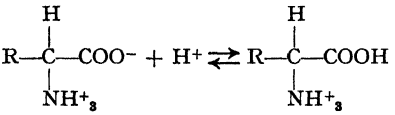
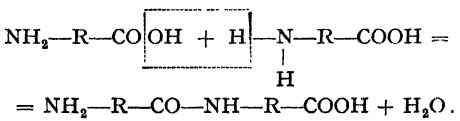
acids obtained from the hydrolysis products of proteins have been proven to be of the same (L) configuration. This conclusion was substantiated by studies on the optical rotation changes on the addition of strong acids. It was shown that the reaction:



is associated with a *positive shift* in the specific rotation in all natural α-amino acids[2]. The specific rotation of all plus-rotating natural amino acids becomes more positive on addition of strong acid; such acids (like aspartic acid) are designated L(+) amino acids. The specific rotation of all levorotating natural amino acids in water becomes less negative upon the suppression of dissociation of the carboxyl (these acids are designated as L(—) acids).

In naturally occurring as well as in synthetic polypeptides the amino acids appear linked through the peptide bonds, —CO—NH—, which are formed in the reaction:



It is obvious that many molecules of amino acids, either the same or different ones, can be linked, and chains of different length can be obtained. Such condensation reactions are not spontaneous, and the synthesis of poly-amino acids, and especially of polypeptides composed of many different residues of amino acids arranged in a definite order, is a difficult task. It is, however, obvious that the properties of the semi-colloidal products obtained will depend chiefly on the following two factors: (1) number of amino acid units in the chains, and (2) the composition of the R. When the number of mers in the chains is small, the resulting peptides do not differ much from the original amino acids. When the number of constituting units is large, the substance acquires colloidal properties. The properties of the larger peptides will depend also on the nature of the radical R. Inspection of Table 74 shows that R may be very different. For example, R in leucine and isoleucine is relatively large and lipophilic. R in the dicarboxylic acids (aspartic acid and glutamic acid) contains one free carboxyl group, hence in a poly-

peptide composed of glutamic acid residues, acid carboxyl groups will be attached to the sides of the chains, and an acidic polyelectrolyte will result. In polypeptides or poly-amino acids composed of lysine, the basic ε-amino groups of lysine residues will make the substance a basic (positively charged) polyelectrolyte. In polypeptides containing cysteine the reducing sulfhydryl groups —SH will be present in the molecules.

Furthermore, of importance is the fact that chains composed entirely of glycine or alanine are smooth, permitting easy orientation and crystallization. Such chains will resemble nylon and similar polyamides (Chap. 19). For example, it would be expected that solubility in water will decrease with increasing chain length. This is due largely to hydrogen bonding between the —NH— and —CO— of two adjacent peptide chains. The solubility of glycine polypeptides indeed does decrease with increase in chain length. The polypeptides containing dissociable groups in the residues (R) are soluble in water. Polypeptides composed of three or more different amino acids, even without free dissociable groups, are more soluble than those with more smoothly built chains. This is due to the fact that heterogeneous structures for purely geometric reasons are not favorable for aggregation.

Of importance is the fact that several amino acids are polyfunctional, and chemical cross-linking and ring formation are possible between chains. The sulfur bridges of cystine are very common in proteins.

The reactions of the basic amino groups and acid carboxyl groups are the most important ones in the step by step syntheses or polycondensation in order to prepare peptides[3]. The formation of a peptide bond is secured by, first, replacing the OH of the carboxyl with chlorine by treatment of the amino acid with phosphorus pentachloride or thionylchloride. The acid chloride forms peptide bonds with the amino groups of other amino acids easily, especially when the intramolecular reaction of the chloride with the amino group of the same molecule is inhibited (by substituting the active hydrogen). Another possibility is to replace the OH of the carboxyl group with an azide group. Carbobenzoxy chloride, C_6H_5—OCOCl, is the best blocking agent for the amino group, as it can be split off easily after the peptide is synthesized.

Emil Fischer and Abderhalden (1900–1916) succeeded in building up remarkably large polypeptide molecules by step by step syntheses. The largest of these polypeptides were composed of 18

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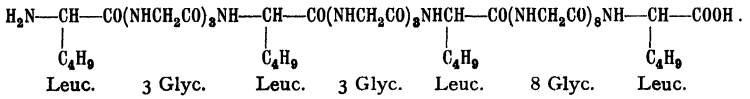
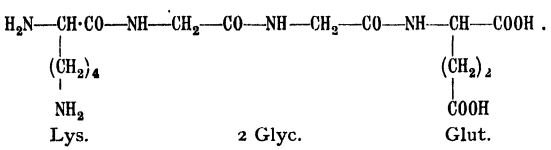
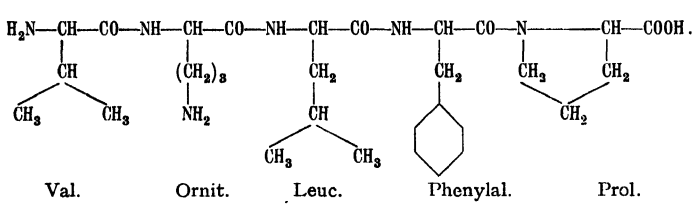
or even 19 amino acid residues. Due to the many steps involved, such syntheses are very time consuming and expensive, and only the cheapest amino acids, such as glycine and leucine were used in the preparation of the large polypeptides. For example, the 18 amino acid polypeptide was a L-leucyltriglycyl-L-leucyltriglycyl-L-leucyloctaglycyl-leucine of the following structure:



After the introduction of the carbobenzoxy method of Bergmann and Zervas[4] many complex polypeptides were synthesized, although they did not exceed in size the classical glycine-leucine polypeptides of Fischer and Abderhalden. For example, a tetrapeptide L-lysyl-glycylglycyl-L-glutamic acid was synthesized by Prelog and Wieland[5]. This peptide is interesting as an example of one containing both an acid carboxyl and a basic amino group in addition to the end-amino and end-carboxyls:



An even greater variety of side groups is present in the L-valyl-DL-ornithyl-L-leucyl-D-phenylalanyl-L-proline, a pentapeptide which contains the residues of ornithine $H_2N(CH_2)_3CH—(NH_2)COOH$, and the unnatural (D-) phenylalanine[6]:



The most brilliant achievement in the field of peptide synthesis, however, is the recent synthesis of the hormone oxytocin by Du Vigneaud *et al.*[7] This polypeptide is composed of the residues of

leucine, isoleucine, tyrosine, proline, glutamic acid, aspartic acid, glycine and cystine in equimolar ratios. Three of the free carboxyls are in the form of amides. The unique feature in the structure of this substance is that five of the amino acid residues form a large ring. The molecular weight of oxytocin is 1007, and its solutions are not colloidal.

Although the polypeptides mentioned above are not macromolecular substances, their constitution illustrates the structural features of the proteins. The peptide linkage appears to be the only essential mode of joining the amino acids into large peptides and proteins[8]. Bridges and cycles are occasionally formed between the chains.

3. Simple, Semi-colloidal Proteins

There is no sharp borderline between polypeptides and proteins, as there is no sharp dividing line between micromolecular substances and macromolecular colloids. The molecular weight of 10,000 is usually agreed upon as the lower size limit of the realm of colloids. The polypeptides mentioned thus far have much lower molecular weights, hence they are micromolecular. The molecules are able to penetrate the pores of ordinary semipermeable membranes easily. However, substances have been found which can be classified either as polypeptides or proteins. The situation is complicated by the fact that some of these substances associate in dimers or higher order polymers having molecular weights over 10,000. Generally, however, these substances are semi-colloidal, and the molecular weights are in the limits of 2000–10,000.

One of the most important groups of such semi-colloidal proteins (which may also be called large polypeptides) are the *protamines*. The chief source of the protamines is fish sperm, in which these substances are bound to deoxyribonucleic acid. Protamines, as the name indicates, are basic nitrogenous compounds. Complete acid or enzymatic hydrolysis yields chiefly basic amino acids, especially arginine. Many arginine residues appear in the chains in which the α-amino groups and carboxyl groups of the arginine residues are linked mutually or with other residues. The free remaining amino groups of the guanidyl residues of the arginine radicals confer alkalinity to the compound. Proline and arginine appear as end groups in most protamines[9], proline with its basic imino group, and arginine with its carboxyl on the other end of the molecule:

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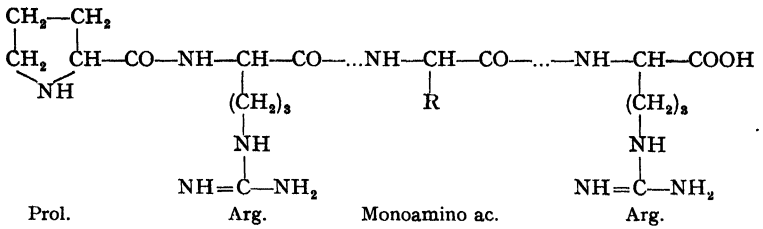

According to the analyses of Felix *et al.*[9, 10], the number of arginine (Arg.) residues is between 35–50 in various protamines. Glycine, alanine, serine, valine and proline are the most common monoamino acids. The order of the units has been partly established in some cases by means of partial enzymatic degradation and other methods (see next chapter). The amino acid composition of a number of protamines is shown in Table 75.

TABLE 75

MOLECULAR RATIOS OF AMINO ACIDS IN SEVERAL PROTAMINES

(after Felix[9])

	Iridine (Salmo irideus)	*Truttine* (Salmo trutta)	*Fontinine* (Salmo fontinalis)	*Salmine* (Salmo salar)	*Clupeine* (Clupea harengus)	*Sturine* (Acipenser sturio)
Glycine	2	2	2	3	—	2
Alanine	2	2	2	2	4	5
Serine	3	3	3	5	6	3
Threonine	—	—	—	—	2	1
Valine	4	5	5	4	4	—
Proline	5	5	5	5	5	—
Isoleucine	1	—	—	—	1	?
Glutamic acid	—	—	—	—	—	1
Arginine	50	50	50	55	53	35
Lysine	2	—	—	—	—	9
Histidine	—	—	—	—	—	8

While no differences could be found in the amino acids of truttine and fontinine, other species of the Salmonidae showed quite definite variations. According to Felix, the changes in amino acid content reflect species differences, since the brook trout and the char, which have protamines of identical composition, can be crossed. It is also obvious from the data of Table 75 that clupeine, the protamine from herring sperm, differs from the other protamines. Clupeine contains threonine instead of glycine, and its alanine and serine con-

tents are higher than those of the Salmonidae protamines. Sturine differs even more, since it contains relatively large amounts of lysine and histidine, less arginine than the others, and no valine or proline.

This example was presented in order to show the great variety in the realm of proteins. In addition to this variety of different molecular compositions in different species, any specimen of protamine from any source is not a homogeneous substance, but a complex mixture. Even the most highly purified protamine specimens appear to be mixtures of closely related molecules[9].

The method of isolation is essentially an extraction with acidic solvents, which dissolve the protamine originally bound to the nucleic acids[11]. The sperm is dispersed in water, coarse fragments are removed by filtration or centrifugation, and the heads of the spermatozoids are precipitated by adding acetic acid. The precipitate, which contains the protamine-nucleic acid salt, is treated with sulfuric or hydrochloric acid. The protamine is thereby set free, and goes into solution. The protamine salt can be precipitated with alcohol or similar solvents. The crude precipitate is further purified by solution and conversion to the picrate, a protamine salt of low solubility. The protamine can be liberated from the picrate by decomposing the latter with sulfuric or hydrochloric acid, and the picric acid can be removed from the solution by extraction with ether. Further purification can be achieved by means of adsorption.

The highly purified protamine salts (hydrochlorides, sulfates, carbonates) are white substances, easily soluble in water, and having molecular weights of 1000–8000. They are hydrolyzed by boiling with strong acids, and also by the enzymes trypsin, papain and cathepsin. Pepsin, which is a strongly acidic enzyme, however, forms a highly insoluble salt with the protamine, hence no hydrolysis is observed with this enzyme. This is a simple reaction between two oppositely charged polyelectrolytes (Chap. 14). There is a high positive charge density on the surface of protamine molecules, as all the amino groups of the arginyl residues acquire positive charges. Pepsin, on the other hand, has a large number of free carboxyls conferring negative charges to the macromolecule.

Even the most highly purified, many times reprecipitated protamine specimens appear to be mixtures, by the standards of modern methods of characterization. Fortunately, there are highly efficient methods available for the separation and characterization of semi-colloidal peptides and proteins. These include, first, chromatographic adsorption methods, as well as distribution between two

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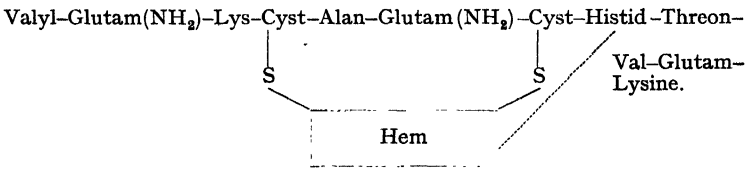
immiscible solvents. Other methods include electrophoresis, the solubility saturation test, and sedimentation in a high speed ultracentrifuge. These methods have revealed the presence of many components even in the most carefully purified specimens. The counter-current distribution method of Craig[12] appeared to be the most efficient one in revealing additional components in various protamines. The method involves stepwise distribution of solute between two immiscible solvents in a series of containers in which the two solvent phases are in contact. After the first equilibration the phases are separated, and equilibrated with fresh solvent, and so forth. If the amount extracted is plotted against the number of transfers in a consecutive series, symmetric maximum curves are obtained for homogeneous substances. Mixtures, however, yield complex curves with several maxima. A 24-fold distribution of a purified methyl ester of clupeine hydrochloride showed distribution curves with as many as 7 maxima, and a 60-fold distribution of one of the middle fractions yielded an even more complex curve[9]. The solvents used in these distribution experiments were a 15% solution of sodium acetate in water as the aqueous phase, and a 5% solution of lauric acid in *n*-butanol as the organic phase. No homogeneous protamine specimens have been prepared thus far.

Another interesting example of a semi-colloidal protein derivative is the *hemoproteide from cytochrome c*[13]. In contrast to the protamines, which are composed entirely of amino acid residues, this hemoproteide contains a hem moiety. Cytochrome *c* is itself an enzyme of molecular weight 13,000 and the hemoproteide is obtained by the enzymatic degradation of cytochrome. The molecular weight of the hemoproteide is 2020, and interesting is the fact that these molecules associate in alkaline solutions to produce larger particles with molecular weights of 10,000–11,000[14]. The composition and sequence of the amino acid residues in the chain is known, and it has also been established that the peptide is linked to the hem through the sulfur atoms of the cysteine residues (in addition, the iron of the hem is bound by a secondary bond to the imidazole of histidine). The structure of the hemoproteide is as follows:



The formula presented, however, does not show the true configuration of the molecule. According to the physical chemical studies of Theorell and associates[14], the peptide chain is not straight, but twisted into a sort of spiral (helix), as shown in Fig. 109.

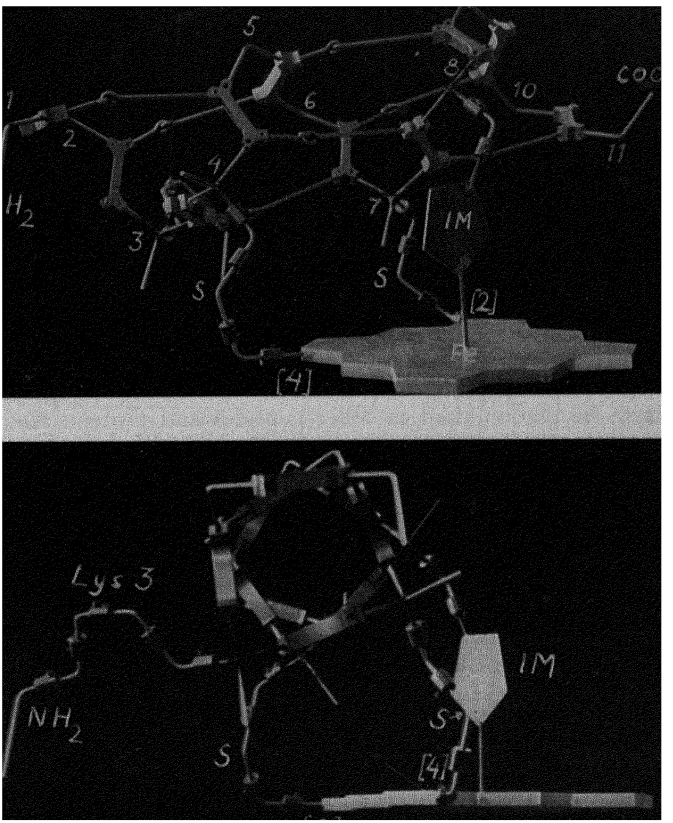


Fig. 109. Two photographs of the molecular model of the hemoproteide from cytochrome *c*. (Courtesy Professor H. Theorell.)

Density and partial specific volume values indicate that the substance has a compact structure in which the hem contributes very

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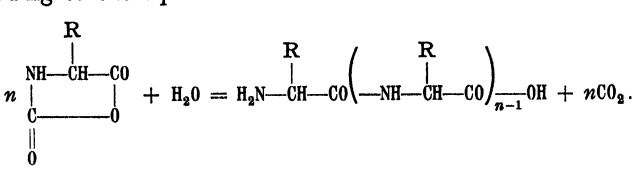
little to the volume. Aggregation in alkaline solutions can be explained easily by this model. This structure was supported also by spectroscopic and magnetic studies. The aggregates were depolymerized upon the addition of histidine, and the substance thus obtained absorbed light in the same way as does cytochrome. This indicates that in the larger molecule of cytochrome *c* more peptides are bound to the hem through the histidine imidazole. The hemoproteide contains 2.76% iron, and cytochrome *c* 0.433%, hence several peptides are bound to the same hem in cytochrome. The stereochemical model of Ehrenberg and Theorell is compatible with this ability to pack more peptides around the hem. The twisting of the peptide chains into the form of the so-called α-helices, and a close packing of the helices around the hem was also indicated in the study of cytochrome *c* by means of X-ray scattering[15].

The construction of geometrically reasonable models, compatible with X-ray structural data, density, reactivity, and other facts, appears to be a very fruitful approach in studying protein structure. According to Ehrenberg and Theorell[14], no geometrically reasonable models could be built if other arrangements instead of the helical structure of the peptide were assumed. The helical configuration seems to be predominant in other peptides and proteins also, as will be shown in the following chapters.

4. Synthetic Poly-amino Acids[16]

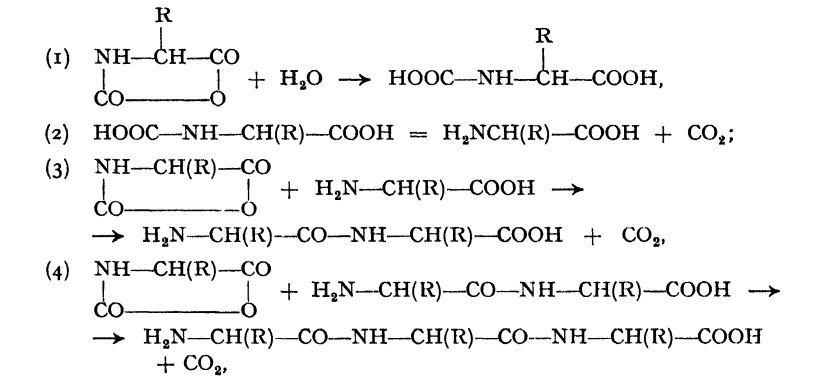
The polycondensation of amino acids does not occur easily, as cyclic micromolecular anhydrides are often formed instead of large molecules (Chap. 4). Derivatives of amino acids and small peptides e.g., esters, often polycondense more readily than free amino acids. The most important monomers used in these syntheses, however, are the N-carboxy-amino acid anhydrides (I) which polymerize according to the equation:



I Poly-amino acid

The monomeric carboxy-amino acid anhydrides are prepared by several methods. According to Leuchs (in 1906–08), the amino acid

is first coupled with carbomethoxy chloride $CO(OCH_3)Cl$, and the carboalkoxy derivative treated with thionyl chloride (see ref. 16). The resulting carboxy-amino acid anhydrides are soluble in cold water without being decomposed, but at higher temperatures carbon dioxide is evolved, and the substance is converted into either an amino acid or a poly-amino acid. Water acts as an initiating agent, the reactions probably occurring as follows:



(1) $\mathrm{NH{-}CH(R){-}CO{-}CO{-}O} + H_2O \rightarrow \mathrm{HOOC{-}NH{-}CH(R){-}COOH},$

(2) $\mathrm{HOOC{-}NH{-}CH(R){-}COOH} = \mathrm{H_2NCH(R){-}COOH} + CO_2;$

(3) $\mathrm{NH{-}CH(R){-}CO{-}CO{-}O} + \mathrm{H_2N{-}CH(R){-}COOH} \rightarrow$

$\rightarrow \mathrm{H_2N{-}CH(R){-}CO{-}NH{-}CH(R){-}COOH} + CO_2,$

(4) $\mathrm{NH{-}CH(R){-}CO{-}CO{-}O} + \mathrm{H_2N{-}CH(R){-}CO{-}NH{-}CH(R){-}COOH} \rightarrow$

$\rightarrow \mathrm{H_2N{-}CH(R){-}CO{-}NH{-}CH(R){-}CO{-}NH{-}CH(R){-}COOH} + CO_2,$

and so forth. Although carbon dioxide is evolved, the mechanism resembles that of chain growth in polymerisation, especially the polymerisation of ethylene oxide.

Waley and Watson[17] have observed the interesting fact that the chain growth of poly-amino acids is promoted by the presence of a poly-amino acid. Similar observations have been made on other polymers (Chap. 18, sect. 6), and can be interpreted as an orientation effect. It resembles the initiation of crystallization by seeding. This promotion of chain growth by the poly-amino acid, however, seems to be due to the chemical effects of the end groups. This conclusion is supported by the fact that the rate of polymerization is a function of the initial number of propagating functional groups (NH_2 groups) of the catalyst, but does not depend on the chain length of the added polymer.

Copolymerization of different N-carboxy-amino acid anhydrides occurs readily, e.g., Woodward and Schramm prepared a copolymer of L-leucine and DL-phenylalanine[18]. Many other poly-amino acids with various residues in the same chain have been prepared[16].

The poly-amino acids with lipophilic side groups are insoluble in water, and those with strongly polar and ionogenic groups at the

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sides of the chains are water-soluble. The molecular weights of these compounds can be determined by chemical determination of the end groups, by sedimentation, viscometry, and other methods, and relatively low molecular weights of 1,600–30,000 have been found (Table 76).

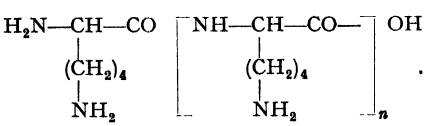
TABLE 76

AVERAGE MOLECULAR WEIGHTS OF SOME POLY-α-AMINO ACIDS
(after Katchalski[16])

	Molecular weight
Polyglycine	5,770
Polyglutamic acid	15,000
Poly-γ-methyl glutamate	30,000
Poly-β-benzyl aspartate	20,000
Poly-L-lysine	4,100
Poly-DL-alanine ethyl ester	1,660
Poly-(DL-phenylalanine-I-leucine)	15,000

Polyglutamic acid is one of the most interesting examples of poly-amino acids, since a similar substance (poly-D-glutamic acid) has been isolated from the capsular substance of *Bacillus anthracis*. This is quite a curious case, since the natural material appeared to be composed of the 'unnatural' D-glutamic acid, while the synthetic poly-L-glutamic acid contained glutamic acid of the natural (L) configuration. It could be shown that no racemization of the L-acid occurred during polymerization, and the poly-amino acid obtained is optically active. Polyglutamic acid is a poly-electrolyte, like other polyacids.

Another poly-amino acid of considerable interest is the water-soluble polylysine. Polylysine is a strong base, because of the many amino groups of the side chains:



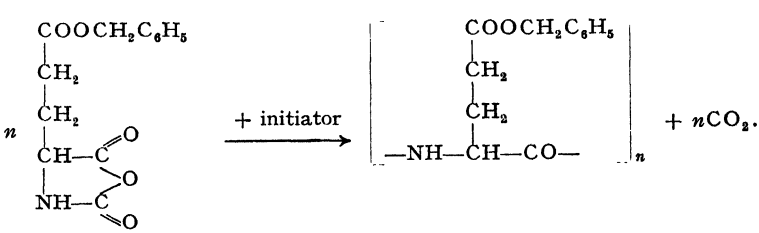
The polymer gives positive biuret and ninhydrin reactions, and a negative picric acid test, the latter indicating the absence of free lysine[16].

Relatively high molecular weight ε-carbobenzoxy polylysines have been prepared by Becker and Stahmann[19] by polymerization

of ε-carbobenzoxy-α-carboxyl-L-lysine in dioxane solution. The molecules were activated by anhydrous ammonia. The resulting carbobenzoxy-polylysines were insoluble in water, and of neutral nature, as the ε-amino groups were blocked by the carbobenzoxy residues. Noteworthy is the fact that the levorotation of the lower molecular polymers was —37° (Na light), of those of $M = 5000$ it was —70°, and of the larger molecules of $M = 41{,}000$ it was —80°. The specific rotation increased with the degree of polymerization (chain length) up to a certain chain length, after which it remained constant. Similar observations have been made by Robinson and Bott[20] on poly-γ-methyl-L-glutamate-DL-phenylalanine copolymer.

The viscosity of the solutions of the poly-amino acids and their derivatives increases with the chain length, as with all other polymers. X-ray diffraction studies on these poly-amino acids indicate that a close structural resemblance exists between these substances and the proteins and polypeptides[21]. Studies on the absorption of infrared light lead to the same conclusion[22]. In spite of this resemblance in general structural features, and partial resemblance in molecular size and shape, natural proteins are (with the exception of substances like poly-D-glutamic acid, which can hardly be classified as a protein) of much more complex composition and structure than the poly-amino acids.

Poly-amino acids which may have widely variable molecular weights, depending on the method of preparation, have been synthesized recently. The poly-γ-benzyl L-glutamates, which are soluble in a variety of solvents, have been thoroughly studied[23]. These poly-amino acid derivatives were prepared by polymerizing γ-benzyl N-carboxy-L-glutamate anhydride in dioxane solution using anhydrous ammonia as initiator. The formation of macromolecules proceeds as follows:



γ-Benzyl N-carboxy-L-glutamate anhydride

Poly-γ-benzyl L-glutamate

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The average molecular weight of the polymer formed depends chiefly on the amount of initiator applied. Sodium hydroxide, sodium borohydride and triethylamine also were found to be useful initiators. Dioxane as solvent could be replaced by many others, and the solvent also was found to affect the polymerization. Poly-γ-benzyl L-glutamates with average molecular weights ranging from 15,000 up to over 500,000 were prepared. The molecular weights and molecular shapes have been studied in various solvents by means of light scattering and viscosity measurements[24]. In some solvents (such as dimethylformamide and cresol) the macromolecules did not associate, and they behaved as if they were rigid rods possessing the α-helical configuration (according to the concept of Pauling and Corey; see sect. 3, Chap. 26). In some other solvents the macromolecular chains appeared as irregular coils. Molecular association also may occur in some solvents, e.g., in pure chloroform or dioxane.

Remarkable is the finding of Doty and associates that the helical macromolecules, which have the shape of rods, could be reversibly changed into the random coil configuration by gradual variation of the solvent composition. For example, in ethylene dichloride the macromolecules had the helical configuration and the shape of long rods; on adding dichloroacetic acid to this solution the properties do not change much when the proportion of dichloroacetic acid is small; however, in the vicinity of 76% dichloroacetic acid an abrupt change in the properties of the solution occurs. Especially sharp is the change in optical rotation. The polymer is dextrorotatory (specific rotation approximately $+15°$) in ethylene dichloride or in chloroform when it has the helical configuration, but it becomes levorotatory (specif. rot. $-14°$) in dichloroacetic acid[25]. A similar transition can be achieved by variation of the temperature of solutions composed of appropriate mixed solvents, e.g., 20 volumes ethylene dichloride and 80 parts dichloroacetic acid. The helix is stable at temperatures over 35°C, the random coil at lower temperatures of 10 to 25°C[25]. Moreover, it is significant that the stable helices of rod-like shape were formed only with polymers which have sufficiently long chains, i.e., high molecular weights, whereas the smaller molecules seemed to have more indefinite shapes of partially organized coils. It is rewarding to look at these interesting phenomena from the point of view of thermodynamics. The transition of the regular helix into a random coil is accompanied by a gain in configurational entropy, since the molecules become

more disordered. However, this gain in configurational entropy, as Doty has shown[25], is more than offset by the loss in entropy due to orientation of the solvent (dichloroacetic acid) molecules, which solvate the uncoiled chains of the poly-benzyl L-glutamate. A very large number of solvent molecules become oriented as they are adsorbed on the polymer chains, hence the entropy of the whole system, in the case of kinked chains in dichloroacetic acid, is smaller than the entropy in the case of rods (helices) in chloroform or dichloroethylene. (Dichloroacetic acid is a better solvent for this polymer than dichloroethylene or chloroform.) The systems containing the rod-shaped helices in this case are more stable, i.e., more probable, than the systems with the kinked irregular chains. Noteworthy is also the fact that the optical rotation changes in the negative direction upon transition of the helices into irregular coils, and that the same negative shift is observed on the denaturation of proteins. This indicates that the helices of the poly-benzyl L-glutamate possess the same configuration as native proteins.

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CHAPTER 26

Globular Proteins

Globular proteins are molecular colloids. They represent the most important group in the class of macromolecular substances called proteins. Most of the proteins of blood, milk, and seeds are globular or corpuscular colloids. Their molecules, or molecular aggregates, have shapes close to the spherical, hence the term 'spheroprotein' is also used.

It was shown in the preceding chapter that proteins have a very complex chemical composition. Complete clarification of the molecular structure is very difficult even with polypeptides and simple proteins having molecular weights of a few thousand. The molecular weights of the globular proteins treated in this chapter are much higher. The difficulties encountered in the elucidation of the molecular structure thereby increase enormously. In spite of this, the structural details are now almost completely worked out for some globular proteins.

Although the globular proteins are classified as belonging to the molecular colloids, they differ from most other molecular colloids (e.g. glycogen, rubber) in several ways. First, in several instances, the molecules of proteins appeared to be composed of 2, 3, or even 4 or more subunits. These subunits are joined by secondary bonds and cohesive forces into a larger entity which can be split into the subunits, for example, by acidifying the solution. However, as these subunits themselves are macromolecular entities, the proteins differ considerably from micellar colloids. Second, pure proteins differ from other molecular colloids in their relatively greater uniformity in molecular size.

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1. Isolation, and Criteria of Purity[1]

Proteins occur in nature in complex mixtures. Blood, milk, egg white, meat, seeds, and various saps all contain a great variety of substances in addition to proteins. Moreover, in all of these sources more than one protein is present, which complicates the isolation and purification. In addition to this, the protein molecules are unstable. At certain higher temperatures or in the presence of certain reagents the configuration of the molecules is changed. The unique folding of the peptide chains may be distorted easily, i.e. the protein can be denatured. These peculiar changes are often irreversible, the protein to be isolated becoming insoluble.

The proteins used for study in the first period of classical colloid chemistry (1910–1930) were mainly ill defined, impure, and more or less denatured. The Danish protein chemist Sørensen was one of the first who took particular care in the purification of proteins. Linderstrøm-Lang (Copenhagen), Pauli (Vienna, Austria), and the enzyme chemists (Sumner, Northrop, and others) continued this line of work successfully. A number of pure proteins were available around 1930, and they were investigated by chemical and physicochemical methods.

The methods of isolation differ considerably from case to case. They differ also depending on the ultimate purpose for isolation of the protein to be separated. For nutritional studies, for instance, the proteins are carefully separated from vitamins and other nonprotein material. The state of denaturation, however, in this case is not critical, since the amino acid composition of native and denatured proteins is the same. The criteria of purity are relatively unimportant when a protein is destined for industrial uses, as for preparation of protein fibers. For physicochemical, chemical, and biochemical studies the proteins must be as highly purified as possible. If a protein is to be isolated for colloid-chemical studies, it is most essential to avoid denaturation. One of the most important factors which controls denaturation is *temperature*. It is obvious that intense molecular bombardment may damage delicate and complex structures such as those of globular protein molecules. Therefore, in order to avoid denaturation, the temperature must be kept as low as possible during the process of isolation. Even room temperatures are often too high, especially if organic solvents are used as reagents in the process of separation. Separation often

must be carried out in a cold room. Home freezers are convenient for work with small amounts.

The isolation procedure is always carried out in several steps, and the processes differ depending on the starting material and the protein to be separated. If the starting material is solid, e.g. ground seeds, the protein must first be extracted. Water, solutions of salts, or weak alkalies are used most frequently for such extractions. The next step is to remove undesirable coarse particles which are present in such extracts. They are usually present even in a fluid starting material, such as milk. The solid particles are removed by filtration or centrifugation. The clarified liquid then can be treated further in various ways, depending on the properties of the mixture. For example, many globulins can be precipitated at once by simply dialyzing the extract against distilled water. Cellophane tubes are convenient for this purpose, and the process can be speeded up by stirring. All micromolecular ingredients pass through the membrane, whereas all proteins and other colloidal components present remain in the tube.

Another common method of separation is the precipitation of the proteins by means of a precipitant, such as ammonium sulfate, alcohol, or dilute acid. Fractional precipitation by gradual addition of the precipitant and mechanical stirring gives the best results. If albumins and globulins are present, the latter are precipitated first, i.e. at a lower concentration of the precipitant. For further purification, the fractions are removed by centrifugation, redissolved, and dialyzed. Treatment with adsorbents and ion exchange resins is sometimes employed[2].

Separation and purification by means of electrophoresis, electrodialysis, and by electrophoresis convection are also important (see Chap. 10). Separation by means of a preparative ultracentrifuge is also possible, if the molecular weights of the components are quite different. When the purification is finally carried out far enough, the protein may crystallize. More often, however, the substance is liberated from water by means of *freeze drying* or *lyophilization*. The aqueous solution of the protein is frozen and subjected to a high vacuum. Water distils from the frozen solution, and the protein remains in the container. Cooling is unnecessary, since the energy needed for the evaporation is taken from the system itself, and thus keeps it in the frozen state. Many proteins can be obtained this way in a solid, soluble form, without being denatured.

The criteria of protein purity have been re-evaluated often in the

last two decades. Crystallinity of a protein is an insufficient criterion, since many crystalline proteins proved to be either mixtures of several components or contaminated with micromolecular ingredients. Egg albumin, serum albumin, and pepsin are examples of globular proteins which, after being carefully purified and crystallized, still proved to be heterogeneous. Another criterion of purity which proved to be insufficient in the light of recent studies is the sedimentation test in an analytical ultracentrifuge. Proteins which show only one peak in the sedimentation diagrams (see Chap. 7) still may be heterogeneous. In the first place, this method is not sensitive enough to detect components which differ little from the chief component. Second, a protein may be composed of a variety of molecules which have the same sedimentation properties but differ in chemical composition and constitution.

Two other physicochemical criteria proved to be more revealing: *electrophoresis* and the *solubility test.* The solubility of a homogeneous substance should be independent of an excess of the substance (Chap. 12). This can at present be verified only in a few cases of crystallized enzymes. However, recent electrophoretic studies have shown that enzymes which appeared homogeneous in solubility tests are inhomogeneous in electrophoresis. The latter method thus seems to be the more sensitive[3]. The reversibility of boundary spreading in free boundary electrophoresis is an especially sensitive criterion. Other tests, such as distribution between two liquid phases[4], can be applied only to proteins of low molecular weight. Finally, valuable information can be obtained by chemical analysis of protein specimens. Sensitive micromethods are now available for traces of carbohydrates, lipids, and other possible contaminants. Amino acid analyses are also capable of rendering useful information on homogeneity. When a particular amino acid is found in very low concentration in a particular protein, the molecular weight of the protein can be calculated from the percentage of this amino acid. Since at least *one* mole (or one residue) of the amino acid should be present in the large protein molecule, the *minimum* molecular weight M of the protein will be equal to $100\ A/a$, where A is the radical weight of the amino acid, and a its percentage (see Chap. 7). In some cases this chemical molecular weight is greater than that determined by physicochemical methods. This indicates that the protein is composed of at least two components, and that one of them is devoid of the trace-amino acid.

2. Size, Shape, and Hydration[5, 6]

The molecular weights of several proteins are shown in Table 77.

TABLE 77

MOLECULAR WEIGHTS OF SOME GLOBULAR PROTEINS DETERMINED BY DIFFERENT METHODS

	M *by osmometry*	M *by sedimentation velocity*	M *by light scattering*	M *by chemical analysis*
Albumin, egg	45,000	44,000	38,000–60,000	44,000
Albumin, serum	65–70,000	70,000	76,000 and higher	
Hemoglobin	67,000	68,000		16,700
Insulin	12,000; 36,000	36,000	12,000 and higher	12,000; 6000
	48,000	48,000		
Pepsin	36,000	35,500		
Excelsin	214,000	295,000	280,000	
Edestin	50,000 (in urea)	381,000	335,000	46,000

Although the agreement between the methods is good in many cases, some discrepancies still exist. These can be explained in part by errors of measurement. Another reason for the discrepancies is that different preparations of the same protein have been used by different authors. The macromolecular preparations of proteins, however, are hardly reproducible, if very high standards for accuracy are considered. For a truly monodisperse protein solution, the molecular weights obtained by different methods should be the same. For polydisperse systems, of course, the number average molecular weight (M_n) obtained by the osmotic pressure and by chemical methods will be smaller than the weight average (M_w) obtained by sedimentation or by light scattering (see Chap. 8). The relatively good agreement between the M_n and M_w values indicates that most of the proteins mentioned in Table 77 are relatively monodisperse colloids. It is also remarkable that in the case of edestin (a globulin from hemp seeds) the chemical molecular weight is practically the same as that determined osmotically, if the solvent is a concentrated urea solution. The large edestin molecules thus seem to be composed of subunits, like insulin. The macromolecules of hemoglobin are stable in solutions. In this instance the four subunits of $M = 16{,}700$ (the value obtained from

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chemical analysis) are bound more tightly than in the case of insulin or edestin.

It was found by sedimentation analyses that in several cases protein solutions are paucidisperse, i.e. there are two, three, or four species of molecules sedimenting in discrete boundaries. The higher molecular weights thereby appeared often as simple multiples of the lowest of them. For example, an insulin solution contains molecules with $M = 12{,}000$, 36,000, and 48,000. The dissociation of the very large hemocyanin molecules occurs in steps at certain pH values, and dissociation products with 1/2, 1/4, 1/8 and 1/16 of the original molecular weight have been observed[7]. These facts led to the assumption that the molecular weights of proteins might be multiples of a definite minimum weight, which seemed to be about 17,000. This assumption, however, proved to be wrong. A number of proteins have molecular weights below 17,000. Furthermore, in several cases, e.g. egg albumin, the molecular weight is not a simple multiple of 17,000.

Although the molecular weights of many globular proteins are known with a reasonably high degree of precision, relatively little is known about the actual sizes and shapes of the molecules in solutions. The molecules of edestin, catalase, and the viruses can be seen in the electron microscope (Chap. 7). The details of the shape, however, are not sufficiently well discernible. It is difficult to decide whether the particles under observation are spheres, flat cylinders, or polyhedrons (see Fig. 110).

Information on the size, shape, and hydration of proteins can be obtained by sedimentation, diffusion and viscosity studies on solutions, as well as by means of X-ray studies on protein crystals. Unfortunately, shape and hydration both influence the sedimentation, diffusion, and viscosity. Streaming birefringence and viscosity are relatively crude methods for investigating small differences in size and shape of globular proteins. Ionization of the protein molecules is also of importance, although the charge effects can be suppressed by adding suitable electrolytes. The frictional ratio f/f_0 is often employed for the evaluation of the shape and degree of hydration (see Chap. 7). The frictional ratio, however, does not represent the axial ratio of the molecules. This ratio only characterizes their resistance against displacement in a liquid. The more asymmetric the molecule, the greater will be the f/f_0. However, it will also be great for a strongly swollen spherical molecule.

The problem of the share of hydration in these effects could be

solved if accurate methods for the determination of hydration were available. Unfortunately, there are no accurate methods available at the present time. The cryoscopic, dilatometric, and solubility methods formerly employed proved to be unreliable[8]. Somewhat more accurate data have been obtained recently by the X-ray analyses of protein crystals, by dielectric dispersion data, by studies

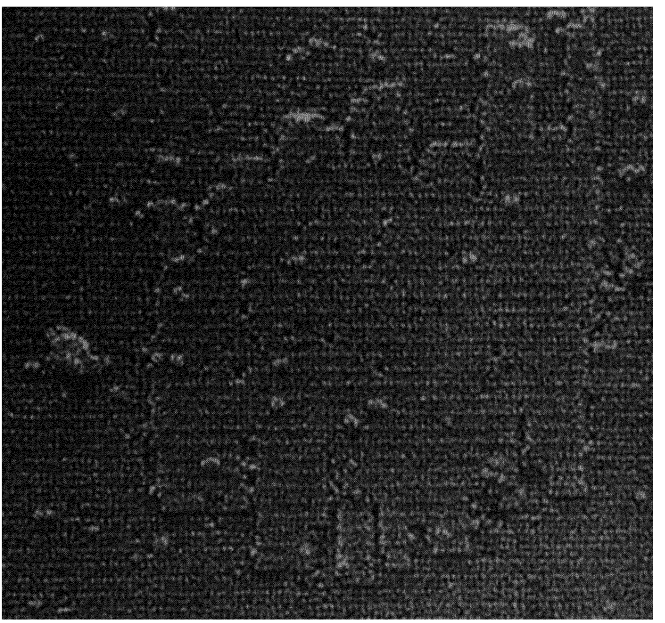

Fig. 110. Electron micrograph showing the regular array of molecules at the surface of a crystal of catalase. This material is an enzyme extractable from liver and has a molecular weight of 250,000 Magnification 139,500 ×. (Courtesy Dr. C. E. Hall, Massachusetts Institute of Technology.)

on the sorption of water vapor by dry proteins, and by sedimentation studies in solvents of different densities. Data obtained by studying the crystals of myoglobin indicate that the molecule of this protein is slightly asymmetric. According to Oncley[9], it is possible to

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calculate the hydration from the frictional ratio, if the asymmetry is known. Estimates from crystallographic data showed that the axial ratio of the myoglobin molecule is about 1.7. The amount of hydrate water, according to the method of Oncley, then is estimated to be 0.08 g water per 1 g of myoglobin[10]. For several other proteins the amount of tightly bound water was estimated to be about 0.1 to 0.3 g per 1 g of protein.

The sorption of water vapor by dry proteins is employed for hydration studies. The protein is exposed to vapor of various pressures, and the amount of adsorbed water is determined by weighing. The sorption at various pressures is expressed graphically (Fig. 111). The lower part of the curve refers to chemically bound

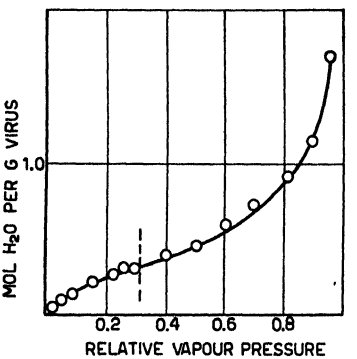

Fig. 111. Water vapor adsorption isotherm for tobacco mosaic virus at 25°, according to Katchman, Cutler and McLaren (*Nature*, 166 (1950) 266). The lower part of the curve, up to the dotted line, shows the amount of the chemically bound water. The upper part shows the amount of water bound by adsorption.

water (by hydrogen bonds), the upper part to water bound by cohesion. Only the chemically bound water is considered as water of hydration, although this approach is somewhat arbitrary. According to sorption studies, one gram of lyophilized egg albumin binds 0.05 grams of water. McLaren and Rowen[11] have estimated that this amount of chemically bound water, if calculated in moles, corresponds to the number of polar groups (such as COOH and NH_2) in the protein molecules. The amount of water which is carried around with the protein molecules in solution probably is greater, since some of the water will be bound by dipole attraction and cohesion.

Since neither the form or hydration is known with a sufficient

degree of precision, all the calculations offered for the molecular dimensions of various proteins are approximate and tentative values (see Table 78).

TABLE 78[12]

TENTATIVE DIMENSIONS AND OTHER DATA FOR SOME GLOBULAR PROTEINS

	f/f_0	*Intrinsic viscosity*	*Assumed model*	*Long axis in A*	*Medium axis in A*	*Short axis in A*
Beef insulin	1.18		prism	44	20	20
Horse myoglobin	1.11	0.031	platelet	54	37	9–14
Horse hemoglobin	1.24	0 038	ellipsoid	65	55	55
Serum albumin	1.30	0.042	prism	145	45	32

3. The Structure and Configuration of the Spheroproteins

The protein molecule is composed of long polypeptide chains. In the fibrous proteins these chains form long bundles, like those of cellulose. In the globular or spheroproteins the peptide chains are folded in some regular fashion. The exact configuration, i.e. the spatial arrangement of this folding, is not yet known in all details even for the smallest molecules of globular proteins. A considerable number of models proposed earlier were abandoned, because they were not in accord with some of the known facts, such as X-ray crystallographic data, molecular weight, density, chemical reactivity. The X-ray diffraction diagrams furnish important information on the arrangement of the structural units, although they cannot provide a complete picture of how the chains are folded, and how the side chains are allocated.

Important progress has been made on the problem of how many peptide chains are present in a protein molecule. This was solved by means of purely chemical methods. Sanger[13] found that 1-fluoro-2,4-dinitrobenzene (FDNB) reacts quantitatively with the free amino groups of proteins. A dinitrophenyl-protein thereby is formed. (Since the reaction is carried out under mild conditions, no hydrolytic cleavage occurs.) The dinitrophenyl-protein is then hydrolyzed with a strong acid, which splits the peptide bonds. In the hydrolyzate then will be found the amino acids, and the dinitrophenyl derivatives of those amino acids which had free amino groups in the original protein. These dinitrophenyl amino acids are bright yellow compounds. They can be separated from the free amino acids by chromatographic methods, and their concentration can

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be determined quantitatively. Furthermore, by analyzing the dinitrophenyl amino acids, it is possible to determine which amino acids have reacted with the FDNB. They will be the amino-end groups of the polypeptide chains. If lysine residues are present as the side chains, they will also react with FDNB, as will the hydroxyl of tyrosine and the imidazole group of histidine. These reaction products can be eliminated, and do not interfere with the determination. Sanger found that upon treatment of insulin in this way, dinitrophenyl glycine, dinitrophenyl phenylalanine, and ε-dinitrophenyl lysine were formed. Quantitative evaluation of the results showed that three free amino groups were present in each molecule of insulin; one of these belonged to the glycine end-residue, the other to the phenylalanine end-residue, and the third was the ε-amino group of lysine. These results, according to Sanger, suggest that the insulin molecule is made up of two open peptide chains. One of them has glycine as the terminal amino acids with the free NH_2-group, and the other has phenylalanine. Many other proteins were investigated by this method. It was found that some proteins, e.g. serum albumin, have only one single chain. The polypeptide chain in this protein must be very long. Since the molecule is essentially globular or slightly asymmetrical, the chain must be folded many times. It was found that some proteins, for example chymotrypsinogen, do not produce any α-dinitrophenyl-amino acids with Sanger's reagent. This indicates that either there are no free terminal amino groups (cyclic structure) or that the reaction with the FDNB is hampered by some steric or other reasons[14].

Several other methods have been employed lately for structural studies on globular proteins, and the amino acid sequence in the simplest proteins, such as insulin and ribonuclease, has been established almost completely. Partial degradation by means of enzymes proved to be a very useful method for this purpose. The partial degradation products then usually are analyzed further by the Sanger method and other chemical procedures.

The problem of the spatial arrangement of the constitutive groups of peptide chains has been thoroughly studied by means of X-ray diffraction, and the atomic distances and bond angles are determined with a moderate degree of precision[15]. However, the folding and configuration of the chains in the molecules of spheroproteins is not quite clear. According to Huggins, Pauling, Corey, Low, and others[15, 16], the peptide chains are twisted in spirals or helices. The helix is stabilized by hydrogen bonds acting between the carbonyl

groups (CO) and the NH-groups of the same chain. In the so-called α-helix of Corey and Pauling[16], each C=O is linked to the N—H group of the third residue beyond it (Fig. 112). Moreover, the

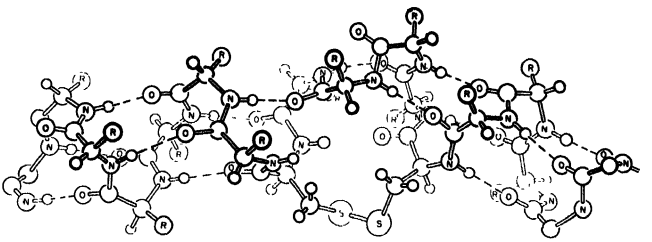
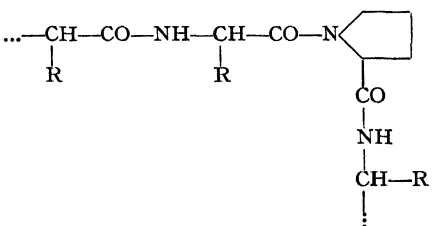


Fig. 112. The α-helix in proteins, according to L. Pauling and R. B. Corey. (Courtesy Dr. R. B. Corey.)

structures are stabilized by hydrogen bonds between the adjacent helices, as well as by the primary sulfur bonds of the cystine radicals of the two chains. Finally, salt bridges between the ionized carboxyls of glutamic and aspartic acid side chains and the ionized ε-amino groups of the lysine residues may play a role in stabilizing the structure.

The macromolecules of serum albumin, hemoglobin, and other globular proteins are composed of folded helices. If there were a straight helix in an albumin molecule, it would have to be about 820 A long[12]. According to Table 78, however, the length of serum albumin molecule is only about 145 A. Hence the helix must be folded in some way. How this folding occurs is not known. It has been indicated that the proline residues might be responsible for the folding, as illustrated below:



The reason for this peculiar role of proline is its structure, i.e. the peptide bond is part of a ring, and there are no H atoms at the

nitrogen of the proline residue. The possibilities of folding have been discussed by Lindley[17] (see Fig. 113).

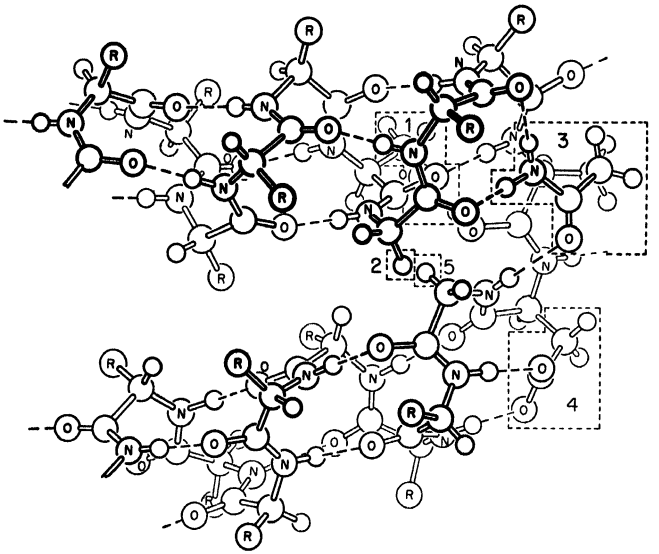


Fig. 113. A hypothetical model of the possible folding of an α-helix according to Lindley (*Biochim. Biophys. Acta*, 18 (1955) 194).

4. Ionization and Ionic Interactions of Globular Proteins

The macromolecules of proteins contain the ionogenic groups —COOH and —NH_2. This is why protein molecules become electrically charged. Ionization of the carboxyls will result in the formation of negative charges, and ionization of the amino groups will produce positive loci on the surface of the molecules. If the number of negative and positive ions of a macromolecule is equal, the macromolecule will not migrate in an electric field. This condition is called the isoelectric state. The isoelectric points of several proteins are compiled in Table 79.

Proteins whose isoelectric point is below 7 are acidic. The number of free carboxyls predominates over the number of free amino groups in such cases. When the isoelectric point is above 7, free amino groups are in excess. Most proteins, such as the ordinary albumins, many globulins, pepsin, and insulin, are predominantly

acidic. The molecules of the acidic proteins contain many glutamic and aspartic acid residues[18].

TABLE 79

ISOELECTRIC POINTS OF SOME PROTEINS

Albumin, serum	4.9	Ribonuclease	7.8
Albumin, egg	4.6	Fetuin, cow	3.5
Albumin, milk	5.1	Globulin, barley	5.7
Hemoglobin	6.9	Thyroglobulin	4.6
Myoglobin	7.0	Hemocyanin (*Eledone*)	4.6
Lysozyme	10.5	Cytochrome (cow)	9.5

The electrochemical nature of proteins is investigated by electrophoretic studies on their mobility (Chap. 10), and by potentiometric studies upon addition of acids and alkalies. Like amino acids and peptides, globular proteins are ampholytic substances, and they act as buffers. They are able to bind a certain amount of hydrogen ions, as well as a certain amount of hydroxyl ions. This binding is expressed by means of titration curves which show the dependence of the pH of the solution upon the added quantity of acid or base (Fig. 114).

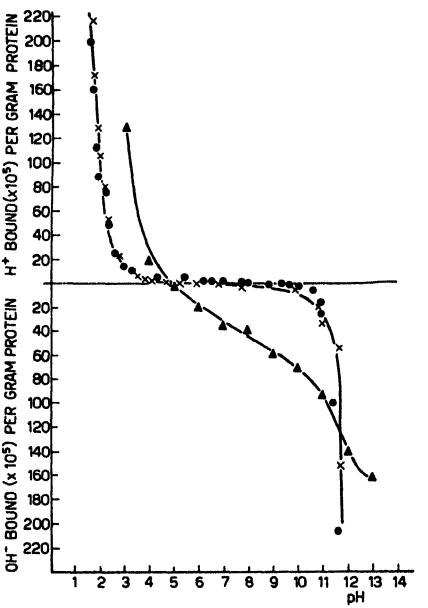


Fig. 114. Acid-base binding curves of two proteins. Discs and crosses – a specimen of Bence-Jones protein. Triangles – serum albumin.

The colloidal stability of proteins is minimal at the isoelectric point. Each particle then carries an equal amount of positive and negative charges, and the particles exert mutual attraction. In non-isoelectric solutions the particles have a net charge and repel each other. For the flocculation of an isoelectric albumin less alcohol is needed than for an acidic or alkaline albumin.

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There is a viscosity minimum of proteins at the isoelectric point, and the viscosity increases on both sides of this point. The optical rotation of albumins and many globulins is nearly constant in the pH limits of 5–8; it increases upon acidification[19]. It is assumed that these variations in properties are caused by changes in protein ionization and expansion of the protein molecules. Viscosity and levorotation of albumin rise strongly also with rising pH in alkaline solutions, especially at pH above 11[20].

Proteins interact readily with various cations and anions[21]. Complex compounds often are formed with the ions of heavy metals. Electrostatic interactions between proteins and other colloidal electrolytes have been studied quite extensively, as outlined in Chap. 14.

5. Examples of Globular Proteins, Serum Albumin

Albumins are distinguished from the globulins by their greater electrophoretic mobility, and by their high solubility in water and salt solutions. Albumins are easily soluble in water, and the colloids flocculate on heating at 55–60° C. Albumin thereby is denatured, and the precipitate cannot be redissolved in water. Serum albumin was chosen as an example because of its exceptional importance in clinical research. Highly purified and crystallized preparations of serum albumin have been prepared and studied.

The best method for the preparation of pure serum albumin is that of Cohn and associates of Harvard University. Many other plasma fractions can be obtained at the same time as by-products. The methods were developed during 1940–1950, one of the goals of the work being the preparation of human plasma fractions for medical purposes[22]. The fractionation is based on the fact that various plasma proteins can be selectively precipitated by ethanol-buffer mixtures. The precipitability depends on the concentration of the alcohol, and on the pH and ionic strength of the solution. The precipitation should be carried out at —5° C, in order to avoid denaturation. Fibrinogen and most of the globulins are removed first by precipitating with a reagent which has a pH of 4, and contains 25% ethanol. Most of the albumin remains in the supernatant. The best method to precipitate albumin, according to Cohn[23], is by means of zinc acetate, in the presence of alcohol. This albumin precipitate is purified by extracting it with a buffer containing barium ions. The insoluble zinc salt of the albumin is thereby converted into a soluble barium salt. In order to remove

the barium, the albumin solution is treated with a cation exchange resin. After that the albumin solution is liberated from electrolytes and other micromolecular ingredients by dialyzing against distilled water. Special mixed bed ion exchange resin columns can also be used for deionization[24]. The pure albumin then can be liberated from water by freeze drying.

The purity of the albumin preparations can be checked by various chemical and physicochemical methods. Conductivity tests and ash analyses are used for testing for electrolytes. Traces of carbohydrates and lipids can be determined by chemical methods. The content of nitrogen, sulfur, and of the amino acids are other chemical characteristics. Pure serum albumin contains 16.0 % N, and 1.93 % S. The amino acid composition of bovine and human serum albumins is presented in Table 80. The presence of other protein components

TABLE 80

AMINO ACID COMPOSITION OF BOVINE AND HUMAN SERUM ALBUMINS

(From G. R. Tristram, *The Proteins* (H. Neurath and K. Bailey, Editors), Vol. I A, 1953, p. 215.)

	Bovine albumin		*Human albumin*	
	% of amino acid	*number of residues per mol.*	*% of amino acid*	*number of residues per mol.*
Alanine	6.25	48		
Glycine	1.82	17	1.60	15
Valine	5.92	35	7.70	45
Leucine	12.27	65	11.00	58
Isoleucine	2.61	14	1.70	9
Proline	4.75	29	5.10	31
Phenylalanine	6.59	24	7.80	33
Tyrosine	5.06	19	4.70	18
Tryptophan	0 68	2	0.2	1(?)
Serine	4.23	28	3.34	22
Threonine	5.83	29	4.60	27
Cystine/2	5.73	28	5.60	32
Cysteine	0.3	2	0.70	4
Methionine	0.81	4	1.30	6
Arginine	5.90	23	6.20	25
Histidine	4.0	18	3.50	16
Lysine	12.82	60	12.30	58
Aspartic acid	10.91	65	8 95	46
Glutamic acid	16.5	78	17.0	80
Amide N	0.78	(39)	0.88	(44)

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in the albumin is checked by means of electrophoresis. The most convenient method is electrophoresis on paper[25], followed by staining with bromophenol blue, and measuring the color density of the paper strips with a densitometer (Fig. 115). If the albumin specimens are not quite pure, small peaks of the α and β globulins appear in the optical density diagrams (Fig. 116).

There are some differences between the bovine and human albumins. Although the molecular weight is the same, the number of amino acid residues in human albumin appears to be less than in the bovine. Something in the human albumin is missed in the analytical determinations. Horse serum albumin differs from both bovine and human albumin. There is some experimental evidence that not only the albumins of various species differ, but that even the albumins of various individuals of the same species are not quite the same. Differences in some physicochemical properties appear also in diseases[26].

Pure serum albumin appears inhomogeneous on electrophoresis, especially at pH 3–4. Another kind of inhomogeneity of serum albumin was demonstrated by chemical methods. It was shown that only a part of all albumin molecules contain free sulfhydryl groups (—SH) reacting with mercury[27]. The albumin component which contains these reactive sulfhydryls was named *mercaptalbumin*. Mercury ions combine with two molecules of mercaptalbumin:

$$2\text{Alb—SH} + \text{Hg}^{++} = \text{Alb—S—Hg—S—Alb} + 2\text{H}^{+}.$$

This mercury mercaptalbumin dimer crystallizes quite readily, and many times recrystallized bovine mercaptalbumin mercury dimer is now commercially available. Dimerization of mercaptalbumin occurs also on standing even in absence of mercury ions. This can be demonstrated easily by sedimentation experiments in the analytical ultracentrifuge. A second component appears in solutions in time, and the sedimentation constant of this component indicates that this is a dimer. There is other experimental evidence which shows that there are more than two or three types of albumin in a pure preparation. It seems plausible that many different kinds of albumin molecules exist in any preparation, but they do not differ much in their molecular size, shape, or chemical composition[28]. Thus an albumin preparation might be compared with a population of individuals which are alike but not quite identical. The evidence is growing that albumin, and other apparently homogeneous proteins, are such "populations" of similar but not identical entities[28].

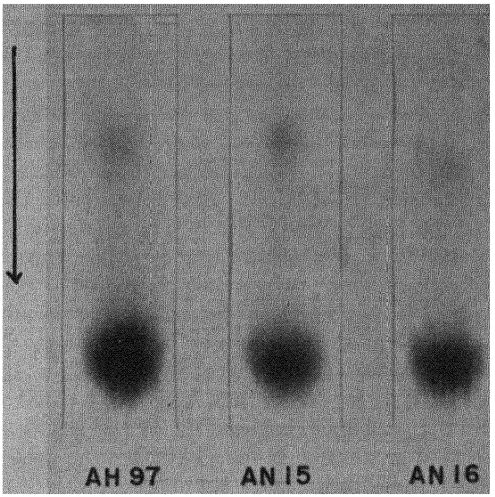

Fig. 115 Electrophoretic migration of albumin on strips of paper. The protein migrated in the direction shown by the arrow. The strips were stained with bromophenol blue. All three albumin specimens contain some globulin impurity.

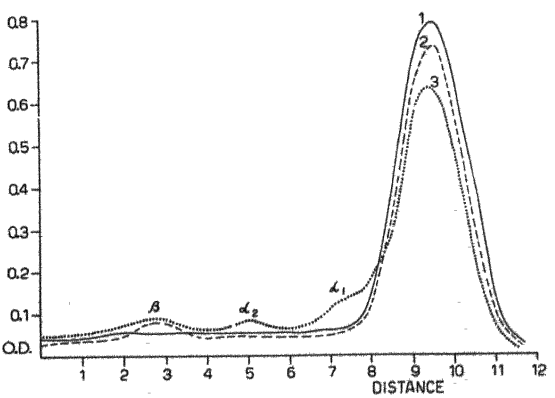

Fig. 116. Electrophoresis diagram of albumin obtained by densitometric scanning of paper strips containing the electrophoretically separated and stained protein On the abscissa is plotted the distance of migration, on the ordinate the optical density. Curve 1, pure, crystallized human serum albumin; curve 2, a highly purified albumin specimen; curve 3, crude albumin fraction containing the α and β globulins.

Serum albumin combines with various micromolecular and colloidal substances, and it is distinguished in this respect from other proteins by being very reactive[21, 22]. Electrolytes are bound at the charged and polar sites, and heavy metal ions often form chelates. Various dyes are also bound to the albumin molecules[21]. The reactions with other colloidal electrolytes depend on the charge of the reacting components. In solutions which are more acidic than the isoelectric point of the albumin, the molecules of the latter are positively charged. Such positive albumin combines with any other negative particles, such as ordinary soaps. It has been shown that serum albumin can also be precipitated by various synthetic polyelectrolytes, such as polymethacrylic acid. After removal of the precipitant, the albumin appeared to be in the native state[29]. Albumin carries negative charges in solutions more alkaline than the isoelectric point, and it reacts then easily with cations, including those of colloidal dimensions, such as the cationic detergents.

It has been shown that a molecule of bovine serum albumin contains 100 carboxyl groups and 58 amino groups. Moreover, the amphoteric nature of the macromolecules is influenced by the 16 imidazole, 19 phenolic and 22 guanidyl groups of the amino acid residues. The dissociation constants of the supposedly free carboxyl, amino and phenolic groups were found to be abnormal; its seems that these groups participate in some kind of internal binding in the macromolecules[30].

The macromolecules of albumin are able to bind other molecules not only by electrostatic attraction, but also by hydrogen bonding and cohesion. For example, aliphatic acids (and their salts) with relatively long carbon chains of 8 carbons combine with albumin more readily than does acetic acid. According to Luck and associates, serum albumin molecules bind caprylates, whereby the latter stabilize albumin against heat coagulation[31]. The caprylate molecules are able to tighten somehow the delicate, folded helices. Under this caprylate protection albumin solutions can be sterilized without being denatured. When caprylate is added to plasma, and the latter heated, all other proteins except albumin (and some very stable glycoproteins) precipitate, and a relatively pure albumin can be obtained from the supernatant[32]. Albumin binds even such non-ionic compounds as testosterone[33]. This indicates that the lipophilic and non-ionic parts of the protein molecule are important in these interactions.

Albumin interacts also with other proteins. For example, at

pH 6 albumin has a negative charge but lysozyme is positive. Reversible association takes place between the albumin and lysozyme under such conditions. It has been shown that this association is decreased by increasing the ionic strengths of the solution[34].

The details of the shape and configuration of the albumin molecules are unknown. The ease with which the very diverse physiolog-

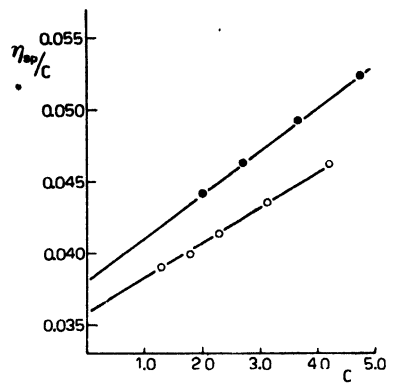

Fig. 117.

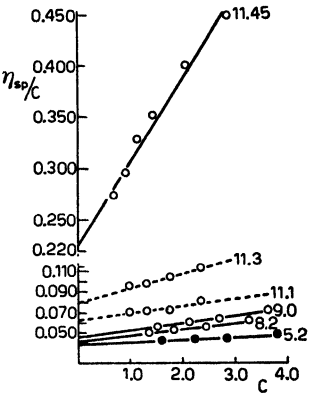

Fig. 118.

Fig. 117. The dependence of the reduced viscosity on concentration (c grams per 100 cc solution) of two albumins. Discs – crystallized human serum albumin in NaCl 0.15 M/l. T = 28.6°C. Open circles – cryst. bovine albumin in NaBr 0.25 M/l at the same temperature. (B. Jirgensons, *Makromol. Chem.*, 16 (1955) 192.)

Fig. 118. The viscosity of human serum albumin in alkaline solutions. The intrinsic viscosity values (intercepts on the ordinate) are low in weakly alkaline solutions, and they increase considerably at pH larger that 11.1. The slopes likewise become larger. (After B. Jirgensons, *Makromol. Chem.*, 18/19 (1956) 48.)

TABLE 81

PHYSICOCHEMICAL CONSTANTS OF ALBUMIN

(in solutions of low ionic strength, and pH of 4.5–9).

Molecular weight	67,000–70,000
Sedimentation constant s_{20} in S	4.1—4.6
Partial specific volume	0.73
Intrinsic viscosity	0.033–0.042
Refractive index increment	$1.86 \cdot 10^{-4}$
Diffusion constant D_{20}	$6.0 \cdot 10^{-7}$
Optical rotation at pH 5 $[\alpha]^{25}_{5461}$	—78°
Optical rotation at pH 5 $[\alpha]^{25}_{D}$	—63°
Optical rotation at pH 9 $[\alpha]^{25}_{D}$	—59°

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ical interactions take place suggests that the molecules must have a certain degree of flexibility[35]. This is substantiated also by the viscosity data, i.e. the increase of the reduced specific viscosity with concentration[36]. It is also plausible that the various components of the microheterogeneous albumin have somewhat different molecular shapes. In Table 81 are compiled some of the most important constants of serum albumin (see also Fig. 117 and 118).

6. Examples of Globulins

Many globulins are found in blood plasma. According to their electrophoretic mobility they are denoted as α, β, and γ globulins, and several components have been found in each of the mentioned groups. Some of the globulins of blood plasma are soluble in water. They are called pseudoglobulins. Those insoluble in water are called euglobulins (the 'true' globulins). They are soluble in plasma because of the electrolytes of the plasma. The molecular weight of most of the serum globulins is higher than that of the albumins. For example, the various γ globulins have molecular weights of 120,000–180,000. Some of the lipid-containing globulins are even larger particles. Data on these substances are presented in Table 82[37].

TABLE 82

THE GLOBULINS OF SERUM

	Sediment. constant s_{20}	*Frictional ratio* f/f_0	*Mol. weight*	*Intrinsic viscosity*
α_1 Globulin	5 0	1.38	200,000	0 066
α_2 Globulin	9	1.58	(300,000)	0 092
β_1 Glob. (metal binding)	5 5	1.37	90,000	0.055
β_1 Glob. (lipoprotein)	20		10^6	
γ Globulin I	7.2	1.38	156,000	0 060

The globulins differ from the albumins in their amino acid composition, and they differ also among themselves. The α and β globulins contain either lipids or carbohydrates. The γ globulins of plasma have a somewhat lower sulfur content than albumin has. The hydroxyamino acid content of the γ globulins is higher than that of albumin. It has been shown by means of the modern methods of separation that none of the globulins mentioned in

Table 82 are homogeneous. For example, γ globulin has been separated in several subfractions which have mobilities between $-1.25 \cdot 10^{-5}$ and $2.25 \cdot 10^{-5}$ at pH 8.7, and isoelectric points between 5.7 and 7.3[38].

Globulins, as well as albumins, are found in various tissues, in egg white, in milk, in saps of plants, and especially in seeds. The seed globulins are insoluble in water, but they are soluble in dilute salt solutions or dilute alkalies. Some of their physicochemical constants are compiled in Table 83[39]. Some seed globulin molecules are composed of subunits. The globulin of peanut has a molecular weight of 330,000. These molecules dissociate into halves of greater asymmetry depending on pH and on the salt concentration[40]. Similar reversible dissociation phenomena have been observed with many other plant globulins.

TABLE 83

EXAMPLES OF PLANT GLOBULINS

	Sedimentation constant s_{20}	*Diffusion constant* D_{20}	*Molecular weight*
Legumin, pea	12.6	$3.94 \cdot 10^{-7}$	330,000
Vicilin, pea	8.1	4.26	180,000
Arachin, peanut	14.6	3.86	330,000
Excelsin, Brazil nut	13.3	4.26	295,000
α Globulin, barley	2.5	7.4	29,000
β Globulin, barley	6.2	4.9	110,000
Gliadin, wheat	2.1	6.7	28,000
Zein, corn	1.9	4.0	51,000

Crystallized β lactoglobulin (from milk) is one of the most thoroughly investigated globulins. The protein appears homogeneous in sedimentation, but it is inhomogeneous on electrophoresis[41]. Components which have different solubilities were isolated by fractional crystallization of this globulin[42]. Moreover, it has been shown that several preparations have different chemical compositions. Differences in chemical and physicochemical constants were also found in other maximally purified globulins[43].

7. Hemoglobins and other Respiratory Proteins

Hemoglobin is red, as the molecules absorb certain waves of visible light. This absorption occurs in a part of the molecule which is called

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hem. This is a relatively small part of the whole large molecule, a ferroporphyrin complex of quite well known structure. It is combined to the large protein part—globin. The hem portion is the important part in the respiratory functions, as the binding of oxygen occurs at the iron atom of the hem. The spatial arrangement of both components (hem and globin) seems to be responsible for the fact that hem combines only with globin and not with any other protein.

Hemoglobin has been prepared in a crystalline state, and its properties have been studied extensively. The molecular weight of hemoglobin is 67,000–68,000, and the molecule is built up of four subunits, each having one hem with one iron atom. The hems probably lie flat on the surface of the large globin component, as they are all easily accessible to oxygen.

The hemoglobins of various species differ in their crystalline shape, solubility, electrophoretic mobility, and in amino acid composition, although the differences are not very great. Differences have also been found between human foetal and adult hemoglobins (Hb). Adult oxyhemoglobin (HbO), carbonylhemoglobin (HbCO), and methemoglobin (an irreversibly oxidized Hb) all crystallize in isomorphous orthorhombic crystals, whereas the corresponding foetal hemoglobins are monoclinic. There are also differences in electrophoretic mobility. Moreover, foetal hemoglobins are more stable against denaturation by alkali than are adult hemoglobins. Several types of electrophoretically abnormal hemoglobins are found in the blood in several diseases (Chap. 31).

Bragg and Perutz and associates studied single crystals of hemoglobin by means of X-ray diffraction[44]. According to these studies, a molecule of hemoglobin is of ellipsoidal shape, and it has the dimensions of 60 · 40 · 40 A when dry. Hydrated molecules have the dimensions of (65–70) · 55 · 55 A, and the volume is 115,000 A^3. There is also experimental evidence that the polypeptide chains are coiled into cylindrical rods possessing the α-helix configuration[45]. Noteworthy are the studies on concentrated hemoglobin solutions by means of low angle X-ray scattering. Riley and Herbert used this method, and they concluded that the molecules of human hemoglobin are flat cylinders or spheres[46]. The hydrate layer was estimated to be about 3 A thick.

Many other proteins resembling hemoglobin are known. Myoglobin, the pigment of red muscles, contains the same porphyrin group (hem) as hemoglobin, but it differs in the globin component.

The molecular weight of myoglobin is only 17,000. Some species of lower animals have respiratory proteins which do not contain the porphyrin group, but the oxygen-binding iron is attached to the protein in a different way[47]. Other species of lower animals (crustaceans, mollusks) have respiratory proteins which contain copper instead of iron. The copper is probably in the cuprous state, and blue compounds are formed upon combination with oxygen. The hemocyanins of lobster and crayfish contain 0.17% copper, those of snail (*Helix pomatia*) 0.25%. The molecular weight of these hemocyanins is very high, and the particles are composed of subunits, as reversible dissociation has been observed[7].

8. Examples of Enzymes

Enzymes are catalytically active proteins. Most enzymes contain a non-protein component in their molecules. The non-protein component or prosthetic group in the enzymes is very small in comparison with the protein moiety. Despite its smaller size, the prosthetic group or coenzyme is the physiologically active part of the enzyme molecule. The function of the protein component, which is called an apoenzyme, is the formation of suitable spatial conditions for the attachment of the substrate, i.e. the molecules to be activated by the enzyme. The catalytic activities of the enzymes are very specific, i.e. a particular enzyme, e.g. pepsin, is able to promote only a definite kind of reaction, such as hydrolytic cleavage of other proteins. Neither coenzyme nor apoenzyme separately are able to catalyze reactions. They must be linked as a whole enzyme or holoenzyme. Some enzymes contain prosthetic groups (coenzymes) with metallic atoms (iron, copper, zinc, manganese) as the active centers. Many enzymes now have been prepared in a highly purified crystalline state, and they have been studied thoroughly by chemical, physical, and biochemical methods[48].

The number of enzymes is very large, since practically all physiological reactions are catalyzed by specific enzymes. Many enzymes have not yet been isolated, but their activities are known through studies on the reactions they promote. Because of the importance of these reactions, the enzymes are classified according to their functions, rather than on their molecular weight and chemical composition. There are proteolytic (protein splitting) enzymes, such as pepsin, trypsin, chymotrypsin, papain; enzymes which catalyze oxidation-reduction reactions, others which facilitate hydrolytic

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decomposition of polysaccharides, or promote esterification, etc. Many enzymes do not possess a coenzyme component, and the function of the prosthetic group is exerted by some active groups of the protein, such as the sulfhydryl —SH, or a metal bound directly to the protein.

Pepsin is an example of a proteolytic enzyme. It was prepared in the crystalline state by repeatedly dissolving crude pepsin (from gastric mucosa) and precipitating it with acid or magnesium sulfate[48]. It was also shown that crystalline pepsin can be separated into fractions of different physiological activity. Pepsin dissolves in water easily, and the solutions are acidic. The acid reaction of pepsin is caused by a high content of the acidic amino acids, such as glutamic acid, aspartic acid, and tyrosine. The catalytic activity seems to be associated with the hydroxyls of tyrosine. The activity disappears when the hydroxyls are blocked by acetylation, and reappears upon saponification. The molecular weight of pepsin is 36,000 and the molecules are very stable in acidic solutions. The isoelectric point of this protein is about pH 2.5–3. Pepsin is formed in the tissues from another protein—pepsinogen, which has a somewhat higher molecular weight than pepsin. The conversion of pepsinogen into pepsin occurs autocatalytically in the presence of traces of pepsin.

Lysozyme is another example of a well investigated, crystallized enzyme[49]. Lysozyme occurs in egg white, tears, sputum, saliva, and in many tissues. It is a lytic enzyme, being able to promote the disintegration of the cell walls of micro-organisms. It acts on some polysaccharide components occurring in the membranes of bacteria. Lysozyme, like pepsin, does not possess a prosthetic group. In contrast to pepsin, it is a basic protein, and the molecules carry a positive net charge at the ordinary pH values of 3–10. The charge is reversed only at pH 11.8. The molecular weight of lysozyme is approximately 16,000. Analytical data indicate that the molecule contains 20 free basic groups and about 22 carboxyl groups not linked in peptide bonds; however, 18 of these carboxyls do not appear free but as amides. This gives an excess of 16 amino groups per molecule, which is in good agreement with titration data.

Many enzymes contain a coenzyme, and these coenzymes often are derivatives of the physiologically active substances known as vitamins. An example is the *yellow enzyme* which is composed of a protein of molecular weight 80,000 and of lactoflavin-phosphoric acid as the prosthetic group[50]. The coenzyme is linked to the

protein by phosphoric acid and the alloxazine NH of the coenzyme. Yellow enzyme is widely distributed in cells and tissues. Its function is to take up hydrogen from compounds rich in H, and to transfer it to atmospheric oxygen. The yellow enzyme itself is built up from riboflavin, which is first phosphorylated, and then linked to a protein.

Other examples of enzymes with prosthetic groups are: carboxylase, the dehydrogenases, and ferricytochrome. Carboxylase has the diphosphoric ester of vitamin B_1 as the prosthetic group. This enzyme converts α-ketonic acids into aldehydes and carbon dioxide. The molecular weight of this enzyme is 75,000. The dehydrogenases have functions similar to those of the yellow enzyme. The coenzyme is composed of several heterocyclic groups, a pentose, and pyrophosphoric acid. Ferricytochrome is an enzyme with a coenzyme component resembling the hem in hemoglobins[51] (see Sect. 3 in Chap. 25). A peroxidase which was isolated from horseradish contains a similar porphyrin coenzyme component; the apoenzyme in this case is a glycoprotein with a carbohydrate content of 20%, and with a molecular weight of 44,000. The catalases which promote decomposition of hydrogen peroxide are also porphyrin enzymes with tri-valent iron.

Extensive studies have been carried out on the kinetics of enzymatic reactions, and on the mechanism involved[52]. Chemical changes often are accompanied by colloid-chemical processes, such as disaggregation of particles of the enzymatically attacked biocolloids. Chemical derivatives of enzymes have been prepared, as in the case of pepsin, and the importance of various constitutive groups has thus been studied. The substrates, for example the proteins to be cleaved, also have been chemically modified and the influence of such modifications has been studied[53] (see Sect. 11).

9. Protein Hormones[54]

Insulin is one of the most important protein hormones. The chemical structure of the insulin molecule is now almost completely solved[55]. Recently acquired evidence indicates that the chemical molecular weight is 6000 (more accurately 5733), and that there are two polypeptide chains in the molecule. The chains are linked by two disulfide bridges. The A chain starts with glycine, which carries the free NH_2, followed by isoleucine, valine, and other residues, and it ends with aspartic acid. The B chain starts with phenyl-

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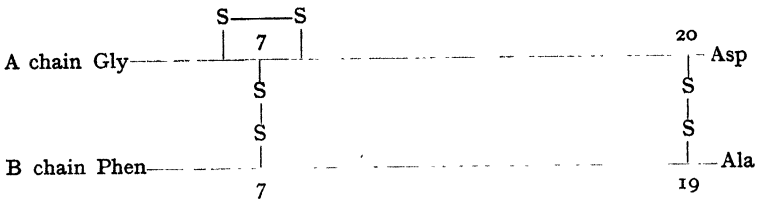
alanine and ends with alanine carrying the free end-carboxyl. The complete sequence of the residues is now almost completely established. The disulfide bridges link the 7th residue of the A chain to the 7th residue of the B chain, and the 20th residue of the A chain with the 19th residue of the B chain, as follows:



Lindley and Rollett[55] have proposed a model for the insulin molecule (see Fig. 119). The B chain is supposed to be a straight helix; the A chain is somewhat shorter and bent. The resulting molecule thus appears only a little asymmetric. The model is in accord with the chemical data of Sanger and Tuppy, and it seems to be consistent also with the physicochemical and crystallographic properties of the protein.

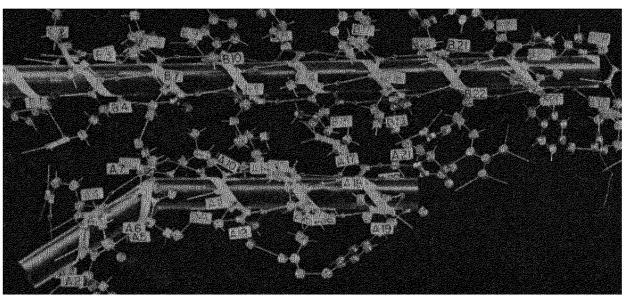


Fig. 119. Proposed model of the 6,000 molecular weight unit of insulin according to H. Lindley and J. S. Rollett (*Biochim. Biophys. Acta*, 18 (1955) 183).

In its solubility properties insulin resembles the globulins. It is practically insoluble at its isoelectric point, which is 5.3. Insulin is soluble in alkali, acid, and in some salt solutions. The reversible aggregation phenomena observed with insulin are very interesting.

Insulin aggregates when heated in acidic solutions at pH 2. A birefringent thixotropic gel is formed upon cooling, and the presence of long insulin fibrils could be shown in these gels in the electron microscope[56]. These fibrils are about 16,000 A long and only 140 A thick. They are formed by linear aggregation of the originally nearly isometric molecules. and by lateral association of these long strings. Treatment with dilute alkalies cleaves the fibers, the viscosity of the colloid decreases, and the insulin even regains its physiological activity.

Some of the hormones produced by the pituitary gland are proteins of moderate molecular size. The *lactogenic hormone* prolactin, and the *growth hormone* belong to this group. Prolactin stimulates the mammary gland and corpus luteum function. The molecular weight of prolactin is 33,000, and its isoelectric point is at pH 5.7. The substance is remarkably stable against moderate heating, acids, and organic solvents. The growth hormone has received much attention recently, because of its importance in the growth of neoplastic tumors of the liver[57]. This hormone was isolated in a pure state by Li and Pedersen[58]. It has a molecular weight of 50,000 in acidic solution at pH 2.3, and 45,000 at pH 9.9. In more strongly alkaline solutions the molecules are split, and a molecular weight of 29,000 was found at pH 11.5. The growth hormone is practically insoluble in water; its isoelectric point is near neutrality. The hormone is composed of amino acids only, which are arranged in two chains. The terminal residues with $-NH_2$ groups are alanine and phenylalanine[59].

10. Denaturation of Proteins

Definition. Description of the phenomena

Denaturation is defined as an increase in disorder in the configuration of the macromolecules of the native proteins. Any treatment of a protein, such as heating, exposing to radiations, treatment with chemicals, or even intense shaking of solutions may cause denaturation. The occurrence of denaturation is manifested by changes in some properties of the protein observed. The changes occurring on boiling an egg are characteristic of this process. The originally globular molecules of egg albumin collide under the strong molecular impacts on boiling, and stick together, as they become partly uncoiled in these collisions. The mesh of the entangled spirals also encompasses the liquid, and the egg white finally sets.

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In dilute solutions of albumin such jellies cannot be formed, because of the lack of solid material, and precipitation then occurs.

It must be pointed out that a decrease in solubility and precipitation do not necessarily involve denaturation. Proteins are precipitated with ammonium sulfate without being denatured. Albumin can be precipitated even with alcohol without denaturation, if the precipitation is carried out at a sufficiently low temperature. *Precipitation and denaturation are two different processes*, and only in some cases can denaturation be judged by estimating the turbidity or amount of precipitate. In many cases proteins are denatured in solutions without being precipitated. For example, many globulins are denatured upon mixing the solutions with guanidine salts, but neither precipitation nor aggregation occurs. Some proteins even disaggregate, when dissolved in concentrated urea or guanidine salt solutions. For this reason, the solubility criterion generally is not a proper measure for the estimation of denaturation. Several other criteria must be applied. It can be expected that many properties of the protein, such as viscosity, reactivity, biological specificity, and optical rotation, should change upon distortion of the globular molecules. The viscosity must increase, if globular, relatively compact molecules are transformed into expanded, fibrous entities. The increase in viscosity was indeed observed in many instances of various denatured proteins[60]. The reactivity of proteins also increases upon denaturation[60], and various chemical methods have been used to estimate this increase in reactivity. In many cases, the sulfhydryl groups (—SH) of proteins are more reactive after denaturation. Since these groups are easily detectable by means of various oxidizing agents, this method of estimating denaturation is applied very often. Denatured proteins are more easily attacked by proteolytic enzymes than are native proteins. When the protein under study is itself an enzyme or hormone, its biological activity decreases upon denaturation, if the native configuration is important for activity.

It is interesting that enzymes may or may not lose their biological activity upon denaturation by spreading on surfaces. Crystalline catalase, if spread as a monomolecular film at the air-water interface, retains its enzymatic activity[61]. The film can be compressed into a fiber which is insoluble, whereas the native, crystalline enzyme is soluble. The insoluble enzyme, however, is active, although somewhat less than the native enzyme. This example shows that considerable alteration in physicochemical properties

may not necessarily involve loss of biological activity. Pepsin, on the contrary, is inactivated after spreading on surfaces[62].

Optical rotation proved to be one of the most generally applicable methods for the estimation of denaturation[63]. The method is simple and relatively sensitive. All proteins are optically active, and they are levorotatory. The levorotation increases upon denaturation (Table 84, Fig. 120). This is a consequence of distortion of the original configuration.

TABLE 84

THE INCREASE IN VISCOSITY AND LEVOROTATION UPON DENATURATION OF SOME PROTEINS

	Specific rotation—$[\alpha]_D$ *degrees*		*Reduced viscosity of 1% solutions*	
	Native	Denatured	Native	Denatured
Albumin, serum	58–64	70–108	0.043	0.1–0.23
γ Globulin	45–53	60–100	0.06	0.1–0.2
Bence-Jones protein	42–49	53–98	0.045	0.08–0.16
Lysozyme	46–50	63–84	0.031	0.06
Pepsin	58–64	77	0.050	0.1
Arachin	41	48–74	0.047	0.06–0.158
Amandin	45	51–72	0.054	0.08–0.15
Legumin	42	49–92	0.030	0.08–0.39

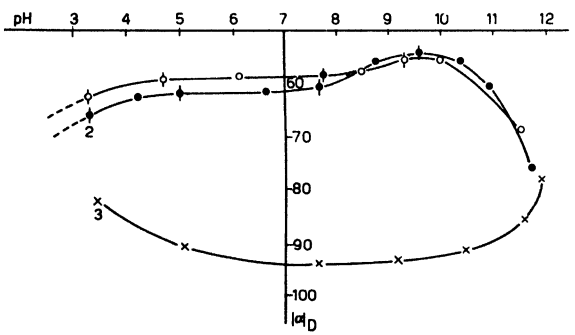

Fig. 120. The optical rotation of native and denatured (lower curve) human serum albumin at various pH. The levorotation of native albumin increases in strongly acidic or strongly alkaline solutions. The levorotation of the denatured albumin shows a maximum in neutral solutions, and the levorotation of the acidic or alkaline solutions has smaller numerical values.

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If the protein is chemically changed under very mild conditions, in which the original configuration of the molecules is preserved, such alterations do not involve denaturation. For example, if a protein reacts with a dye without losing its native configuration, it is not denatured. Likewise, it is not denatured if the amino groups are acetylated without distorting the macromolecules. Hydrolytic cleavage is not involved in denaturation. If the peptide bonds are split by some drastic treatment, the change is called degradation or decomposition.

Reversibility of denaturation

It was found that some enzymes are denatured reversibly, if spread on surfaces or heated. The criterion of reversibility in these cases was the restoration of the enzymatic activity. Denaturation of ferrihemoglobin with acid is another reversible process[64]. In this instance the criteria of denaturation have been absorption of light, solubility at the isoelectric point, and acid-base binding capacity. Serum albumin seems to be reversibly denatured by urea or guanidine salts. The optical rotation and viscosity increase considerably in the presence of these agents, and they return to normal (almost completely) after removal of the denaturants by dialysis.

The reversibility of denaturation, however, is a disputable phenomenon. It has not been established that the reversibility described by various authors could be proven if more criteria were applied. It is obvious that the distorted chains will be able to return to the original configuration only if the degree of distortion is small. The reversible dissociations into subunits, as in the case of hemocyanins, do not involve denaturation.

The mechanism of denaturation

Denaturation seems to be a very diverse phenomenon, and the idea has often been put forward that the mechanism of denaturation may differ from case to case. This diversity, however, is much reduced, if the actual denaturation is distinguished from other processes which often accompany denaturation (such as precipitation, dissolution, gelation). If denaturation is defined as an increase in disorder or as distortion of the original configuration, the essential mechanism is always the same. It will involve disruption of hydrogen bonds and other secondary linkages of the polypeptide chains. In addition to this, in some cases the sulfur bridges may be disrupted. As a consequence of these disruptions of the bonds

which hold the helices in the original configuration, the macromolecules will uncoil; they will become more disordered than the native molecules. Some residues, such as the —SH, originally hidden in the interior of the large molecule now become accessible. All this leads to an increase in reactivity and viscosity.

Important data on the mechanism of denaturation has been obtained by means of X-ray analyses of native and denatured proteins. According to Astbury[65], the characteristic X-ray diffraction patterns of the native proteins are completely changed upon denaturation. The spectrum becomes nearly identical with that of β-keratin or stretched wool, i.e. of that of the linear proteins (Chap. 27). This indicates that the macromolecules of the spheroproteins become uncoiled upon denaturation.

The increase in viscosity upon denaturation, however, is not always due to a complete unfolding of the globular molecules. The example of insulin shows that linear aggregation of slightly distorted globular molecules may produce long fibrils, which is reflected in a tremendous increase in viscosity. Barbu and Joly[66] have presented several more examples of this kind. The initial denaturation these authors call 'activation'. They showed that such 'activated' or denatured molecules of several albumins and globulins aggregate and form fibrils under specific conditions[67].

Other interpretations are plausible in other cases in which viscosity increases upon denaturation. Doty and Katz studied serum albumin denaturation by means of light scattering[68]. Concentrated urea was used as the denaturing reagent. Their work led to the conclusion that serum albumin molecules do not unfold in concentrated urea but only swell. Riley and Arndt came to the same conclusion regarding the heat denaturation of serum albumin[69]. They investigated the system by means of X-ray scattering both in solid state and in concentrated solutions. The results indicated that the original configuration was but partially distorted on denaturation.

The mechanism of denaturation may be somewhat different in surface denaturation of proteins. When a solution of egg albumin is shaken, fibrous particles are formed in the solution. This is due to denaturation at the water-air interfaces followed by aggregation. Denaturation occurs also if solutions of spheroproteins are spread on surfaces. Monolayers of the proteins are formed on the surfaces under suitable conditions. The pressure exerted by the spreading layer can be measured by means of the surface film balances

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(Chap. 6), and the thickness of the protein film can be determined. These studies have revealed that the thickness of protein monolayers often is less than the thickness of the original native molecules. It seems then that the layers are composed either of unfolded sheets of polypeptide chains, or that they represent original flat subunits, which glide apart upon denaturation[70].

A protein can be denatured to various degrees[71]. When γ globulin is denatured at room temperature with 1.5 M/l guanidine hydrochloride, the optical rotation increases gradually, and then becomes constant. If the temperature then is raised to 50° C, the optical activity increases sharply, and again becomes constant (see Fig. 121).

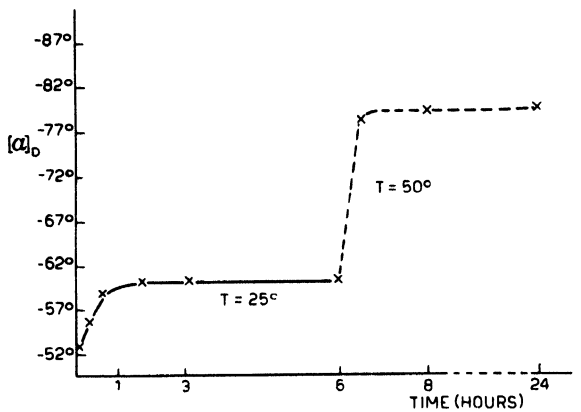

Fig. 121. The change of optical rotation with time in the denaturation of bovine γ globulin with guanidine hydrochloride (1.5 M/l) at 25°C and at 50°C; pH = 5.1. (B. Jirgensons, *Arch. Biochem. Biophys.*, 41 (1952) 333)

Two different explanations can be offered for this phenomenon. The first is that under mild denaturation (room temperature) only a part of the native globulin molecules is affected, whereas the rest remain unaffected. Another explanation would be that all molecules are affected, and that denaturation occurs in stages. The first is the "all or none" hypothesis, which is favored by some protein chemists. The fact that proteins appear to be composed of several components, which may have different stabilities toward a denaturing agent is sometimes mentioned in support of this hypothesis. Although γ globulin is composed of numerous components, they do not differ much in molecular structure and configuration, and the possibility of a step-wise denaturation seems more likely.

According to Kauzmann[72], who observed similar stages in the denaturation of ovalbumin with urea, the stages are due to more or less complete unfolding, as illustrated in Fig. 122 and 123.

Evidence on the abrupt character of denaturation of some proteins has been presented recently by Tsyperovich and Loseva[73].

Proteins can be classified into several groups in their resistance to denaturation. The proteins which have a relatively low molecular weight, such as ribonuclease, insulin, and lysozyme, are very resistant. The albumins are less stable, and most globulins are less stable than the albumins. Generally the stability decreases with increasing molecular weight and complexity of the proteins, but exceptions also are known. The field thus far is very little explored, and it is obvious that thorough studies on these denaturation phenomena may contribute to the problem of protein structure and configuration.

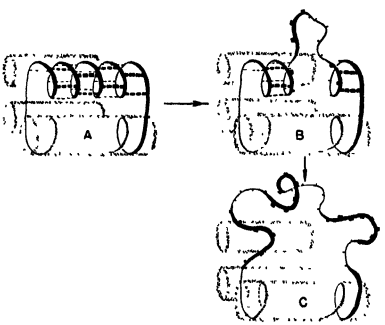

Fig. 122.

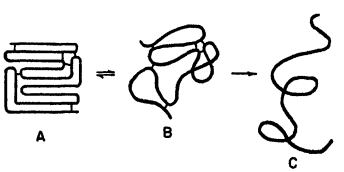

Fig. 123.

Fig. 122. Gradual unfolding of a globular protein according to W. Kauzmann A Native protein; B initial stage of unfolding; C partially denatured protein. (From *The Mechanism of Enzyme Action*, W. D. McElroy and B. Glass, Editors, The Johns Hopkins Press, Baltimore, 1954.)

Fig. 123. Partial and complete denaturation of a globular protein according to W. Kauzmann. (From *The Mechanism of Enzyme Action*, W. D. McElroy and B. Glass, Editors, Johns Hopkins Press, Baltimore, 1954.)

Interesting considerations on the importance of various bonds in denaturation and stability, chiefly on the basis of energetics, have been put forward by several authors[74].

Fibers from denatured globular proteins

Conversion of globular proteins into fibrous material is desired in manufacturing artificial protein fibers. Casein of milk, zein of

corn, soybean and peanut proteins are used as starting materials. The process involves solution of the starting material in suitable solvents, whereby the proteins are denatured to some extent. The concentrated viscous solutions then are treated further in various ways, and fibers are extruded by the same methods as in the manufacture of rayon. After extrusion, the fibers are hardened in order to improve their mechanical properties[75]. A consideration of the relationship which exists between the strength of the fibers and chain length of the protein material has shown that better fibers can be prepared from long linear molecules. No suitable fibers can be produced from proteins which have molecular weights below 10,000, such as salmine. The denatured proteins used for the fibers usually have molecular weights of 12,000–60,000. It seems that the mechanical properties are not appreciably improved if the molecular weight is raised over 24,000. Attempts have been made to improve the fibers by cross-linking them by means of certain reagents[76]. The denaturation involved in the transformation of the globular molecules need not necessarily lead to complete unfolding of the globular macromolecules.

11. Chemical Modification of Proteins[77]

Chemical modification of proteins usually leads to denaturation. Extreme precautions must be taken if chemical derivatives are to be prepared and denaturation is to be avoided. For example, deamination of proteins cannot be accomplished completely if the original configuration of the macromolecules must be preserved. The deamination with nitrous acid is expected to proceed as follows:

$$\text{Protein}(NH_2)_n + n HNO_2 = \text{Protein}(OH)_n + n N_2 + n H_2O.$$

Complete deamination can be achieved only when proteins are treated in this way in weakly acidic solutions at room temperature. However, proteins are denatured under such treatment[78]. It has been shown[79] that under milder conditions, when the original configuration is preserved, only incomplete deamination can be achieved. Moreover, the reaction appears to proceed not only as indicated in the equation, but nitrous acid reacts also with other groups of the protein, such as the sulfhydryls, and the tyrosine radicals.

The amino groups and sulfhydryls react readily with many other reagents. The amino groups can be acetylated, methylated, or

similarly modified. The reaction with fluorodinitrobenzene was described in Section 3. The sulfhydryl or thiol groups (—SH) react with oxidizing agents, such as iodine, nitrous acid, and ferricyanide. It can be shown that iodine reacts specifically with —SH when the solution is weakly acidic, when the temperature is low, and when iodides are present in excess. Moreover, an excess of iodine should be avoided. Excess iodine, especially at higher temperatures, iodinates the aromatic radicals of the protein.

Active acylating agents, such as ketene, react with all active hydrogens of proteins, such as H in amino groups, thiols, and hydroxyls. Epoxides react with these, and also with the carboxyls of proteins. Aromatic isocyanates react with amino and thiol groups.

The coupling of proteins with diazonium compounds is important for immunological studies (see Chap. 30). Direct diazotization of several proteins has also been accomplished. It was shown that only half of the tyrosine residues of pepsin can be diazotized, and that pepsin thereby loses about one half of its original activity.

Reduction of proteins is accomplished by various means, whereby either the —S—S— bridges of cystine, or the carboxyls are affected. The reduction of the disulfide bridges is achieved by means of cysteine, glutathione, thioglycolic acid, and similar reducing agents. Partial denaturation is associated with such reductions, since the —S—S— bridges often participate in linking the polypeptide chains. Biologically active proteins, e.g. enzymes, usually lose their activity upon such treatment. It is also difficult to restore the original molecule by oxidation, as there is little chance of making the separated sulfur atoms meet each other again. Moreover, the general configuration of the macromolecule probably is damaged by both reduction and following oxidation. The reduction of carboxyls, according to Fromageot[80], can be accomplished by dissolving a protein in ethylmorpholine, and treating the solution with $LiAlH_4$ in a nitrogen atmosphere. This reaction appeared to be important in determining the carboxyl end groups in the peptide chains of proteins.

Apart from denaturation, the colloid chemical properties of proteins are always affected if something happens to the ionogenic groups of the proteins. Thus proteins become more acidic upon deamination; their isoelectric point is shifted to the acid side. However, if only deamination has occurred, and the protein is still native, the other colloid-chemical properties, such as viscosity, do not change appreciably.

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A very promising new approach in the synthesis of protein derivatives is the method of Becker and Stahmann[81] who showed that N-carboxyamino acid anhydrides react with proteins. The feaction occurs in aqueous solution at pH 7.4 according to the rollowing scheme:

1)
$$\text{Protein—NH}_2 + \begin{array}{l}\text{NH—CH(R)—CO}\\ \text{CO}\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\text{O}\end{array} =$$
$$= \text{Protein—NH—CO—CH(R)—NH—COOH}$$
N-carboxyamino ac. anh.

(2)
$$\text{Protein—NH—CO—CH(R)—NH—COOH} =$$
$$= \text{Protein—NH—CO—CH(R)—NH}_2 + \text{CO}_2;$$

(3)
$$\text{Protein—NH—CO—CH(R)—NH}_2 + n \begin{array}{l}\text{NH—CH(R)—CO}\\ \text{CO}\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\text{O}\end{array} \longrightarrow$$
$$\text{Protein—NH—[—CO—CH(R)—NH—]}_n\text{—CO—CH(R)—NH}_2 + n\text{CO}_2 .$$

Polyamino acid chains are "grafted" at each free amino group of the protein in these reactions. Crystalline bovine albumin and chymotrypsin were treated with N-carboxyglycine anhydride. Amino acid analyses showed that 171 moles of glycine were attached to one mole of albumin, and 65 moles per mole of chymotrypsin. Most remarkable was the fact that the enzymatic activity of chymotrypsin was not impaired by this treatment, and that the antigenic properties of the albumin were practically unchanged.

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CHAPTER 27

Fibrous Proteins

The study of fibrous proteins is even more difficult than the study of globular proteins. This is due to the insolubility of many fibrous proteins, and to the complex composition of the tissues from which they must be isolated. The fibrous structural tissues of the animal body, as indicated in previous chapters, may contain other fibrous structural materials, such as polysaccharides (hyaluronic acid, chondroitin sulfuric acid, chitin) together with the proteins. Most of the fibrous proteins are insoluble in water, neutral salt solutions, and similar mild reagents. Drastic methods of extraction will cause degradation. In spite of these difficulties, great progress has been made in the study of fibrous proteins in recent decades.

The classification of fibrous proteins is equally difficult. The insoluble *keratins* (wool, hair, nails, horn) actually are space polymers of indefinite molecular weight. Chemical similarity in amino acid composition and similarity in structure as revealed by X-ray analyses, however, indicate close relationship between the visibly fibrous wool and the bulky material of horn. All keratins have in common a high content of the sulfur amino acid cystine. *Silk* differs strikingly from the keratins in that it does not contain any cystine. Another important group of structural proteins comprise the *collagens* with their characteristic high proline and hydroxyproline contents and specific X-ray diffraction patterns. *Myosin* of muscle, and *fibrinogen* of blood, as well as the mucoproteins, represent three different types of soluble fibrous proteins.

According to Astbury[1], fibrous proteins can be classified into two large groups: (1) the keratin-myosin-epidermin-fibrinogen

(k-m-e-f) group and (2) the collagen group. This classification is based on structural analysis by means of X-rays. The first group can be subdivided further into the α- and β-keratins, depending on the configuration of the chains, as revealed by analysis of the X-ray diffraction patterns. It must be emphasized that these structural similarities do not necessarily mean that the proteins have the same chemical composition and solubility properties, hence this classification according to X-ray patterns is of limited practical importance.

In this chapter attention will be focused chiefly on the colloid-chemical properties of the fibrous proteins: swelling and solubility, size, shape and interactions of the macromolecules. The colloid chemistry of these systems is of considerable interest for such industrial applications as the manufacturing of gelatin and glue, tanning of hide, and wool and silk technology. Colloid-chemical data are of interest also in the histochemistry of tissues in connection with various problems of physiology and pathology.

1. Silk[2]

Natural silk is an extracellular fiber produced by the silk worm (*Bombyx mori*). Examination of the fiber shows that it is composed of an inner core—the silk fibroin—and an outer coating called sericin. The latter is an ill-defined adhesive protein material which can be removed by boiling the fiber in a soap solution. The remaining pure silk fibroin is the chief ingredient of the fiber, which determines the mechanical and optical properties of the material. Chemical analysis of the products of hydrolysis shows that silk fibroin is composed chiefly of glycine (44%) and alanine (30%), and contains no sulfur. Considerable amounts of serine (16%) and tyrosine (13%) also have been found[3], whereas the content of the other amino acids is low. Structural analysis by means of X-ray diffraction has revealed that the fibers are composed of crystalline and amorphous regions. The oriented polypeptide chains of the crystalline sections are composed chiefly of glycine, alanine and serine residues. The repeating distance along the chain is 6.95 A, comprising two amino acid residues, most probably glycine-alanine or glycine-serine. Other dimensions of the unit cell are about 9.5 A and 10–12 A[4]. The distance of 6.95 A indicates that the peptide chains are almost completely stretched in the fiber, since a completely stretched chain has a repeating unit[5] of 7.27 A. The macromolecular chains are

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mutually interlinked by hydrogen bonds. The crystallinity and mechanical properties of the fiber indicate that the macromolecular chains must be very smooth and of a regular structure, hence the amino acid residues with bulky side groups (such as tyrosine, phenylalanine, glutamic acid) must be in the amorphous part of the fibroin (see Fig. 124–126).

Silk swells to only a small extent in water, and it is insoluble in dilute alkalies and acids. Hydrolytic degradation occurs upon heating with strong acids or alkalies. In concentrated formic acid the fiber swells and contracts. This contracted silk exhibits a rubber-like elasticity and is amorphous. Concentrated solutions of some inorganic salts are able to dissolve silk; for example, the protein

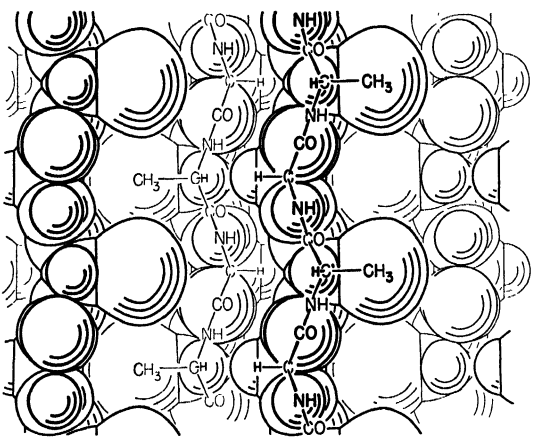

Fig. 124. Polypeptide chains in silk fibroin according to R. B. Corey and L. Pauling (Convegno Antonio Baselli: le macromolecole dei viventi, Istituto Lombardo d scienze e lettere, Milano, *Rend. Sci.*, 89 (1955) 10).

dissolves in lithium bromide[6], as well as in some concentrated thiocyanates[7].

Silk fibroin forms soluble complex compounds with cupric ethylenediamine[8]. The complex is very unstable, as all copper can be removed by dialysis. The protein remains in a colloidal solution which, if sufficiently concentrated, sets to a gel. The protein is incompletely precipitated on acidifying the fresh solution. The molecular weight of the protein in such solutions was found to be 33,000. However, this is not sufficient evidence that this is the

molecular weight of native silk fibroin, since hydrolytic cleavage probably occurs on solution in the alkaline cupric ethylenediamine hydroxide. The question as to whether the polypeptide chains in natural silk fibroin are of indefinite length or whether they are composed of macromolecules of a certain size is not settled. Long spacings have been detected by X-ray analysis[9]. The data, however, cannot be unequivocally interpreted as yet. Electron microscopy has not revealed a definite periodicity in the structure of the fibrils. However, electron microscopy has shown that silk fiber, partly digested by trypsin, is composed of rather distinct microfibrils about 100 A in diameter[10].

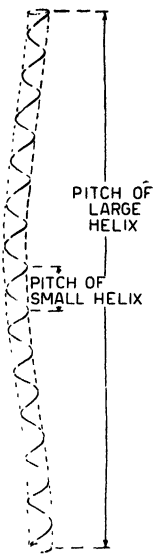

Fig. 125.

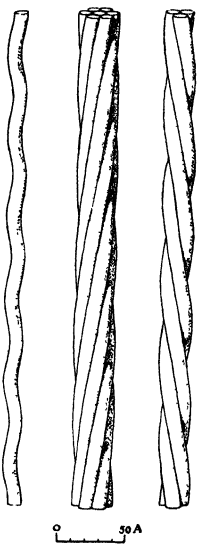

Fig. 126.

Fig. 125. A compound helix of a fibrous protein according to L. Pauling and R. B. Corey.

Fig. 126. Submicroscopic structure of fibrous proteins. Six compound α-helices (see Fig. 125) twisted around a seventh straight α-helix to form a seven-strand cable (Courtesy Professor L. Pauling and Dr. R. B. Corey.)

Silk, as an ampholyte, is able to bind a certain amount of alkali and acid. It was found that one equivalent of acid unites with 4000 g of silk[4]. Silk exhibits an affinity for acid dyes, this affinity being due to the presence of basic groups in the amorphous part of fibroin and sericin.

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2. Wool and other Keratins[a]

The keratins of wool, hair, nail, horn, and feather are proteins of high sulfur content, cystine being the major sulfur amino acid. Between 7 and 14% of cystine has been found in various keratins[11]. The keratins are rich also in glutamic and aspartic acids, arginine, proline, serine, threonine, and leucine; they contain very little or no glycine or alanine residues. The methionine, histidine and tryptophan contents also are very low. The beaks and claws of birds, shells and scales of reptiles and other animals are composed of keratinous material of a similar composition.

The histology of all these materials is very complex. Mammalian hair, including wool, is composed of three concentric zones. The surface layer is called cuticle, the next inside layer is called the cortex, and the core, the medulla. It is possible that the keratins of the various zones differ somewhat, although nothing definite is known at present. The cuticle of wool has a scale structure.

Most of the information on the structure of keratins was obtained by means of X-ray diffraction studies, wool and hair being the objects most thoroughly investigated. Wool fiber in the natural state exhibits a characteristic diffraction pattern, known as the α-keratin structure (Fig. 127). If hair or wool is allowed to soften in hot water or dilute alkali, it can be stretched considerably, and the stretched fiber exhibits another X-ray diffraction pattern, known as the β-keratin (Fig. 128). In unstretched hair the arc on the meridian corresponds to a spacing[12] of 3.3 A. Generally, the β-keratin or stretched hair pattern resembles that of silk. The other spacings found in the stretched hair diagram are 4.5 A and 9.8 A respectively. The spacing of 3.3 A was interpreted by Astbury[12] as the repeating distance along the fiber axis, i.e., the distance between peptide bonds; the spacing of 4.5 A was thought to be that of the peptide chains from each other, and the spacing 9.8 A as the distance of the planes in which the chains lie. According to Huggins[13], Pauling and Corey[14], the chains are turned into spirals (helices). The structure is stabilized by intramolecular hydrogen bonding between parts of the same chain, and also by intermolecular hydrogen bonding between the C=O.....H—N groups of adjacent chains. Moreover, the chains of the keratins are thought to be interlinked by sulfur bridges of cystine residues.

It was found in a study of the X-ray diffraction of various keratinous materials that some of them appear naturally as ex-

tended β-keratins. This is the feather keratin subgroup, including claws, shells and scales. All these materials exhibit spacings of 3.1 A, and the 3.3 A regular β-spacing can be obtained by stretching these keratins.

It is now generally recognized that the α-keratin structure must be one of coiled and cross-linked peptide chains, thus resembling the structure of weakly vulcanized rubber. In the β-configuration the chains are almost completely extended. A very remarkable discovery was that hair steamed for a short period in the stretched condition contracts after the removal of the stress to a length even shorter than the original. The heat treatment apparently removes some of the bonds securing the natural configuration, and

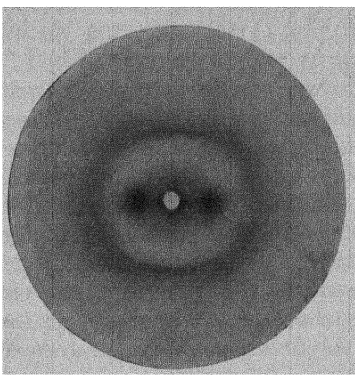

Fig. 127.

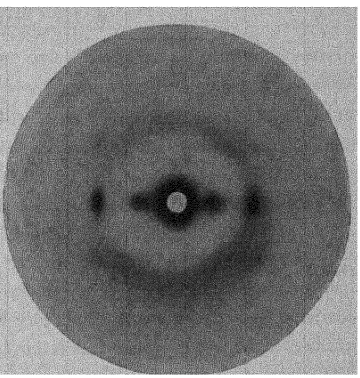

Fig. 128.

Fig. 127. X-ray diagram of α-keratin (unstretched Cotswold wool). CuK_α rays. Specimen-to-film distance = 4 cm. (Courtesy Professor W. T. Astbury.)
Fig. 128. X-ray diagram of β-keratin (Cotswold wool strectched by 80%). CuK_α rays. Specimen-to-film distance = 4 cm. (Courtesy Professor W. T. Astbury.)

the chains return to a more disordered state, in accordance with the second law of thermodynamics. A more drastic treatment of the extended fiber results in a loss of elasticity, i.e., the stretched keratin does not contract after removal of the tension (permanent set). This change, which is very important for handling wool fabrics and hair, is due to the formation of new cross-linking bonds of the heat-denatured structures[12]. "Cold permanent waving" of hair can be achieved by means of certain reducing agents, such as thioglycolic acid HS—CH_2—COOH or its salts[15]. Hair which is swollen in solutions of these reducing agents exhibits a rubber-like behavior.

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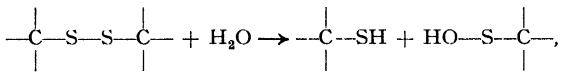
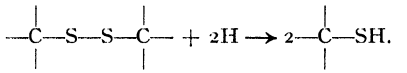
On longer treatment, a permanent set is achieved, as in the heat treatment.

These phenomena have been explained more or less completely by the assumption that the sulfur bridges R—S—S—R between the chains are cleaved under the influence of heat or the reducing agent[16]. The hot steam treatment probably causes hydrolysis:



while reduction may occur as follows:



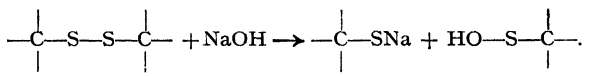
When the sulfur bridges interlinking the chain, and other linkages, are partly removed, the chains attain some freedom to slip along each other and become more flexible. Due to the fact that the —SH and —S—OH groups are very reactive, new linking will occur in the deformed fiber. Moreover, intra- and intermolecular bonding will occur through the formation of hydrogen bonds (possibly even new salt bridges between R—COO^- and R—NH_3^+, as well as new ester bridges between the carboxyls and hydroxyls of the hydroxyamino acids between chains). Consequently, a new stable structure (permanent set) of the fiber will result. In a short treatment, however, the original structure of the fiber is destroyed but the new links are not yet formed, hence the fiber contracts strongly on removal of the tension.

Although the structural changes can be studied by means of X-ray diffraction and infrared spectral analyses, these optical methods are unable to answer all questions. One of the greatest limitations of these methods is that only a relatively small part of the keratin of hair is highly oriented, the largest portion being amorphous. Hence the changes observed in the spectral patterns do not necessarily involve all changes occurring in the fiber during the treatment[17]. The study of the mechanical properties of keratin fibers under various conditions is of practical importance, although such studies provide limited information about the structure.

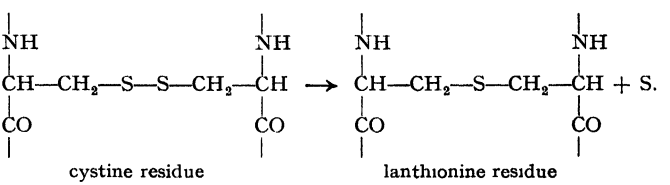
Extensive studies have been conducted on chemical modification, swelling, and solution of keratins. The swelling of hair or wool fiber is anisotropic, the fiber increasing only 1–2% in length but

35–40% in width. The uptake of the first portion of water is an exothermic process associated with orientation of the bound water (decrease in entropy). The uptake of further amounts of water (over 4%) is a less exothermic process, i.e., a very loose sorption without noticeable orientation[18]. Wool is able to bind more acid than silk, one equivalent of acid being bound by 1200 g of wool. This indicates that wool must have more free amino groups than silk, which is in accord with the data on the amino acid composition of these fibrous proteins. The alkali-binding capacity of wool is almost the same as the ability to bind acids. From these and other facts, Speakman concludes that the isoelectric point of wool must be[19] between 5 and 7. The limiting values of alkali bound to the macromolecules of wool, however, are difficult to determine, since wool is very sensitive to hydrolytic cleavage by alkali[20].

The minimum swelling of wool is observed in a medium with a pH of 5–7, corresponding to the assumed isoelectric point. The swelling is enhanced by increasing the acidity of the solution. The same effect occurs in alkaline solutions, which cause not only swelling but degradation, associated with a diminution in mechanical strength. For this reason, alkaline solutions are avoided in the modern treatment of wool textiles (washing, etc.). Neutral or weakly acidic synthetic detergents instead of alkaline soap solutions are used for washing the fibers and fabrics. It is not known with certainty which bonds are attacked and cleaved by the partial degradation with alkali. It is possible that some of the peptide bonds, due to the peculiar spatial configuration of the chains, are weaker than others. The same may be true with respect to the cystine bridges interlinking the chains[21]. Dilute alkali is able to hydrolyze the most labile bridges according to the equation:



Moreover, it can be shown that alkali causes elimination of one sulfur atom from the disulfide, as follows:



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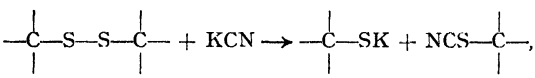
A new amino acid, named lanthionine, with one sulfur atom less than cystine, can be isolated after hydrolysis of the keratin[22].

The swelling of wool is also facilitated by urea, guanidine salts, sulfides, sulfites, cyanides, and salts of thioglycolic acid. The influence of these practically neutral reagents is explained by: (1) either cleavage of the sulfur bridges, or (2) rupture of the hydrogen bonds stabilizing the fiber structure. While the sulfides, sulfites and thioglycolates split the sulfur bridges, thereby facilitating expansion, the guanidine salts are known as hydrogen bond cleaving agents. The greatest swelling was observed when both reducing and hydrogen bond cleavage agents were present. Potassium cyanide in the presence of concentrated guanidine hydrochloride produces lateral swelling to more than 100%[23]. The cystine disulfide bridges are cleaved according to the equation:



and the hydrogen bonds are split simultaneously by the action of the guanidine salt. Noteworthy is the observation that the nitroprusside test for free sulfhydryl is negative in wool treated with potassium cyanide in the absence of guanidine salt (at pH 10), but the reaction is positive when the guanidine salt is present[23]. This proves that the hydrogen bonds are as important in the formation of stable chain structures as are the sulfur bridges.

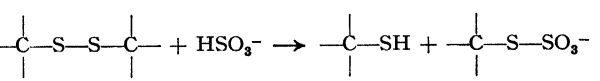
Wool and other keratins dissolve on heating with sodium sulfide[24], saturated lithium thiocyanate[25], in mixtures of detergents and reducing agents[26], or water-alcohol mixtures containing salts and thioglycolic acid[27]. The solutions in detergents and reducing agents can be extruded into fibers showing the β-keratin X-ray diffraction spectrum. Two main species of particles have been found in the solutions by means of electrophoresis, and molecular weights of about 55,000–75,000 and 35,000–40,000 respectively have been estimated.

The amount of keratin dissolved appears to be dependent on several factors, such as the alkalinity of the reagent, its reducing capacity, its ability to loosen the hydrogen bonds, temperature, etc. The particle size of the original keratin also is important, finely ground keratin being dissolved to a greater extent than coarse material. The molecular weights found for the dissolved keratin, however, do not represent the true molecular weight of

native keratin, since the cross-linked macromolecules are partially degraded upon solution.

Partial degradation and dissolution of keratin occurs also on oxidative treatment. If wool is treated with peroxyacetic acid, it can be dissolved in alkali (with only about 7–10% left as an insoluble membrane)[28]. The solution was electrophoretically rather homogeneous, and the keratin had a molecular weight of about 70,000. The keratin precipitated from this solution showed the α-keratin X-ray spectrum, indicating the presence of the coiled chain configuration. Upon treatment of the precipitated material with formic acid, however, the β-keratin spectrum was obtained.

Mercer and Olofsson[29] have studied the colloid chemical properties of keratins dissolved in concentrated urea (in the presence of reducing agents). The solutions were found to be polydisperse, the molecular weight of the major component being about 84,000. It was also found that the particles in these solutions were very asymmetric. Fibrous keratin can be obtained from these solutions. An even more extensive degradation occurs, if the urea solution is made more alkaline, in which case lower molecular weights of only 8000 were estimated, and no fibers could be drawn from this highly degraded keratin. The colloid chemical properties of feather keratin dissolved in urea and bisulfite were studied by Woodin[30]. The concentration of urea was 5 M, that of bisulfite 0.1 M, and the pH was regulated with phosphate buffer, and was 6–9 in most cases. The cystine and cysteine contents also were determined in this study. In the swelling and dissolution reactions the urea loosens the hydrogen bonds, and the bisulfite ion (in nearly neutral solution) splits the cystine bridges as follows:



Measurements of osmotic pressure, turbidity (by light scattering), sedimentation rate and viscosity indicated that, in infinitely dilute solution, feather keratin has a molecular weight of 10,000. The reduced viscosity was higher at pH 8.5 than at pH 6, but the intrinsic viscosity obtained was the same in both instances, namely 0.15 (Fig. 129). From these data the axial ratio of the dissolved macromolecules was estimated to be about 13 : 1. Both disulfide and sulfhydryl groups were found in the molecules of dissolved keratin. It was also found that the macromolecules associate in more con-

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centrated solutions, and it was concluded that this association involved the formation of intermolecular —S—S— bonds at the expense of intramolecular bonds. The particles formed in such association are more asymmetric than the original molecules.

In conclusion, it can be said that keratin represents an interesting case of space polymers composed of partially oriented and strongly interlinked chains. The intermolecular linkages in this instance can be cleaved and rejoined. Although the exact composition and con-

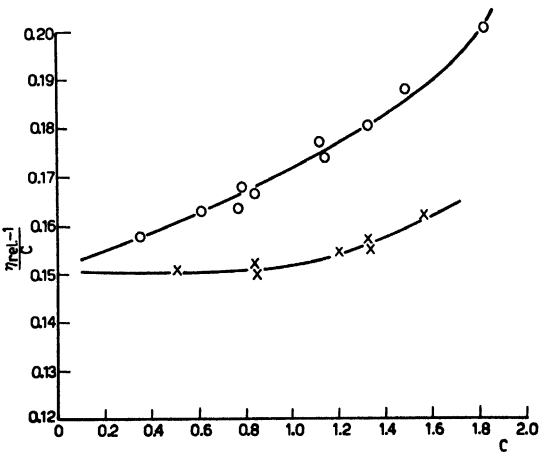

Fig. 129. Viscosity of dissolved feather keratin. On the abscissa is plotted the concentration of the colloid (g per 100 ml), on the ordinate the reduced specific viscosity. Upper curve (circles) – HS-keratin in 5 *M* urea, 0.1 *M* bisulphite, 0.05 *M* phosphate, pH 8.5. Lower curve (crosses) – keratin in 5 *M* urea, 0.1 *M* NaCl, 0.05 *M* phosphate, pH 6. (From. A. M. Woodin, *Biochem. J.*, 57 (1954) 105.)

figuration, as well as modes of interlinking of the chains are unknown, the folding in the crystalline portion is partially resolved, and the importance of sulfur bridges and hydrogen bonds in the stabilization of the structures has been ascertained.

3. Epidermin[31]

Chemical studies on amino acid composition, ease of hydrolytic decomposition, as well as optical and other investigations of keratinous materials from various sources, all indicate a great variety of these structural proteins. Block classified all keratinous structural proteins into true keratins (eukeratins) and pseudokeratins[32].

The true keratins generally have a higher cystine content than the pseudokeratins. Moreover, the molecular ratio of arginine to lysine in true keratins is about 3 : 1, whereas in the pseudokeratins it is 1 : 1. Finally, the latter are attacked more easily by proteolytic enzymes than are the former. Some data, as reported by Block, are compiled in Table 85.

TABLE 85

AMINO ACID COMPOSITION OF VARIOUS KERATINS
(according to Block[32])

Protein	*Lysine*, %	*Arginine*, %	*Cystine*, %	*Molecular ratio, lysine/ arginine*
Human hair	2.5	8.0	16.5	5 : 14
Finger nails	2.4	8.4	12.0	3 : 15
Sheep wool	2.3	7.8	10.0	4 : 14
Egg shell membrane	2.1	7.4	6.4	5 : 15
Goose feathers	1.0	4.8	6.4	4 : 14
Snake epidermis	1.4	5.4	5.3	3 : 12
Quills, porcupine	2.2	7.3	7.8	4 : 11
Human skin	4.7	5.9	3.8	3 : 3
Neurokeratin	2.7	3.8	3.4	3 : 3

Examination of Table 85 shows an interesting relationship between the chemical composition of the keratinous material and its origin. In the highly differentiated species (human) are found true keratins of the hair and nails, and a different (pseudo-) keratin of the skin. Snake epidermis (a less differentiated species), however, is chemically in between the nail-hair keratin and skin epidermis substance. It is also interesting to note that the keratinous materials in bird feathers and egg shell membrane are chemically similar, and differ considerably from such materials as sheep wool, nails or human skin. Finally, noteworthy is the fact that brain and nerve tissues contain considerable amounts of pseudokeratins.

The keratinous material of mammalian skin is called epidermin. It was found that the chemical and physico-chemical properties of the epidermin differ considerably, depending on the method of extraction and the sample used. The surface layer of skin is composed of dead, keratinized cells which have grown out of the live deeper layers. It is impossible to dissect and separate the dead keratin

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from the live cells, hence all preparations of epidermin are rather ill defined. However, it has been shown[31] that the surface layer is richer in disulfide bonds and poorer in —SH bonds than is the lower zone. The proteins can be extracted by concentrated urea solutions, and the extracts can be liberated from the urea by dialysis. Gelation or partial aggregation and flocculation may occur on dialysis of the protein. At least two different proteins seem to be present in these extracts.

According to Mercer and Olofsson[29], the major component of epidermin dissolved in urea has a molecular weight of 60,000. The system is polydisperse, but it becomes more homogeneous on adding some bisulfite, which splits the disulfide bonds, thereby reducing the molecular weight. The particles in solution appear to be very asymmetric.

The finding of Rudall[31] that one of the keratin components of skin is non-fibrous is also noteworthy. The outer layer of epidermis is especially rich in this component, which has a sulfur content of 2.8%. The fibrous component contains less sulfur (only about 1%), and is found predominantly in the inner layer.

Epidermin films exhibit the α-keratin X-ray diffraction pattern. Stretching under ordinary conditions does not change the pattern, even on maximum extension of about 200–300%, when the films break. The films could be stretched even more in saturated ammonium sulfate, whereby the α-keratin pattern gradually changed into the stretched chain β-keratin spectrum[31].

4. Collagen and Gelatin[2, 33]

The collagen group of fibrous proteins is as diverse as the previously described keratin group. The group is characterized (1) chemically, chiefly by its peculiar amino acid composition, and (2) structurally, by the characteristic X-ray diffraction pattern the protein exhibits. The various collagens appear in skin (under the keratinous epidermis), cartilage, tendons and bone. Tissues of lower animals also contain a variety of collagens, though they have not as yet been studied in detail.

All collagens appear fibrous both on microscopic and electron microscopic examination (Fig. 130). The fibers seen in the electron microscope are composed of a considerable number of linear macromolecules of indefinite length. The cross-striations on the fibers do not indicate a composition of spherical subunits, but are a result

of a more dense accumulation of the staining or shadowing material at certain portions of the fiber. It is assumed that these portions contain amino acid residues with more affinity for the staining material (e.g., phosphotungstic acid) than the other periodically appearing portions. The collagen fibers are relatively resistant to solvents, but on prolonged boiling with water they gradually dissolve. The purified, hot water degraded collagen is called *gelatin.* An even more degraded impure material obtained by boiling bones and connective tissues is called *glue.* The characteristic property of gelatin which makes this protein indispensible for photographic plates and films, as well as in food product industry, is gelation of the aqueous solutions. A characteristic property of the macrofibers

Fig. 130. Electron micrograph of collagen.

of collagen is their shrinkage on warming. The contracted material is rubbery in behavior, whereas the native fiber can not be extended appreciably.

The amino acid composition of collagens from various sources has been studied extensively[34]. Characteristic of all collagens is a high content of glycine (15–30%) and proline (8–17%). Moreover, the collagens contain a specific amino acid—hydroxyproline, generally between 7 and 13%. The collagens do not contain tryptophan, and the sulfur amino acid content is low. The absence of tryptophan and the low content of methionine and some other indispensible amino acids lower the food value of these proteins

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greatly. The so-called *elastins* represent a subgroup of collagens which differ from the ordinary collagens in amino acid composition, mechanical, and other properties. Elastins contain large amounts of glycine, alanine, valine, and proline, but very little hydroxyproline. The aortas of mammals are the best sources of this collagen. It should also be mentioned that collagen contains about 0.2–0.3% hexosamine, indicating the presence of a small amount of a polysaccharide (see Chap. 24).

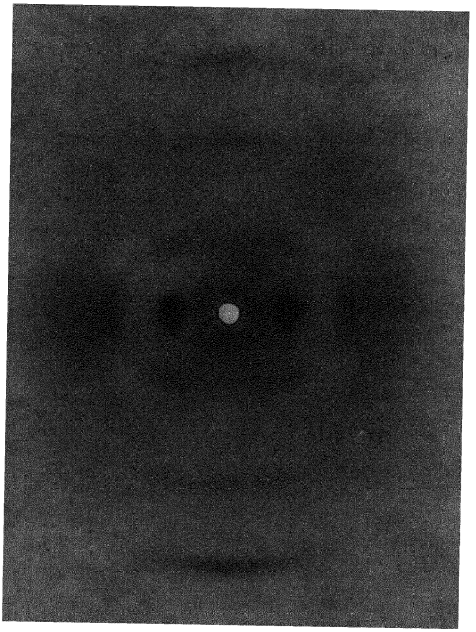

Fig. 131. X-ray spectrum of collagen (rat tail tendon). CuK_α rays. Specimen-to-film distance = 6 cm. (Courtesy Professor W. T. Astbury.)

The X-ray diffraction pattern of the collagens is very characteristic. It differs considerably from that of the keratins. The collagens show more reflections (see Fig. 131), the meridional spacing of 2.86 A and the equatorial spacing of 10–11 A being the most important and characteristic. The 2.86 A spacing represents the repeating unit in the axial direction. The equatorial spacing depends

on the water content of the material, and it changes on swelling. The wide angle X-ray diffraction of various collagens and gelatins has been studied extensively since 1920 (Herzog and Jancke, Katz, Astbury) and a large volume of data has been collected. The correlation of these data with the configuration of the chains, i.e., interpretation of the spectroscopic data in terms of structure, has been less satisfactory. The helical models of Bear[33] and Pauling and Corey[2,14] seem to be very close to the true configuration[35]. The low angle scattering of X-rays has also been studied recently[36]. These studies have revealed characteristic periodicities in the axial direction, all the spacings being orders of a fundamental unit of 640 A. This latter figure is the same as the dimension of the banding revealed by the electron microscope. It is well established that the macromolecules of collagen are linear spirals. Their configuration must differ, according to X-ray and other data, from the configuration of silk and keratin, but it is not known as yet what this difference is. In contrast to the keratins, the collagen molecules are not cross-linked by sulfur bridges. Hydrogen bonds definitely are important in both *intra-* and *inter*molecular stabilization of the structures. Moreover, the interlinking of the side chains by salt bridges and ester bonds must be considered. The basic amino groups of the side chains of arginine and lysine may become linked with the free carboxyl groups of the aspartic and glutamic acid residues in the chains:

$$R—NH^{+}_{3}\ldots{}^{-}OOC—R.$$

A similar linking is possible between the acid carboxyls and the alcoholic hydroxyl groups of the hydroxyamino acids.

Two types of swelling have been observed with collagen fibers: (1) lateral swelling accompanied by longitudinal contraction, and (2) normal lateral swelling without contraction of the fiber. The swelling is minimal at pH 5–9, and it increases strongly with increased acidity or alkalinity[37]. This was explained on the basis of osmotic effects caused by the immobility of the large collagen ions (Donnan effects), and by the rupture of intermolecular links by the acid and alkali. Strangely enough, there is a swelling maximum in acidic solutions[37] near pH 2–3 (Fig. 132). If the swelling is explained as due to the Donnan effect, it follows then that the suppression of ionization of the free carboxyls at the very low pH values of 0.5–1.5 is the cause of this maximum. Lateral swelling without contraction, however, is produced by neutral salt solutions,

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which would indicate rupture of hydrogen bonds rather than cleavage of salt and ester bridges. Hence, the acid-alkali swelling differs considerably from the salt-caused swelling. It seems likely that in the swelling of collagen with various reagents either the polar groups of the amino acid side chains or the hydrogen bonds may be involved. The acids and alkalies may either cleave the salt and ester bridges or just enhance the ionization. The presence of the swelling maximum in acid solutions, and the absence of it in strongly alkaline solutions indicates that in acidic and weakly alkaline solutions the uptake of water is governed by ionization, whereas in strongly alkaline solutions (pH about 12.5–13) cleavage of primary bonds also occurs. More of the intermolecular space in

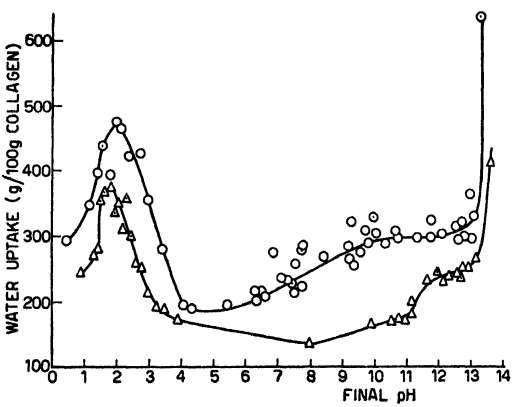

Fig. 132. The swelling of ox-hide collagen before (triangles) and after (circles) treatment with alkali. On the abscissa is plotted pH, on the ordinate the uptake of water (grams per 100 grams collagen). (According to J. H. Bowes and R. H. Kenten, *Biochem. J.*, 43 (1948) 369.)

the gel thereby becomes available, and more water can fill this space. According to Bowes and Kenten[37], up to 800 g of water can be absorbed by 100 g of collagen of ox hide in strongly alkaline solutions.

Cleaned and extracted pieces of hide, sinews, and similar preparations of collagens have been mainly used for the study of these structural proteins (apart from the soluble gelatin). Attempts to bring collagen into solution without degrading it have also been made. Most popular at the present time is the method of Orekhovitch[38]. According to this method, collagen can be extracted from

the skin by means of citrate buffers of pH 3–4.5 at low temperatures. The extracted protein was named *procollagen*, since it was believed to be the precursor of the true collagen of skin. The extracted material has practically the same amino acid composition as native collagen. The structure of the fibrous material precipitated from solution also resembles native collagen as revealed by examination under the electron microscope, X-rays, and infrared light[39]. Needle-like particles have been observed in the precipitates. They are birefringent, as is native collagen, although they are not truly crystalline.

Various figures for the molecular weights of the dissolved collagen (or procollagen) have been reported depending on the preparation and method used for size determination. According to Bresler and associates[40], the molecular weight is about 70,000; the particles are asymmetric and have the dimensions 380 A in length and 17 A in width. The data were obtained chiefly on the basis of sedimentation and diffusion measurements. Much higher values for the molecular weights have been obtained by light scattering[41]. Jackson and Fessler[42] achieved extraction of collagen from skins of young rabbits by 0.067 *M* phosphate at pH 7.0. The intrinsic viscosity of this collagen was 47, the axial ratio was estimated 40 : 1, the molecular weight approximately 1,000,000. Values between 3 and 10 million have been found by the light scattering method, and the entities in the solutions appeared to be highly coiled threads. The discrepancies may be due partly to the method of determination, and partly also to differences in the preparations themselves. One important point has been neglected in the discussion of the configuration of the macromolecules (or their associations): the fact that highly oriented, paracrystalline precipitates are formed from the dissolved material. Orientation of highly kinked threads upon precipitation is unlikely. Hence, the entities of the dissolved collagen must have an oriented chain configuration. The very possibility of extracting a soluble fraction of the same chemical composition as the original collagen indicates that some sections of the collagen fibers must be more labile toward solvents than others. The material extracted probably is composed of fragments of fibers retaining their original configuration. It is not known how many helical chains are present in these fragments, or how long they are. The collagen colloids show only one component on electrophoresis.

Attempts to determine the chemical molecular weight of collagen by means of chemical analyses have also been made[43]. Amino acid

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analyses of carefully purified specimens of hide collagen hydrolyzates have shown that the minimum molecular weight must be 39,000. The molecule then, on the basis of chemical data, would contain 2 residues of histidine and methionine, and 3 residues of tyrosine. The largest component is glycine (136 residues), followed by proline (51 residues), hydroxyproline and alanine (41 residues each). Acid and base binding experiments indicated that the free reactive basic and acid groups are those of the side chains of the amino acid residues, since the amount of acid bound corresponds to the amount of diamino acids, and the amount of alkali bound to that of the dicarboxylic acids.

The colloid chemical properties of gelatin gels and solutions have been thoroughly studied by various methods. Gelatin is an amphoteric linear polyelectrolyte. The solutions are polymolecular (polydisperse), the average molecular weights being generally in the limits 15,000–150,000[44]. These averages, when determined by the same method, depend on the method of preparation of the gelatin, i.e., on how much the original collagen is degraded. Being a strongly hydrophilic colloid, gelatin is not easily precipitated by salts. Fractionation of gelatin, however, can be accomplished by means of alcohols, acetone, and similar non-solvents for proteins. Coacervation and gelation also may occur with highly concentrated additives[45]. For the purpose of fractionation, dilute solutions are taken, and the precipitant is added gradually with stirring. Such fractionations have been performed in many laboratories, and the molecular weights, size distribution, and other properties of fractionated material have been studied[46]. It has been found that for the precipitation of a gelatin of molecular weight 65,000 from a 1% solution, the mixture should contain about 60% alcohol. More alcohol will be needed for the precipitation of the smaller molecules. If V is the relative volume of alcohol, and M the molecular weight (M_w), then, for a 1% solution of isoelectric gelatin the following relation holds[47]:

$$V = 0.367 + \frac{1.04 \cdot 10^4}{M}$$

The number average molecular weights determined by osmotic pressure measurements are smaller than the weight average weights determined by sedimentation or light scattering. Even greater values are obtained by the sedimentation equilibrium method (M_z values).

Data on unfractionated gelatins from various sources are presented in Table 86[48].

TABLE 86

AVERAGE MOLECULAR WEIGHTS FOR VARIOUS GELATIN SPECIMENS
(after Williams[48])

Gelatin	*Intrinsic viscosity*	M_n	M_w	M_z
Knox P–20	0.35	37,600	95,000	237,000
Knox P–111–20	0.36	38,000	95,000	250,000
Knox P–180	0.175	17,900	24,000	61,000
Knox MFG-2	0.195	19,300	34,000	60,000

The differences between the various molecular weights are great indeed, as are the variations between gelatins from different sources. The differences between the average molecular weights by various methods indicate polydispersity. These differences are smaller when well fractionated specimens are compared, although even then the weight average molecular weights are greater than the number averages. Gelatin differs in this respect strongly from the rather monodisperse globular proteins (see Table 77, p. 437).

As a polyelectrolyte, gelatin exhibits all the viscosity abnormalities of this type of colloid, and comparable data can be obtained only with solutions containing salts. According to Table 86, intrinsic viscosities between 0.17 and 0.35 have been obtained. More data are presented in Table 87.

TABLE 87

INTRINSIC VISCOSITY AND MOLECULAR WEIGHT OF GELATINS
(after Williams[48])

Gelatin	*Intrinsic viscosity*	M_w	M_v
Limed pigskin gelatin	0.461	120,000	99,600
Limed ossein gelatin	0.361	94,300	78,000
Acid pigskin gelatin	0.350	83,000	72,500
Oxypolygelatin	0.157	35,000	28,000

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In Table 87, M_v represents the viscosity average molecular weight, which is smaller than the weight average but larger than the number average. The oxypolygelatin mentioned in this Table is a modified protein intended as a blood plasma substitute[49].

According to Williams[48], the intrinsic viscosity depends on the viscosity average molecular weight (M_v) as follows:

$$|\eta| = 2.9 \cdot 10^{-4} \cdot M_v^{0.64}.$$

Pouradier and Venet[50] found for fractionated gelatin another expression:

$$|\eta| = 1.66 \cdot 10^{-5} \cdot M_n^{0.885},$$

in which case the molecular weights were calculated on the basis of osmotic data. The agreement between the two equations is poor. This seems to be due largely to the fact that the M_v values differ from those for M_n, even when compared on fractionated material, since the latter is not monodisperse.

Extensive light scattering studies on gelatin have been done recently by Boedtker and Doty[51]. According to their findings, the macromolecules of gelatin are not associated in 0.15 M sodium chloride solution at 40° C, or in 2 M potassium thiocyanate at lower temperatures. Weight average molecular weights of 96,000 and 111,000 have been obtained for the unaggregated protein. Aggregation occurs at lower temperatures (in absence of such dispersing agents as thiocyanate) in which case particle weights of many millions were obtained.

The macromolecule of gelatin in solution seems to be a random coil, with the solvent being entrapped to a considerable extent[48, 51, 52]. At sufficiently low temperatures the coils may aggregate without causing gelation, if the concentration of the colloid is sufficiently low. It was concluded from light scattering studies[51] that the aggregates are cross-linked, as they dissociate only slightly on dilution. The gelation seems to be attributable to the simultaneous growth and interlocking of aggregates arising from crystallite formation at a low temperature and at a sufficiently high concentration of gelatin[51].

The viscosity of gelatin solutions has a minimum at the isoelectric point which is near pH 5. The viscosity increases as the net charge on the chains increases, this being due to mutual repulsion of the chain segments and expansion of the coil. Acid and base binding experiments indicate that there is one acid binding group and one

base binding residue for about every 8–12 amino acid residues. Streaming birefringence studies showed that the particles in solution can be oriented, hence they are asymmetric, or the flexible coils are deformed easily and become elongated on flow.

Gelation phenomena and properties of gelatin gels have been studied extensively, and good reviews are available[53]. The essential factors which determine gelation are: (1) average molecular weight of the gelatin, (2) gelatin concentration, and (3) temperature. The more degraded the gelatin, the higher are the concentrations needed to produce gels at a specific temperature. This gelation temperature, i.e., the maximum temperature at which gels can be formed, depends on the two other factors mentioned, hence the quantitative relations are complex. A 1% solution of good quality gelatin forms jellies at 20° C or even at a somewhat higher temperature, and firmer gels are obtained with 2–4% gelatin. Gelatin gels have a network structure, the chains being partly oriented into para-crystalline strands, as found by X-ray studies (Hermann and Gerngross, 1932). The nature of the bonds interlinking the chains, however, is still obscure. It has been found[54] that the guanidine groups of the arginine residues are important in gelation, and that the ε-NH_2 groups of lysine are not essential. This is substantiated by the fact that deaminated gelatin is able to form jellies, although the viscosity of the deaminogelatin is lower than that of the original gelatin[55]. Hydrogen bonds seem to be the most important links in the formation of the network on gelation.

Important information on gelatin solutions and gelation has been obtained by means of optical rotation studies. The specific rotation of gelatin solutions is about –100°, and it depends on pH, temperature, and salts present[56]. The rotatory power of the warm solutions increases sharply on cooling, values up to –1000° being reached on gelation. However, if sufficient water is removed from a warm solution (40°) so that gelation occurs at this temperature, the protein shows the low specific rotation values of the soluble gelatin[57]. It has also been shown that the ordinary cold gels have an infrared spectrum similar to that of collagen, whereas the spectrum of the warm gel is different. These observations support the view that the configuration of the macromolecules of gelatin probably changes on gelation. The lower rotating "warm gelatin" is slow in forming inter-chain hydrogen bonds; on lowering the temperature, this modification is changed into the optically very active modification capable of interlinkage and network formation.

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Finally, it should be mentioned that gelation phenomena are reversible, and that the melting points of the gels roughly correspond to the gelation temperatures. In Table 88 are presented data illustrating the dependence of the melting points on concentration and molecular weight[58].

TABLE 88

MELTING POINT, CONCENTRATION AND MOLECULAR WEIGHT OF GELATIN[58]

Specimen		*Molecular weight* M_w	*Concentration %*	*Melting point, ° C*	
				Chilled at 0°	*Chilled at 15°*
P6–00		72,100	2.03	27.0	29.2
	ditto		5.96	29.9	30.5
L3–80		52,700	2.09	19.5	23.7
	ditto		6.14	23.0	25.9
L1–120		48,000	2.77	18.7	22.5
	ditto		5.77	21.0	24.4
P6–180		33,400	2.01	10.9	
	ditto		5.92	16.0	did not gel

It is obvious that the gels melt more easily the lower the molecular weight and concentration. Moreover, the melting point depends on the method by which the gel was prepared. The gels formed at 15° are more stable than those obtained at 0°. This is due to the fact that at a moderately low temperature the chains may assemble in a better linked meshwork than at a very low temperature, since certain segments of the chains must meet in order to become linked.

5. The Linear Proteins of Muscle[59]

The colloid chemistry of muscle proteins is very complex and somewhat confusing, as various things have been designated by the same terms, and different terms have been assigned to quite similar objects. In this section only the linear or fibrous *myosins* will be mentioned, the other constituents and the major functions and structural characteristics of the tissue being left for Chap. 29.

Proteins are extracted from fresh minced muscle by salt solutions of high ionic strength of 0.5. The globulin precipitated on about 20-fold dilution of such extracts with water was originally called

myosin (v. Fürth, 1919). Physical-chemical studies on this protein showed that it is a polydisperse fibrous protein of high molecular weight. The solutions appeared to be very viscous, and they exhibited double refraction of flow[60]. It was discovered later by Szent-Györgyi that this globulin is composed of two different proteins[61]. The original complex protein then was renamed *actomyosin*, and for the fibrous component the name *myosin* was retained. The other component was found to be essentially a globular protein, although capable of forming asymmetric aggregates. This component was named *actin*. Another fibrous protein component of muscle tissue, called *tropomyosin*, was discovered in 1946 by Bailey[59]. In contrast to actomyosin, this protein is soluble in water, although it can be extracted from skeletal or cardiac muscle only with salt solutions. The solutions of the protein are very viscous, and the viscosity is suppressed by salts. It crystallizes from salt solutions in the form of translucent, birefringent plates[62].

The amino acid composition of the fibrous muscle proteins differs strongly from that of the collagens and keratins. The most characteristic chemical feature of the myosins is their high content of dissociable side groups, i.e., a high content of lysine, arginine and glutamic and aspartic acids. Ionogenic carboxyl and amino groups are present in 45% of the residues of tropomyosin, in 25% of all residues of actin, and in 34% of the residues of myosin. Hence, each third amino acid residue of the myosin chains contains a dissociable group. Consequently, the macromolecules of these proteins are *highly charged*, more so than any other proteins. These analytical results agree reasonably well with the data on acid-base binding. The sulfur content of the myosins is moderate (about 1%), while the glycine content is very low. Noteworthy is the fact that α-amino groups could not be determined with certainty either in myosin or tropomyosin by the Sanger method (Sect. 3, Chap. 26). This would indicate that (1) the free α-amino end group in the myosins is substituted, or (2) that the macromolecule has no free end groups, i.e., it is cyclic. Although the second possibility is preferred by some investigators, it should be considered with caution. Due to the large molecular weight of these proteins, the methods of end group determination become less reliable than in the case of lower molecular proteins. Moreover, as mentioned, the α-amino end groups may be blocked, or the Sanger reaction may be inhibited by some as yet undiscovered causes.

The molecular weight of pure myosin was found to be 850,000

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(±30,000) by sedimentation and diffusion[63], and 840,000 (±33,000) by osmotic pressure measurements[63] in the same laboratory. The molecules seem to be very thin rods about 2300 A long and only 23 A wide. According to the angular dissymmetry of light scattering, the length of the rods[64] is 1500 A. In alkaline solutions of pH 10.7 the large myosin molecules are split into subunits of molecular weight approximately 170,000, and smaller subunits of the relative weight of 16,000 are split off by concentrated urea or guanidine salt solutions. Bailey concludes[59] that the myosin particle is built up of about 5 units of molecular weight 165,000, which are associated with several subunits of $M = 16{,}000$. Both the larger and the smaller subunits are asymmetric, the larger possessing the axial ratio of 30 : 1 and the smaller 12 : 1. Osmotic pressure measurements on tropomyosin solutions indicated that this protein has a particle weight of 135,000, and it falls to about one-half of this value at high ionic strength of 0.3. In neutral solutions of concentrated urea or in acid solutions (pH 2.1) tropomyosin has a molecular weight of 53,000. No N-terminal groups were found in these molecules, and it was concluded that they are cyclic in constitution although the actual shape most probably is cylindrical[59].

Actin is known in a relatively low molecular modification (G-actin) and in a very high molecular fibrous modification (F-actin). According to Mommaerts[65], the molecular weight of G-actin is 57,000, as determined by light scattering. These units seem to have a cylindrical shape, and they have a strong tendency to associate into long fibers (F-actin). The smaller units are stable only in acid or alkaline solutions, and in the absence of salts, when strong electrostatic repulsion prevents association[65]. Salts suppress this electrostatic repulsion, and purely linear association occurs, if the solution is alkaline. At neutral or acid reaction, however, linear as well as lateral association takes place. In muscle tissue actin is associated with myosin, and the reactions between the proteins, as well as the reversible aggregations, are strongly influenced by substances such as adenosine triphosphate.

Fibrils and rodlets in various myosin preparations have also been observed in the electron microscope[66]. Moreover, myosin "crystals" are broken up into threads of uniform thickness by ultrasonic waves[67]. Generally, myosin is denatured easily by heat, dehydration with organic solvents or even by freeze-drying, while tropomyosin is more stable. If a solution of myosin is squirted into distilled water, the protein precipitates in the form of threads. These myosin

threads are birefringent, and they display the characteristic α-keratin diffraction pattern when examined with X-rays. This indicates that there is some similarity in the folding and twisting of the polypeptide chains in myosin and the α-keratins. However, the α-keratin pattern of myosin does not change to the β-keratin spectrum on stretching the fiber.

Beautiful electron micrographs of tropomyosin have been published by Astbury and colleagues[68]. These fibrous particles can be presented as typical examples of linear colloids (Fig. 133). The particles appear as moderately bent stiff strings. They are of indefinite length, and about 200–300 A thick. These fibrils are not

Fig. 133. Electron micrograph of tropomyosin fibrils deposited from aqueous solution. (Courtesy Prof. W. T. Astbury.)

the macromolecules but they are aggregates of the macromolecular units. Disaggregation of the fibrils occurs readily, e.g., by the action of potassium chloride.

The salt solutions of myosin, actin, or the complex actomyosin set easily, and the gels may be thixotropic, depending on the conditions (concentration of the protein and salt, pH). Firm gels can be obtained with as little as 0.5–1.0% F-actin.

One of the major difficulties in the study of these proteins is the fact that they are hard to purify. Although the best preparations of myosin or G-actin appear homogeneous in the ultracentrifuge,

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several components are revealed on electrophoresis. The major portion of the well purified myosin, however, is electrophoretically homogeneous[64]. This difficulty in purification is due partly to the fibrous nature of the macromolecules.

Myosin is split by trypsin into two components, H-meromyosin and L-meromyosin, the former having a molecular weight of 232,000, and the latter 96,000[69]. These fragments differ from the subunits mentioned previously. H-Meromyosin is rich in reactive sulfhydryl groups and is enzymatically active (as adenosine triphosphatase). H-Meromyosin also contains more glycine, tyrosine, phenylalanine and proline but less histidine, arginine, and glutamic acid than L-meromyosin. Both components are more soluble than myosin, but the L-meromyosin deposits on dialysis in the form of birefringent sheaths. The axial ratio of the H-meromyosin is 15 : 1, and that of the L-meromyosin 33 : 1. Szent-Györgyi concludes that one myosin molecule consists of four L-meromyosin and two H-meromyosin units, and that the former represent the contractile part of the myosin molecule.

In conclusion it may be said that in spite of considerable progress in the study of the various components, the problem of the fibrous muscle proteins is not yet solved. It is now known with certainty what is the true molecular weight of the various myosins and actins, and how they interact in the muscular contraction and relaxation processes. The fibrous nature and the high charge density are well established facts, and together with the data on the reversible aggregation of actin, have served as a basis for extensive speculation. The long and thin myosin molecule seems to have a double chain structure capable of disaggregation in several ways, as well as of interaction with actin with a resultant change in shape. The mechanism of these processes, for instance that of the aggregation of the G-actin into F-actin, is still quite obscure, as are the structural details. Although no terminal α-amino groups could be found, the presence of C-terminal groups was proved[70]. Myosin has been shown to have at least one isoleucine C-terminal group in a particle weight of approximately 300,000. Similarly, a C-terminal phenylalanine was found in actin[70]. The α-carboxyl end group residue of tropomyosin was found to be the same (isoleucin) as in myosin, although the sequence of the next following amino acids in the two proteins is different[70]. These findings, of course, do not support the hypothesis that the macromolecules of myosin and tropomyosin are long chains with joined ends.

6. Fibrinogen

In contrast to the previously mentioned structural proteins, fibrinogen is found in blood plasma. It is found there in the amount of 0.3–0.4%, its physiological function being to form clots and prevent loss of blood in case of injury. In this important process, fibrinogen is the chief material which undergoes aggregation and polymerization into highly branched networks encompassing large amounts of liquid (see Chap. 30).

Fibrinogen is one of the least soluble plasma proteins, hence it appears in the first fraction in the common methods of plasma fractionation. For example in the cold ethanol fractionation procedure it is precipitated with 8% ethyl alcohol at pH 7. It is purified by redissolving in a phosphate or citrate buffer and reprecipitating with ammonium sulfate. The protein is liberated from salts and other micromolecular impurities by dialysis.

As in the case of myosin and other muscle proteins, fibrinogen contains large amounts of dicarboxylic amino acids (27%), and the basic residues of lysine (9%), histidine (2.6%) and arginine (7.8%). The other amino acids are present in moderate amounts. Due to the high content of dicarboxylic and basic residues, the side groups of many residues are ionogenic, and the charge density on the surface of the macromolecules of fibrinogen is high.

The weight average molecular weight of fibrinogen, according to recent determinations, is 330,000. Sedimentation and diffusion measurements, as well as light scattering, have led to the same result[71]. Fibrinogen is prone to aggregation, and the larger values formerly obtained for the molecular weight are explained as being caused by aggregation. The solutions exhibit streaming birefringence, and they are viscous. The intrinsic viscosity of fibrinogen is 0.25. This value is much lower than that of myosin (2.1), but considerably higher than that of the other plasma globulins (0.04–0.08). The molecule of fibrinogen seems to be of rod-like shape, the rods having a length of approximately 700 A, and an axial ratio of 20 : 1. The particle may be split into subunits of various sizes and shapes. Although fibrous particles have been observed in the electron microscope in most cases[72], beads also have been seen in some preparations[73].

Fibrinogen is denatured easily at interfaces, and also by heat. It coagulates at 56° C, and thereby becomes denatured. This instability invalidates considerably the conclusions on the true nature of

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native fibrinogen, since the preparations studied in most cases (e.g., the specimens under the electron microscope) may be denatured. The beads observed in the electron microscope may be such disaggregation units, although fibrous products also may be formed on denaturation followed by aggregation. This is the case in the formation of fibrin clots from fibrinogen under the influence of thrombin. Depending on the conditions, highly translucent gels of finely dispersed fibrin, or more coarse and opaque systems which synerize easily, can be obtained[53]. The liquid can be squeezed out of the gel, and the fibrous meshwork of the gel then remains as a membrane. Gelation has been observed in solutions containing as little as 0.004% of fibrinogen, although more fibrinogen is needed in order to form firm jellies. This astoundingly high gelation capacity is explained partly by the fibrous nature of the meshwork, and partly by the high charge density and polarity of the framework. Due to this, the framework is able to hold large amounts of water, bound by secondary bonds and encompassed mechanically.

The clotting of blood will be discussed in Chap. 30, and it will be shown how very complex this important process is. Systems simpler than blood have also been studied in order to understand the various steps and factors involved. An important finding was that of Ferry and associates, that the formation of fibrin from fibrinogen may be regulated by some additives, e.g., hexamethylene glycol[74]. Aggregates composed of about 15 molecules of fibrinogen could be found to be present as intermediates in fibrin clot formation. Partial uncoiling and even chemical changes seem to occur, and the association of the asymmetric macromolecules may be chiefly linear, or lateral as well, depending on the conditions. The reversibility of the aggregation depends on pH, as well as on the additives present in the solutions[75].

7. Fibrous Mucoproteins

The mucoproteins or *glycoproteins* contain about 5–20% carbohydrate, and the macromolecules may be of various sizes and shapes. The mucoproteins found in blood plasma are of a relatively low carbohydrate content, and the macromolecules are of the same size and shape as most other blood proteins. The molecular asymmetry is relatively low, except for some of the globulins appearing together with fibrinogen in the fractions first precipitated, which differ from fibrinogen in being non-clottable. The sedimentation

constant of these globulins is about twice as great as that of fibrinogen, but the axial ratio is 10 : 1, i.e., the particles of these globulins seem to be less asymmetric than fibrinogen[76]. A decrease in solubility on cooling is a characteristic of these proteins, hence they are sometimes called *cryoglobulins*. The molecular size and shape seem to be different in various individual cases, especially in the diseased state, when abnormal globulins with large and asymmetric particles (called *macroglobulins*) have been found (see Chap. 31).

Very large and asymmetric mucoproteins have also been found in human urine and in egg white. The urinary mucoprotein can inhibit the agglutination reactions between red blood cells and some viruses, as it combines with the viruses. The molecular weight of this protein was estimated at several millions, and the axial ratio at 170 : 1. The solutions of this protein are very viscous[77]. It is interesting that a mucoprotein of similar ability to prevent virus hemagglutination was isolated from egg white. The molecular weight was found to be the same as for the previously mentioned urinary protein, and the axial ratio was estimated to be 90 : 1[78].

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CHAPTER 28

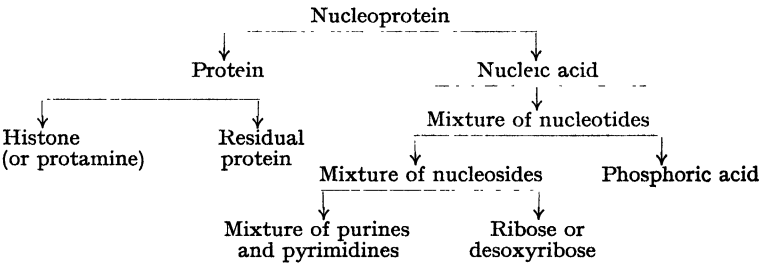
Nucleic Acids, Nucleoproteins, and Viruses

Nucleoproteins are the important constituents of cell nuclei. They were discovered by Miescher (in 1868), and their chemical nature was disclosed later by Kossel, Emil Fischer, Levene and others. In the last three decades, further important contributions toward the elucidation of this problem have been made by Todd, Chargaff, and Caspersson, among many others. Of great importance was the work of Stanley, who isolated the tobacco mosaic virus, obtained it in a crystalline state, and showed that the virus was a nucleoprotein. It was also discovered that nucleoproteins are present not only in viruses and in the chromosomes of nuclei but also in the cytoplasm. Nucleoproteins are universal constituents of living cells, both animal and plant. They are capable of reproducing themselves, and retain the characteristics of the species in cell growth, division and multiplication. No wonder then that so much attention has been paid to these substances. Complete solution of the nucleoprotein problem may well be the clue to the mystery of reproduction.

Nucleoproteins are very complex molecular colloids. The macromolecule of a nucleoprotein is composed of *two* parts: (1) *nucleic acid* and (2) *protein*. The former is an *acid polyelectrolyte*, the latter a *basic polyelectrolyte*. Both components are colloidal, and they are joined by ionic bonds and secondary linkages. The protein moiety is complex, and only a part of it can be cleaved off easily from the nucleic acid. The "residual protein" is bound to the acid very tightly. Nothing much is known about this "residual protein". The loosely bound protein has been somewhat more thoroughly investi-

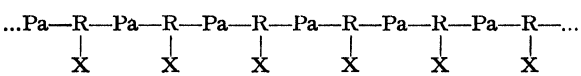
gated. In the case of fish sperm nucleoprotein, the protamines comprise this portion of the protein (see Chap. 25). The nucleoproteins from other sources contain the so-called *histones*, basic proteins of more complex composition than the protamines.

The nucleic acid moiety is also complex. Hydrolytic decomposition of the macromolecules yields a mixture of *nucleotides*, the latter being split further into *nucleosides* and *phosphoric acid*. The nucleosides appear to be N-glycosides of ribose or desoxyribose and some purines or pyrimidines. The course of cleavage, and the constitutive components are shown in the following diagram:

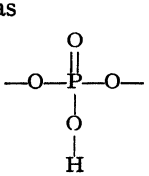


1. Nucleic Acids[1]

Nucleic acids are acidic polyesters. The repeating units in the linear macromolecule are the phosphoric acid residue (Pa) and the ribose or desoxyribose residue (R). The purines or pyrimidines are attached at the sides of the phosphoric acid-sugar chain through N-glycosidic linkages to the aldehyde carbon atom of the sugar. Denoting by X the heterocyclic purine or pyrimidine, the pattern of the nucleic acid macromolecule can be illustrated as follows:



The orthophosphoric acid residue (mer) is the simplest. This mer (Pa) appears in the chain as



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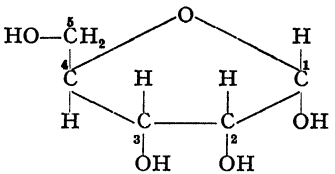
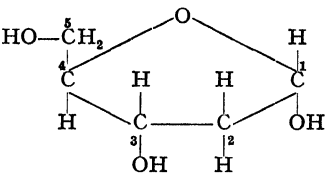
the —O— atoms being linked to the sugar, and the remaining hydrogen being able to dissociate and make the substance acid.

The mers of the sugar (R) also are relatively simple, most common being those of α-D-ribofuranose or of α-D-2-desoxyribofuranose. The presence of other sugars, however, is strongly suspected.

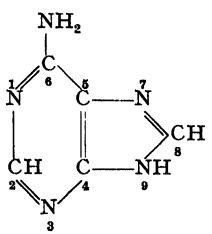


α-D-Ribofuranose

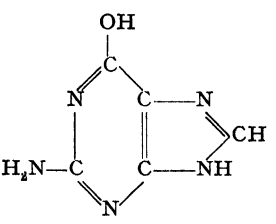


α-D-2-Desoxyribofuranose

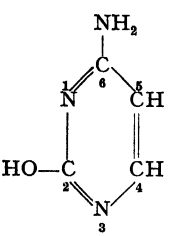
The variety in the heterocyclic side groups (H) is greater, the following heterocyclic compounds being found: adenine, guanine, cytosine, uracil, thymine (5-methyluracil) and (in small amounts) 5-methylcytosine:



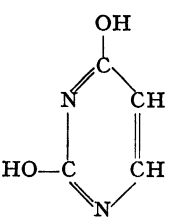
Adenine



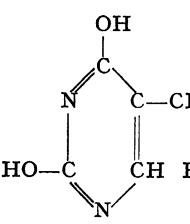
Guanine



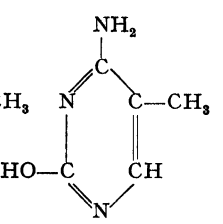
Cytosine



Uracil



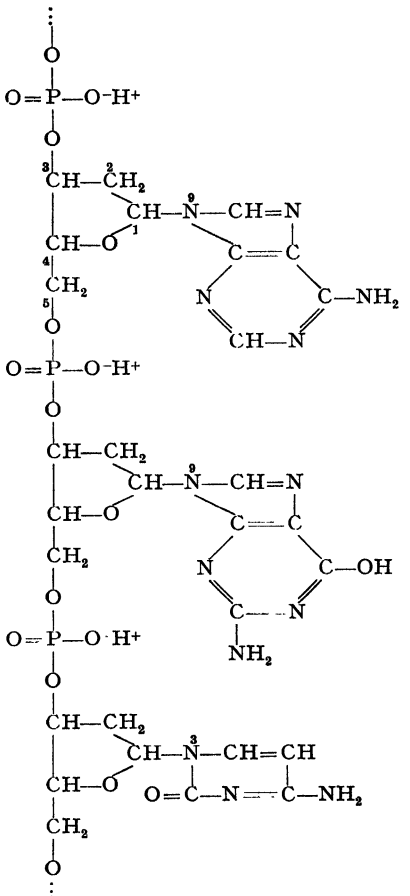
Thymine



5-Methylcytosine

The glycosidic linkage is formed with N-9 in the purine ring of adenine or guanine, and with N-3 in the pyrimidine ring. A section

of the linear macromolecule of a nucleic acid then can be illustrated as follows:



The nucleic acids, according to their chemical composition, are classified into two large groups: (1) the *ribonucleic acids*, and (2) the *desoxyribonucleic acids*. The sugar component of the former is ribose, and that of the latter is 2-desoxyribose. (The section of the nucleic acid chain illustrated above contains 2-desoxyribose residues.) Both ribonucleic and desoxyribonucleic acids contain adenine, guanine

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and cytosine. Uracil is found only in ribonucleic acid, whereas desoxyribonucleic acid contains thymine and methylcytosine. Ribonucleic acid (RNA) is found in yeast, calf thymus cytoplasm, sarcoma and carcinoma tissues, and in some viruses, to mention just a few sources. Desoxyribonucleic acid (DNA) is found chiefly in the nuclear nucleoproteins, for example of fish sperm, calf thymus, and also of some bacteria and viruses. The RNA and DNA preparations from various sources are characterized by the ratios of the components, for example, the ratio of adenine to guanine or that of cytosine to thymine. The constituents are now determined chiefly by paper chromatography, after they have been split off the macromolecules[2]. Each of the pyrimidines or purines travels on paper a certain distance with a suitable solvent, and the location of the spots is best determined by means of ultraviolet light. All the purine and pyrimidine derivatives found in nucleic acids have characteristic absorption spectra in the ultraviolet, as have the undegraded acids themselves. The partition and identification can be achieved also by means of fractional adsorption and elution on columns. Some illustrative data on the composition of various specimens of RNA and DNA are presented in Table 89[3].

TABLE 89

THE COMPOSITION OF RIBONUCLEIC (RNA) AND DESOXYRIBONUCLEIC ACIDS (DNA)

Source	*Adenine*	*Guanine*	*Cytosine*	*Uracil*	*Thymine*	*Methylcytosine*
1. Yeast, RNA	0.26	0.31	0.20	0.23		
2. Yeast, RNA II	0.28	0.31	0.20	0.20		
3. Beef pancreas RNA	0.21	0.49	0.21	0.08		
4. Fowl sarcoma RNA	0.14	0.46	0.28	0.12		
5. Calf thymus cytoplasm, RNA	0.19	0.36	0.28	0.17		
6. Tobacco mosaic virus, RNA	0.28	0.25	0.20	0.27		
7. Human DNA, average	0.31	0.23	0.21		0.25	0.01
8. Human DNA II, average	0.30	0.19	0.19		0.32	
9. Calf thymus DNA	0.28	0.21	0.21		0.28	0.01
10. Beef spleen DNA	0.29	0.21	0.21		0.28	0.01
11. Mouse sarcoma DNA	0.30	0.22	0.23		0.26	
12. Herring roe DNA	0.27	0.19	0.22		0.27	0.03
13. Pneumococcus DNA	0.30	0.21	0.18		0.30	
14. Wheat germ DNA	0.26	0.23	0.17		0.26	0.05

The numbers given express the proportion of purine and pyrimidine bases found in the specimens. Variations between specimens 1 and 2, and 7 and 8, respectively, represent the results found on similar specimens in two different laboratories. Differences in composition indeed are conspicuous, and in the group of ribonucleic acids they are more pronounced than in the group of desoxyribonucleic acids.

One of the best starting materials for the preparation of *ribonucleic acid* is yeast. The yeast is treated first with alkali, which splits the protein-RNA complex, and the ribonucleic acid can be separated by precipitating with acetic acid. Ribonucleic acid is a slightly yellowish powder, difficultly soluble in water and dilute acids but soluble in alkalies. The water-soluble salts of the acid can be precipitated from the aqueous solutions with alcohol. The macromolecules are degraded by heating with strong acids, alkalies, or by enzymatic hydrolysis at ordinary temperatures. Ribonuclease is a well-investigated enzyme which attacks RNA. The nucleic acids form insoluble compounds with basic dyes, as well as with some heavy metal ions.

Concentrated solutions of the soluble salts of ribonucleic acid exhibit double refraction of flow, and the viscosity of the solutions is high[4]. Gelation also is observed in concentrated solutions. All these observations support the view that the macromolecules are fibrous. The chains of the ribonucleic acids, however, are not extremely long, since the viscosity of dilute solutions is only moderate. Sedimentation constants of only 2–3 have been found for the ribonucleic acids from yeast[5], although larger constants of 8.6 *S* have been found for the ribonucleic acid from tobacco mosaic virus[6]. Since the diffusion constants are difficult to evaluate, the molecular weights obtained by the sedimentation velocity and diffusion method have given only approximate results. Values between 8000 and 300,000 have been mentioned, and it is likely that the variations are due to the following: (1) differences in the samples (various degrees of degradation), (2) variations due to the limitations and characteristics of the methods of measurement, and (3) aggregation[7].

The *desoxyribonucleic acids* have been investigated more thoroughly than the ribonucleic acids. The former have larger molecular weights, and their fibrous nature is more pronounced than that of the latter. Calf thymus gland is the common starting material[8]. Fresh glands are frozen and minced in a cooled mincing machine. The material is extracted with a 1% solution of sodium

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chloride, in order to remove soluble proteins and ribose-containing nucleoproteins. The centrifuged and washed residue then is extracted with 5% sodium chloride, which brings the desoxyribonucleic acid-containing nucleoprotein into solution. The viscous solution is filtered through a coarse filter, and the nucleoprotein is precipitated by diluting with water. According to Signer and Schwander[9], the extraction is accomplished best by the use of 10% sodium chloride in the presence of fluoride which prevents enzymatic degradation by the desoxyribonuclease present in the tissue. The nucleoprotein is purified by redissolving in sodium chloride and reprecipitating by dilution. The pure nucleoprotein then is split into the desoxyribonucleic acid and protein components by concentrated sodium chloride, in a prolonged treatment (10–14 days at 0° C). The protein (histone) thereby precipitates, and the sodium salt of the desoxyribonucleic acid remains in solution. The sodium desoxyribonucleate is precipitated by alcohol, and purified by redissolving in water and reprecipitating with alcohol. The yield is 5 grams of sodium salt from 700 grams of thymus glands[9].

The first important study on the size and shape of the desoxyribonucleic acid particles was carried out by Signer on a specimen prepared by Caspersson and Hammarsten[10]. Signer found that solutions of the sodium salt of the poly-acid were highly viscous and exhibited streaming birefringence. It was concluded that the particles are thin rodlets having a molecular weight of 500,000–1,000,000. The solutions appear to be strongly polydisperse, even the specimens prepared under conditions in which the greatest possible care was taken to avoid degradation[9]. Recent work on light scattering of the solutions of desoxyribonucleic acids indicates that the particles in solution are not thin rigid rods but gently coiled stiff chains[11]. This conclusion is based chiefly on the angular dependence of light scattering which permits distinction of random coils from rigid rods. According to Doty[11], the weight average molecular weight of various preparations of desoxyribonucleic acid (sodium salts) is 6,000,000, and the number average value is 3,000,000. The contour length of the chains is estimated to be approximately 15,000–20,000 A, i.e., 1.5–2 μ or 0.0015–0.002 mm. The end-to-end length of the coil (diameter), according to these data must be about one-fourth the contour length of the chain. The diameter of the chain itself is very small, and it has been estimated to be 15–20 A. The macromolecular chains have also been resolved in the electron microscope[12], the thinnest units appear-

ing as bent fibers, having a length of about 15,000 A and a width of 15–20 A. It is probable that in the cell nuclei the macromolecules are present as almost straight, gently twisted spirals which are able to change their shape to a limited extent.

If 6 million is accepted as a tentative value for the molecular weight, the number of molecules per cell can be calculated[11]. The amount of desoxyribonucleic acid per cell in calf thymus is estimated to be $7 \cdot 10^{-12}$ g. From this figure, the molecular weight, and Avogadro's number, the number of nucleic acid molecules per cell is found to be 1,400,000. Each of the 48 chromosomes of these cells thus contains approximately 30,000 macromolecules of nucleic acid.

Molecular weights and frictional ratios of desoxyribonucleic acids (i.e., their salts) have been estimated also from sedimentation velocity and diffusion measurements. For example, Conway and Butler[13] found by these methods that the weight average molecular weight was 1½ million, and the frictional ratio was 6.9. Since considerable difficulties are encountered in the evaluation of the diffusion constants for these colloids, Doty has suggested calculation of the molecular weights by using a relation developed by Mandelkern *et al.*[14]. This equation correlates the sedimentation constant (s) and intrinsic viscosity $|\eta|$ to the molecular weight (M) of linear polymers as follows:

$$M = \left[\frac{s_0 \cdot |\eta|^{1/3} \cdot N \cdot \eta_0}{2.5 \cdot 10^6 (1 - \bar{V} d)} \right]^{3/1}$$

where η_0 is the viscosity of the solvent, N Avogadro's number, $\bar{V}$ the specific volume, and d the density of the solution. Applying this equation, M of desoxyribonucleic acid was found to be 5,800,000 in good agreement with the best values found by the method of light scattering[11].

The viscosity of solutions of desoxyribonucleates has been studied extensively. Investigators at Harvard University have found[11] that the reduced specific viscosity is constant in very dilute solutions of sodium desoxyribonucleate (25–75 milligrams per liter), when 0.2 M sodium chloride is used as the solvent. The intrinsic viscosity of this preparation was found to be 53.4. The viscosity of this linear polyelectrolyte depends on the flow gradient, and on the presence of salts[13, 15], the latter strongly diminishing the internal friction. Since the molecules of this polyelectrolyte are relatively stiff, the contraction caused by the decrease in intramolecular repulsion was

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questioned. It was found, however, that at low shear rates[16] and in low concentrations of the colloid, the viscosity is affected very little by the salt. This is in accord with the idea that the shape of these macromolecules indeed is relatively stable, and that the effect of the added salt is due to decreased interaction between the colloidal particles. In the absence of salts the small counter (gegen-) ions (usually Na^+) can complete their ion atmospheres by interacting with two oppositely charged macro-ions, thus increasing the viscosity. In the presence of salts there are enough micromolecular anions available, and the intermolecular attraction between the particles is reduced. Added salts decrease both the *inter*molecular attraction and the *intra*molecular repulsion between the charged segments of the linear macro-ions.

Very interesting is the observation that urea and other protein denaturants reduce the viscosity of solutions of desoxyribonucleic

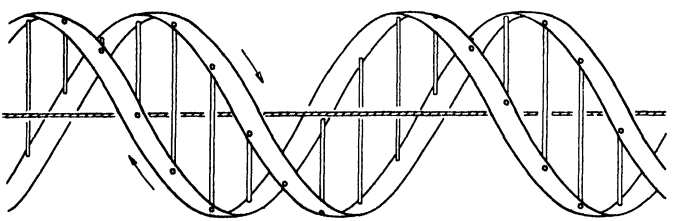

Fig. 134. A molecular model of nucleic acid according to Watson and Crick. The macromolecule is composed of two helices which are wound in opposite directions. The spirals are cross-linked by hydrogen bonds connecting the purine and pyrimidine groups of the side chains.

acid[17]. Urea (6 *M*) causes a considerable decrease in the viscosity of the nucleic acid, and the effect is irreversible in that the orginal viscosity is not recovered when urea is removed by dialysis. When urea is removed, the particles of the nucleic acid assume a more compact form, the frictional ratio being decreased considerably. It was inferred that this is due to the breakage of intramolecular hydrogen bonds on which the configuration of the macromolecules depends.

The idea has emerged lately that nucleic acids have a helical configuration[18]. The fine X-ray diffraction diagrams obtained lately by the study of fibrous desoxyribonucleic acids have given sufficient experimental data for calculation of the spatial arrangement of the constitutive groups (Fig. 134). Aqueous gels and wet crystals

have also been studied, and it was concluded that the structures depend markedly on the water content[19]. The molecular fiber itself appears to be rigid and has a diameter of about 16 A. The presence of particles composed of preferably 7 macromolecules also was ascertained in concentrated gels. Hydrogen bonding plays a considerable role in all the helical models proposed. It is also noteworthy that similar helical models have been suggested for the macromolecules of proteins, and, since the proteins are bound to the nucleic acids in the complex nucleoproteins, both components must have similar configurations. Although considerable progress has been achieved in the clarification of the structural problems, the exact configuration of the macromolecules of nucleic acids is far from being solved.

2. The Histones

Histones are the soluble protein components of the complex colloidal material called nucleoprotein. The histone can be split off by treatment with acid, which forms a soluble salt of histone, or by a prolonged treatment with 10 % sodium chloride solution. The histones can be precipitated from solution with ammonia, and the precipitate does not dissolve in excess ammonia. All preparations of the histones appeared to be polydisperse colloids of basic nature, the isoelectric point being between pH 10 and 11. Although they resemble the protamines in this respect, their chemical composition is more complex. Their nitrogen content is lower (17–19 %) than that of protamines, and they contain almost all the amino acids, except tryptophan and the sulfur amino acids, which either are found in minute amounts or not at all. The variations in amino acid composition of various histone specimens is great, some examples being presented in Table 90.

TABLE 90

EXAMPLES OF VARIATION IN AMINO ACID COMPOSITION OF HISTONES (from Tristram[20])

	Rat liver histone	*Calf thymus histone*	*Rat sarcoma histone*
Leucine, %	10.1	5.2	5.1
Isoleucine	5.7	20.5	17.6
Tyrosine	5.3	3.3	2.6
Arginine	15.9	17.4	16.3
Lysine	11.5	10.2	11.8
Glutamic acid	10.4	4.3	3.3

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The amounts of the other amino acids found in different specimens also vary, even if examined in the same laboratory by the same methods. Attempts to fractionate histones have been only partially successful. Fractionation of calf thymus histone by means of adsorption produced three fractions which showed considerable difference in amino acid composition as determined by the most reliable chromatographic adsorption methods[21]. In Table 91 are presented some data which illustrate the chemical differences between the fractions. The figures represent the per cent amino acid nitrogen of the total nitrogen.

TABLE 91

VARIATIONS IN AMINO ACID CONTENT OF THREE FRACTIONS OF HISTONE

	Fraction A	*Fraction B*	*Fraction C*
Glutamic acid	2.62	5.62	3.86
Alanine	17.66	7.67	12.55
Lysine	36.09	16.97	24.68
Arginine	6.74	19.76	12.65

Grégoire and Limozin obtained two fractions from calf thymus histone[22]. The more soluble fraction, according to electrophoretic mobility data, contained 3 or more basic proteins. The less soluble histone was almost homogeneous on electrophoresis, and it contained 14.2% arginine, 12.4% lysine, and 1.7% histidine. Butler and associates[23] found two components on electrophoresis below pH 7, but three components could be seen when the mobilities were observed in acidic solutions. Sedimentation and diffusion studies on a most soluble fraction (obtained by precipitation with acetone and ammonia) yielded the molecular weight of 18,000, and an axial ratio of 27 : 1. The solutions of this histone jellied at pH 8, a fact which demonstrates the fibrous nature of this protein. Several components of the histones have also been observed in the ultracentrifuge, and molecular weights of 130,000–140,000 and of 11,000–16,000 have been estimated for two of the components[24]. The fast-sedimenting histone in the latter study contained 0.23% tryptophan, 0.67% cystine, and 2.4% methionine, whereas many other preparations have been found devoid of these amino acids. The other (slow) component, which comprised 37% of the total, was found to be free of tryptophan and cystine, and it contained 0.8% methio-

nine. All these examples show that the variety in the histones is greater than the variety in the composition of the nucleic acids. Histones represent one of the most challenging examples of practically unexplored proteins of great biochemical importance.

The so-called residual proteins, which cannot be separated easily from the nucleic acids, have been studied even less than the more easily extractable histones. This protein contains over 1% tryptophan, and its nitrogen and arginine content is lower than that of ordinary histones[25]. In contrast to the histones, it is insoluble in neutral and acid solutions and soluble only in alkaline solutions. According to Butler *et al.*[23], the low tryptophan content of their histone fractions is due to contamination with some other protein, since the chemical molecular weight, calculated from this low percentage of tryptophan should be 200,000, whereas only 18,000 was found by physical methods. It is possible that the residual protein is bound to the nucleic acid moiety by non-polar secondary bonds, since chloroform facilitates its removal[26].

3. The Nucleoproteins[27]

The preparation of desoxyribonucleoproteins has been described in Section 1 (p. 510). The best method of extraction is with the use of sodium chloride, followed by precipitation of the nucleoprotein by dilution of the sodium chloride extract with water. Hence the nucleoprotein behaves like a high molecular globulin. Nucleoprotein can be classified as a complex linear colloid. It can not be decided yet with certainty whether the particles are chemically definite complexes of a certain number of protein molecules bound to the linear macromolecule of the desoxyribonucleic acid, or if they are aggregates of less definite composition. As shown in the previous sections, the molecular weight of protein components is much smaller than that of the nucleic acid component. The weight ratio of both components, however, is nearly the same, hence many protein molecules must be bound to the nucleic acid moiety.

The X-ray scattering of thymus nucleoprotein indicates that the nucleoprotein is not a definite compound of a unique structure, since mixtures of the nucleic acid and protein components showed the same scattering ability as the native complex[28]. It is, however, possible that the method used is not sensitive enough to reveal the differences.

Two important facts should be borne in mind considering the

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colloid-chemical properties of the nucleoproteins: (1) their instability, and (2) variability depending on the source and method of isolation. The first fact is well illustrated in the study of thymus nucleoprotein by Shooter, Davison and Butler[29]. Two components of the nucleoprotein with extremely variable sedimentation constants were observed; the variations were explained as being due to aggregation. The gel-like structure of the native nucleoprotein could not be broken up without damaging (denaturing) the native nucleoprotein. It is obvious that the particles are relatively large and asymmetric, but since they are aggregated in the native state, there is little significance in ascribing to them a definite molecular weight. Stepwise cleavage of calf thymus nucleoprotein is produced by 0.5 *M* sodium chloride, and dissociation into the components is accompanied by an increase in viscosity[30]. This indicates that the original complex is less asymmetric than the components produced by the cleavage. This is in accord with the concept now prevailing that several small helices are linearly associated with a large nucleic acid helix. Crudely then, the particle of nucleoprotein can be thought of as a cylindrical tube with a central cylindrical core of nucleic acid helix along which smaller cylindrical rods of the proteins are attached (cf. Fig. 134, p. 512).

The viscosity of solutions of the nucleoproteins is very high, and intrinsic viscosities of thymus or liver nucleoproteins up to 830 have been reported. Less viscous preparations were extracted from liver by means of 1 *M* sodium chloride. These less viscous nucleoproteins possessed intrinsic viscosities of 29–202, depending on the solvent[31], which are still very high values in comparison with the intrinsic viscosities of ordinary protein solutions (0.03–0.1). The sedimentation constants of the very viscous preparations are about 26–31 *S*, and the material is polydisperse, whereas the less viscous preparations are more homogeneous and have lower sedimentation constants[31]. Electrophoretic mobility studies usually reveal one principal component, and several other components in smaller amount. Part of the protein component may be split off the nucleoprotein complex by prolonged electrophoresis[32].

The variability in the properties of nucleoproteins isolated from various sources is illustrated by the example of a desoxyribonucleoprotein from the sperm of the sea urchin. This nucleoprotein can be extracted by distilled water from the sperm, which was pretreated with citrate or phosphate[33]. In contrast to thymus nucleoprotein, this colloid was precipitated by dilute salt solutions. The nucleo-

protein was composed of 35–37% desoxyribonucleic acid and an equal amount of histone, while the remainder was a non-histone protein. Strong salt solutions of 0.4–1 *M*/l and higher dissolve the nucleoprotein, whereby the nucleic acid–protein complex is partly dissociated. The viscosity increases on dissociation, indicating that the dissociation products are more asymmetric than the original particles. The undissociated particles appeared in the electron microscope as somewhat curved rods approximately 4300 A long and 250–300 A wide.

The nucleoproteins containing ribonucleic acid differ considerably from the desoxyribonucleoproteins. For example, Eggman, Singer and Wildman[34] describe a nucleoprotein which was isolated from the leaves of several species of plants. The nucleoprotein was reasonably homogeneous with respect to sedimentation and electrophoretic mobility, and its molecular weight was estimated at approximately 375,000. However, these units too, like those of the previously mentioned nucleoproteins, aggregated and formed dimers, trimers and larger aggregates. In contrast to the thymus and liver nucleoproteins, these plant nucleoproteins seemed to consist of nearly spherical particles.

As a result of the complexity and lability of the native nucleoproteins, attempts have been made to attack the problem "synthetically", i.e., the reactions between the nucleic acids and various basic polyelectrolytes have been studied. Not only histones and protamines, but many other proteins react with nucleic acids, this being a colloid-colloid reaction (Chap. 14). Warburg and his colleagues[35] made use of these reactions in fractionating mixtures of proteins, some of which had enzymatic activity. The basic proteins, which carry an excess positive net charge, are more readily precipitated by the negatively charged nucleates than are the neutral proteins. The reactions between the nucleic acid and the synthetic basic polyelectrolytes polylysine and polyvinylamine also have been studied recently[36]. It was found that the solubility of the complexes decreased with increasing concentration of the components. It was also shown that the nucleic acid–polylysine complex is similar to the nucleoprotein in its behavior toward the dissociating influence of salt solutions.

4. Viruses and Bacteriophages[37]

Viruses are disease-causing agents of colloidal dimensions. The virus particles are invisible under ordinary microscopes, and they pass

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through even the dense bacterial filters. The early colloid-chemical studies on these particles have been done chiefly by means of ultrafiltration. More information concerning their sizes, shapes and other colloid-chemical properties has been gained recently by direct observation in the electron microscope, as well as by sedimentation studies using the ultracentrifuge. It is now well established that the various viruses are colloidal, and that they are either nearly

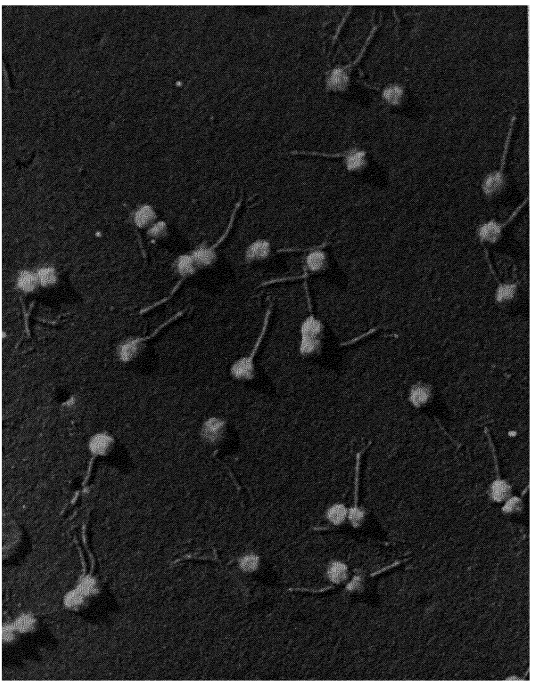

Fig. 135. Electron micrograph of T5 bacteriophage. Magnification 45,000×. (Courtesy Dr. R. C. Williams, Univ. of California.)

spherical or rod-like. The bacteriophages are colloidal agents capable of disintegrating bacteria. Most bacteriophages have somewhat more complex shapes than viruses, i.e., they are composed of a round "head", and a rod-like "tail" (Fig. 135). Data on the dimensions of some important viruses and bacteriophages are compiled in Table 92.

TABLE 92

LENGTH AND DIAMETER (in mμ) OF SOME VIRUSES AND BACTERIOPHAGES

Vaccinia	260 × 210
Herpes simplex	150
Rabies fixe	125
Influenza	115
Staphylococcus bacteriophage	head: 100; tail length: 200
Fowl plague	90
T1 bacteriophage	head: 50; tail length: 120
Pneumonia virus of mice	40
Tobacco mosaic	(200—280) × 15
Poliomyelitis	25
Yellow fever	22
Foot-and-mouth disease	10

A variety of viruses have been isolated recently and obtained as pure paracrystalline or even as truly crystalline substances[38]. Chemical studies have shown that they are composed wholly or to a great extent of nucleoproteins. The particles in solution, although small in comparison with bacteria, are larger than the molecules of most simple proteins. As shown in Table 92, the particle size among various types of viruses varies considerably. The same pertains to the composition and structure. The largest viruses and the bacteriophages resemble the simplest bacteria, and it appears to be difficult to draw a definite borderline between the viruses and the bacteriophages on the one side and bacteria and other microbes on the other. The chief biological characteristic of the viruses remains the fact that they grow and reproduce only in living cells, not in artificial mixtures of amino acids, salts, vitamins, and other nutrients. Hence there is a similarity between the viruses and the chromosomes of the cell nuclei.

The plant viruses are simpler in chemical composition than the animal viruses and bacteriophages. The chief constituent of the plant viruses is ribose-containing nucleoprotein, whereas the animal viruses and bacteriophages contain desoxyribonucleoprotein or both ribonucleoprotein and DNA. For example, tobacco mosaic virus contains 6% ribonucleic acid, whereas cowpox vaccinia has 5.6% desoxyribonucleic acid. Moreover, the vaccinia contains 5.7% lipid, while there is no lipid found in tobacco mosaic virus. Influenza virus contains both ribonucleic acid (3%) and desoxyribonucleic

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acid (2%) and a large amount of lipid. The T2 bacteriophage is almost a pure nucleoprotein (40% desoxyribonucleic acid, 6.6% ribonucleic acid), although it also contains some lipid (2.6%).

The amino acid composition, including the percentage of nucleic acid, of some viruses is shown in Table 93[39]. The rest of the substance is chiefly lipid.

TABLE 93

THE AMINO ACID COMPOSITION OF SOME VIRUSES

(g amino acid per 100 g virus)

	Tobacco mosaic virus	*Tomato bushy stunt v.*	*T3 phage*	*PR8 influenza v.*	*Lee strain influenza B virus*	*Shope papilloma v.*
Alanine	4.1	6.0	3.4	2.0	2.1	2.5
Arginine	8.8	5.7	2.8	4.5	3.6	6.2
Aspartic acid	11.7	8.9	4.4	6.4	6.4	9.5
Cysteine	0.6	0 6				5.4
Glutamic acid	9.9	4.4	4.1	6.8	5.5	10.2
Glycine	1.4	3.7	3.3	1.9	2.2	2.4
Histidine	0	1.1	0.7	1.2	1.3	1.7
Isoleucine	5.7	5.8	1.8	3.5	3.6	3.6
Leucine	8.0	9.2	3.7	4.6	4.7	6.3
Lysine	1.3	3.7	2.4	3.2	4.1	6.2
Methionine	0	1.8	0.7	2.0	1.9	1.9
Phenylalanine	7.5	4.2	1.4	3.3	3.0	4.1
Proline	4.9	2.4	1.5	2.2	2.3	4.6
Serine	6.0	5.1	1.1	1.8	1.8	3.3
Threonine	8.4	8.1	2.0	3.1	3.4	4.4
Tryptophan	1.9	0.5		1.0	0.6	0.9
Tyrosine	3.4	4.1	1.6	2.8	1.9	5.3
Valine	7.8	10.3	2 4	2 9	2.7	4.6
Total	91.4	84.0	37 3	53.2	51.1	83.1
Nucleic acid, %	6	17	50	5	5	9

As the amino acid total varies (due to the varying nucleic acid and lipid content), only the two influenza virus preparations and the bushy stunt virus versus Shope papilloma virus are comparable. The amino acid composition of the influenza viruses appears to be the same, whereas the bushy stunt virus differs strongly from the papilloma virus, e.g., in the content of alanine, cystine-cysteine, histidine, lysine, methionine, and other amino

acids. The sedimentation constants of the viruses and bacteriophages run to several hundreds or even thousands, and the molecular weights to many millions. Crystallinity is not a sufficient criterion for homogeneity, and it seems likely that the apparently similar units revealed in the crystals of viruses by the electron microscope[40] are actually composite particles, and that they may differ from each other even in the same crystal. We must recall here the facts on the complexity of many crystallized proteins (for example, albumin, pepsin) which are non-homogeneous, in spite of crystal-

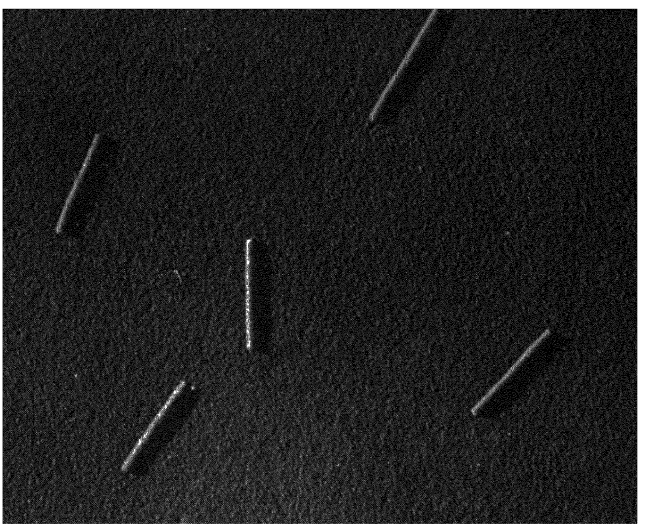

Fig. 136. Electron micrograph of air-dried preparation of tobacco mosaic virus. Magnification 44,000 ×. (Courtesy Dr. R. C. Williams, Univ. of California.)

linity (Chap. 26). The ability of the viruses to mutate also should be mentioned: it appears that some generations of the reproducing viruses differ in some biological properties (e.g., infectivity) or physico-chemical characteristics from the previous generations.

Although the viruses, especially the plant viruses, strongly resemble nucleoproteins, the complexity of the particles should be borne in mind. Many kinds of smaller macromolecules of proteins may

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be present in the large particles thought to be the giant molecules or particles of viruses. Some of the larger viruses have also shown some complexity in the structure and organization of the unit, as revealed by electron microscopy[41].

Important recent studies on the structure of the tobacco mosaic virus have been published by Schramm and associates[42]. It was found that partial disintegration of the virus particles occurs at

Fig. 137. The nucleoprotein strings of the tobacco mosaic virus, the protein fragments, and partially disintegrated rods. Magnification 90,000 ×. (Courtesy Prof. Dr. G. Schramm, Tuebingen.)

pH 10.3. Very high magnification (150,000 times) electron microscopy studies showed clearly that the particles are composed of doughnut-like discs which are pulled on a thin central rod which has a diameter of 34 A (see Fig. 137–139). This central rod is

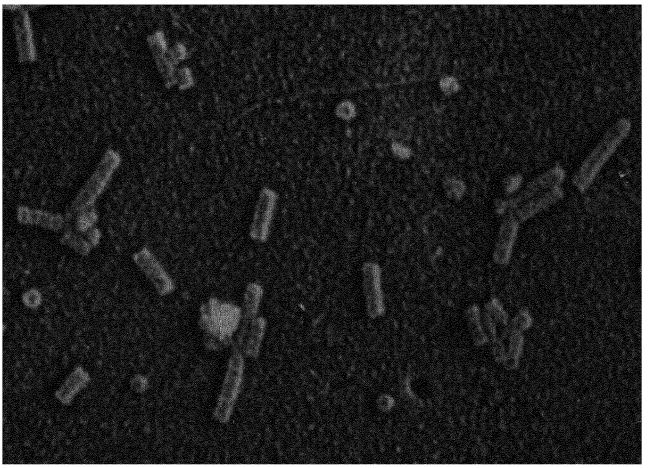

Fig. 138. Same as Fig. 137, magnification 120,000 ×. The doughnut-shaped protein fragments are well visible in this electron micrograph.
(Courtesy Prof. G. Schramm.)

Fig. 139. Same as Fig. 138 (a partially dissociated particle), magnification 150,000×.
(Courtesy Prof. G. Schramm.)

composed of the nucleic acids, whereas the "doughnuts" are proteins. The "doughnuts" have a molecular weight of 90,000, and they aggregate into larger cylindrical particles by being piled up on each other, even without a central nucleic acid rod.

Of paramount importance also are the recent discoveries of Fraenkel-Conrat and Williams[43] on the reactivation of tobacco

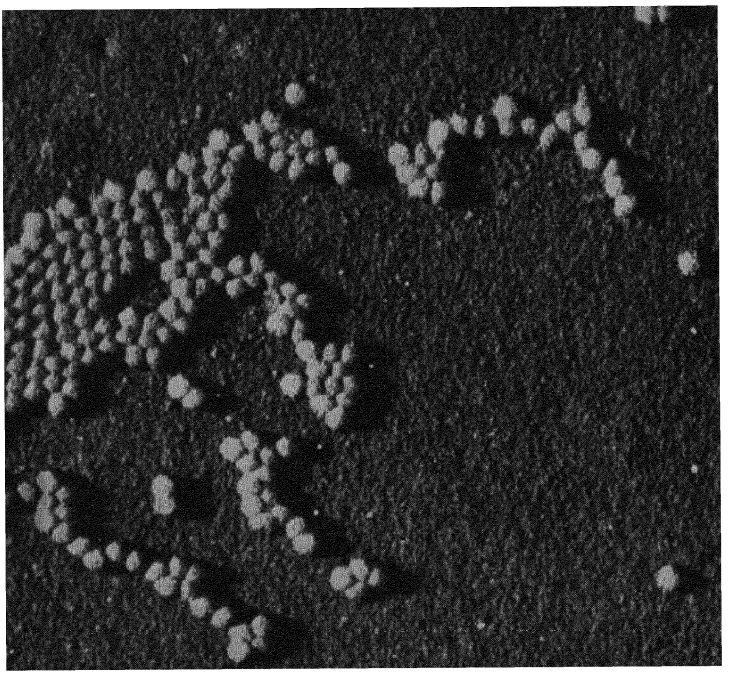

Fig. 140. Electron micrograph of the bushy stunt virus particles. Magnif. 100,000 ×. (Courtesy Dr. R. C. Williams, Univ. of California.)

mosaic viruses. It was shown that the protein and ribonucleic acid components which were obtained by treating the virus with alkali (at pH 10.5) or sodium dodecyl sulfate can combine and reconstitute the active virus. The reconstituted virus particles appeared in the electron microscope as rods of the same dimensions as the original native virus, and the "synthetic" viruses were able to grow and

cause lesions on tobacco leaves. It was shown by combining the nucleic acids and proteins from various strains of the mosaic viruses that only the nucleic acid component is the one which supplies the symptoms characteristic for the particular strain.

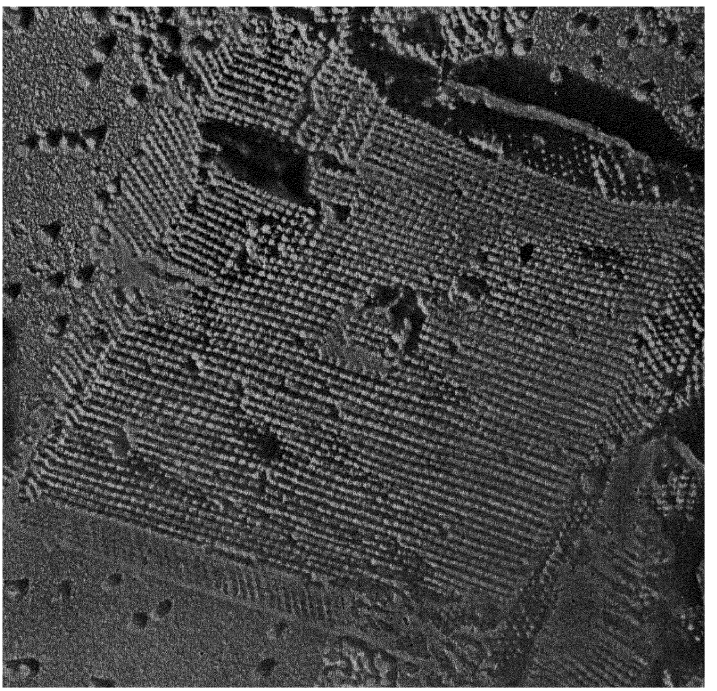

Fig. 141. Electron micrograph of a necrosis virus protein crystal according to Labaw and Wyckoff. There has been some local dissolution of the crystal in its lower right corner. Magnification 50,500 ×. (From L. W. Labaw and R. W. G. Wyckoff, *Koninkl. Nederl. Akad. van Wetenschappen, Amsterdam, Proceedings*, Series B, 59 (1956) 171).

The isolation and purification of viruses is accomplished by the same methods as are used for other proteins (Chap. 26 and 27). Separation is accomplished at low temperatures, and the starting

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material usually is frozen and ground in the frozen state. Fractional centrifugation is often used, removing coarse cell fragments by means of low-speed spinning, and bringing the virus down in the preparative ultracentrifuge. Purification is achieved by repeating such low- and high-speed centrifugations. Precipitation with ammonium sulfate and alcohol at low temperatures is also used, the acidity (pH) of the solutions being an important factor in achieving fractional precipitation, and avoiding denaturation. The

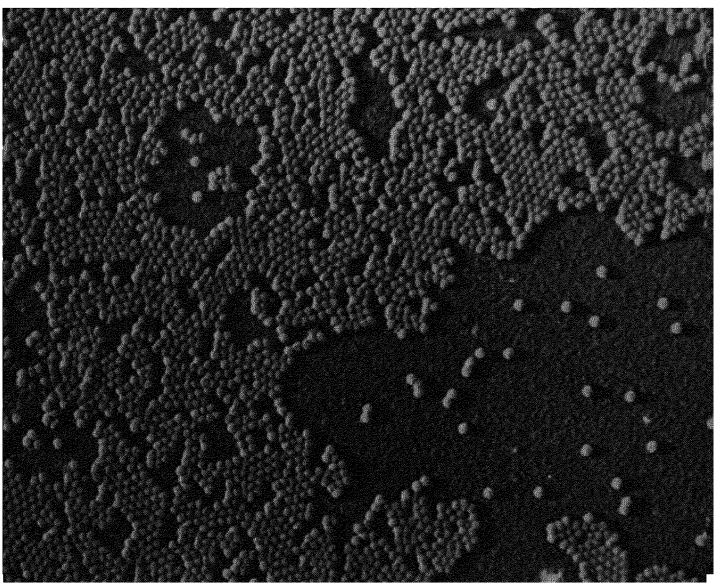

Fig. 142. Electron micrograph of purified MEF-1 poliomyelitis virus. Magnifition 61,000 ×. The average diameter of the particles is 270 A. The mass of the particle in terms of molecular weight is about $6 \cdot 10^6$. (Courtesy Dr. R. C. Williams, Univ. of California.)

crystallinity and high infectivity of the crystallized material are guarantees that no denaturation has occurred during the isolation.

Tobacco mosaic virus is one of the definitely asymmetric rod-shaped particles, and can be considered now as a well-investigated example from the colloid-chemical point of view. Electron microscopy data in this case are in complete accord with sedimentation,

viscosity, and light scattering results as mentioned previously (Chap. 7). Another example of a thoroughly studied, relatively simple virus, is the bushy stunt virus, which was isolated in a truly crystalline state by Bawden and Pirie[44]. X-ray analysis of these crystals also has been performed[45]. According to the X-ray data, the particles in the crystals have a radius of 170 A, corresponding to a "molecular weight" of 24,000,000 (Fig. 140).

The animal viruses and bacteriophages have been studied less from a chemical and physical standpoint than have the plant viruses. The rabbit papilloma virus, for example, gives rather viscous solutions, and it was concluded that the particles are asymmetric. The electron microscope, however, revealed spherical particles possessing a certain organization, and it seems that the high viscosity and frictional ratio is due to the hydration[3]. Owing to the complexity of structure and functions, the animal viruses and bacteriophages scarcely can be classified as complex nucleoproteins, but they seem to belong to the realm of microbiology or virology. The branch of virology is now developing rapidly, the size, shape, and chemical characteristics of the objects having become as important as the biological and pathological aspects[37].

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CHAPTER 29

Colloid Phenomena in Cells and Tissues[1]

Thirty years ago, great hope was placed in the methods of colloid chemistry. It was confidently believed that this new science would provide a solution for most of the problems associated with life processes, such as cell division and reproduction, muscular contraction, and ageing. The great riddles of life, however, have remained unsolved, and, moreover, it became apparent in these last decades that the classical physical colloid chemistry alone is rather helpless in the unwieldy systems of cells and tissues. It must be admitted that purely chemical approaches have been more fruitful both in the domain of metabolic processes and in submicroscopic morphology. The astounding achievements of the chemistry of large molecules have definitely proved that chemical views and methods are as important as physical methods in the study of very complex organic colloids. These techniques open new perspectives in the understanding of the very complicated systems of cells and tissues. The time may not be ripe for integrating the vast information about biochemistry of function with the knowledge about the structure of biocolloids, but progress is apparent. Some highlights of this recent progress in the organic colloid chemistry of cells and tissues will be outlined in this chapter.

1. Biocolloids in a Cell

The branch of virology is an example of recent applications of modern colloid chemical methods. Beginning with the indispensible method of ultrafiltration (Bechhold, Elford, Ferry) important

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achievements were further facilitated by the new methods of centrifugation, fractional precipitation, viscosimetry, light scattering and electron microscopy. In addition, the chemical and colloid-chemical data about nucleoproteins and nucleic acids proved to be useful in the clarification of the virus problem. Studies on viruses and bacteriophages placed the fundamental problem of what is living and non-living in a new light. Some virologists have called the virus particles living macromolecules. We have seen, however, in the preceding chapter that most of the animal viruses and bacteriophages are organized entities, and they can not be called macromolecules. The evidence is growing that some viruses (e.g., vaccinia virus) are composed of a variety of nucleoproteins, lipoproteins, simple proteins and micromolecular substances. There remain, however, the smaller animal and especially the plant viruses. They certainly are capable of reproducing in living tissues, and with this faculty they resemble living cells. The particle weight of these entities is very large, in comparison to proteins, and, on the basis of all we know about the nature of simple proteins, it seems unlikely that even these small virus particles are macromolecules. The multiplication of enzymes is an interesting problem. In a germ cell there must be hundreds of various enzymes in minute amounts, and larger amounts of them are found in the embryo than in the initial germ cell.

It is obvious that these macromolecules of enzymes, simple viruses and nucleoproteins create difficulties in classifying biological entities into living and non-living. If they are classified as living, some polymers also may be claimed as "alive", for example the polymerization of methyl methacrylate in the presence of a polymer (Chap. 18, Sect. 6) strongly resembles the autocatalytic multiplication of enzymes. The chains grow, they may be cleaved, and the cleaved chains may grow further, as long as there is some monomer present. If the small viruses and enzymes are classified as non-living organic macromolecules or their associations, a very artificial borderline will be drawn between these things and the larger viruses and microbes, which are truly living. Any such definitions of life are convenient, artificial schemes incapable of accomodating the whole unwieldy variety of complex macromolecular systems found in nature. It seems that we shall have to admit the existence of systems which possess only a few characteristics of living matter, and hence may be classified at best as 'semi-living'.

Any single microscopic cell of plant or animal tissue is a much

larger and much more complex body than a virus or a particle of nucleoprotein. The two major parts of the cell are: the *nucleus* and the *cytoplasm*. Moreover, most cells possess a *membrane*. Each of these components is very complex. The nuclei of certain cells are covered by nuclear membranes, which have microscopically visible holes. Inside the nucleus can be discerned a smaller *nucleolus* and many *granules* rich in the nucleoproteins. The granular elements of the nucleus can be stained easily with certain basic dyes, and they absorb ultraviolet light strongly at about 2600 A. These important particles are identified as the chromosomes, the carriers of hereditary factors (genes). There is ample experimental evidence that the genes are arranged in a linear order in the threadlike chromosomes. A chromosome, according to the data of the preceding chapter, contains about 30,000 macromolecules of nucleic acid. It is plausible that each macromolecule of the nucleic acid or nucleoprotein represents one gene. We have seen that the nucleic acids differ in composition, chiefly in the ratio of the individual pyrimidines and purines. It is possible that there are other differences between the macromolecules of the nucleic acids, such as variations in the order of the pyrimidine and purine residues along the chains, as well as variations in configuration. Moreover, some differences between the genes or structural units of the same chromosome may be due to the differences in the protein component (histone) attached to the nucleic acid. Both the nucleic acid and the protein moiety may be important as carriers of the individual characteristics of the cell, of the organism, and of the species. Genetics, the science of heredity and hereditary factors, is able to correlate certain characteristics of an organism with certain parts of its chromosomes. Since any species and any individual organism is characterized by a multitude of such features, there must be also a *multitude of individually different macromolecules of nucleoproteins* in the nucleus of a germ cell. Our chemical and physical methods, however, are much too crude to be able to discern differences between individual colloidal particles or macromolecules.

But let us return to the other components of the cell. A certain number of granular and fibrous bodies are found also in the cytoplasm of the cell. Very important among these bodies are the *mitochondria*, microscopic objects of the shape of short rods and fibers. These mitochondria contain most of the enzymes of the cells, hence the metabolic reactions occur chiefly around these particles. Other microscopic and submicroscopic structures include the so-

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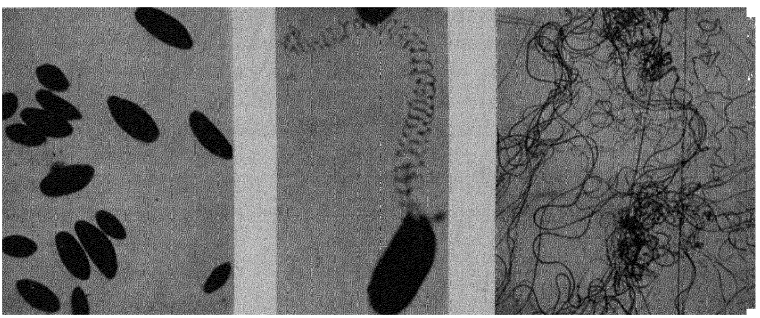
called *Golgi substance* and the *microsomes*. The former is fibrous, and of high lipid content; the latter are of colloidal dimensions ranging in size from 50 to 250 A. The colloid-chemical method of *differential centrifugation* has been successfully applied for the separation and isolation of these cell constituents in larger amounts. The nuclei appear in the heaviest fraction in low-speed centrifugation, the mitochondria in the next lighter fraction, and the microsomes and Golgi substance can be brought down only by ultracentrifugation[2]. Water, lower molecular proteins, and a variety of micromolecular organic and inorganic substances are present in the nuclear sap, and in the cytoplasm.

The consistency and water content of cells may vary within wide limits, depending on the material. Dry spores of low water content can be contrasted to marine organisms containing over 95% of water, although the most common amount of water in cells is 60–80%. The protoplasmic substance in most cells thus represents a jelly. A living cell, however, is a *highly organized* jelly in which a variety of enzymatic reactions occur in highly complex yet regular pathways. The framework of this organized gel of the cytoplasm is composed of linear biocolloids, chiefly proteins, polysaccharides, lipids and other substances. The framework is hydrated and linked in such a manner as to permit reversible changes in consistency and other mechanical properties. Most of the water, however, especially in the cells rich in liquid, is mechanically encompassed in the framework. Important also is the fact that most of the biocolloids found in the cells are *colloidal electrolytes*.

Many examples of biological systems are known in which the changes of properties are determined largely by the changes in particle shape and configuration. The hagfish slime is one of such examples[3]. The Pacific hagfish, *Polistotrema stouti*, when disturbed surrounds itself by a large volume of transparent, elastic jelly. Microscopic examination of the whole process showed that the animal first excretes a milky liquid of low viscosity containing egg-shaped particles about 0.1 mm long. When this secretion is diluted with water and agitated, the particles uncoil and produce a meshwork of fibers; the consistency of the slime thereby increases, and an elastic and transparent jelly is formed. The microscopic picture of the whole change is presented in Fig. 143 I, II, and III. The strands of this jelly are about 0.001 mm in diameter, and they are very long. The amount of the solid material, the fibrous protein named mitin, amounts to only 0.025% in this jelly. This example may

serve as an excellent microscopic model for the uncoiling of globular proteins, the molecules of which of course are much smaller than the microscopic particles in the hagfish slime.

The variety in structure, composition, and function of cells from various sources need not be emphasized. It is impossible in this short review to tabulate the variation in composition, or to list all the substances found in cells. Some important examples have already been mentioned, and other more specialized tissues will be described in the following sections. It must be recognized that, besides the purely biochemical methods of studying the metabolic pathways, and the purely physical methods of radiation biology, electron microscopy, phase microscopy[4], and other optical methods,



I II III

Fig. 143. I: The low-viscous secretion from the slime gland of hagfish. The particles have the length of about 0 1 mm. II: The egg-shaped particles unroll upon agitation. III: Fibers (about 0.001 mm in diameter) formed upon unrolling of the globular particles; the dispersion then sets forming a transparent jelly.
(From J. D. Ferry, *J. Biol. Chem.*, 138 (1941) 265.)

colloid chemistry has also contributed to progress in this field. Especially important have been the colloid-chemical approaches in the understanding of the gel structure of protoplasm, and of the colloid-colloid interaction between nucleic acids and proteins, between the acid polysaccharides and proteins, and between the proteins themselves. Some other important interactions, e.g., between the macromolecular proteins and micellar lipids will be mentioned later. Furthermore, the interpretation of the physical data found, e.g., by optical methods on various complex cell ingredients, has been greatly facilitated by the knowledge about the

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behavior of simpler colloidal systems. This is true, for example, with respect to birefringence studies on biological materials[5], as well as in the study of these materials by means of X-rays[6].

Significant practical problems may be solved when the cellular processes are better understood. Such a problem is the treatment of cells to produce certain hereditary changes. Mutations can be caused by radiations, chemicals and other means, but the mechanism of the change is unknown. Another important practical problem is the control of malignant growth. A number of factors known and partially known may cause uncontrolled, wild growth of certain cells which may invade vital organs, causing illness and death. Although purely biochemical factors are now considered foremost in the fight against neoplastic diseases, colloid-chemical aspects about the reactions between the various biocolloids of the cell also may be important. Both of the above problems depend directly on the solution of the problem of reduplication which so far has escaped a satisfactory explanation[7]. It is clear, however, that whatever the mechanism of the reproduction of the chromosomes and the whole cells may be, the colloid-chemical factors affecting the interactions between the nucleic acids and proteins, as well as the configuration of the macromolecules involved must be important.

2. The Ground Substance and Connective Tissues

Ground substance and connective tissues comprise the structural matrix of the animal body, forming and supporting other tissues and organs. A variety of large, fibrous macromolecules of proteins and polysaccharides are the major structural elements of these tissues which, like single cells, are as yet but partly explored. Microscopic examination has revealed three major constituents in these tissues: (1) collagen fibers, (2) elastic fibers, and (3) ground substance (Fig. 144). Cellular elements (fibroblasts) also can be discerned in the collagen fibers. The smooth collagen fibers are more or less oriented, and, as outlined in Chap. 27, they are composed of linear macromolecules of the collagen. It can be extracted from the tissue easily leaving behind the other ingredients.

The elastic fibers of connective tissues are composed of fibrous proteins (elastins) which differ from collagen in amino acid composition, resistance to hydrolytic cleavage, and in mechanical properties. The fibers are amorphous in examination with X-rays, and the

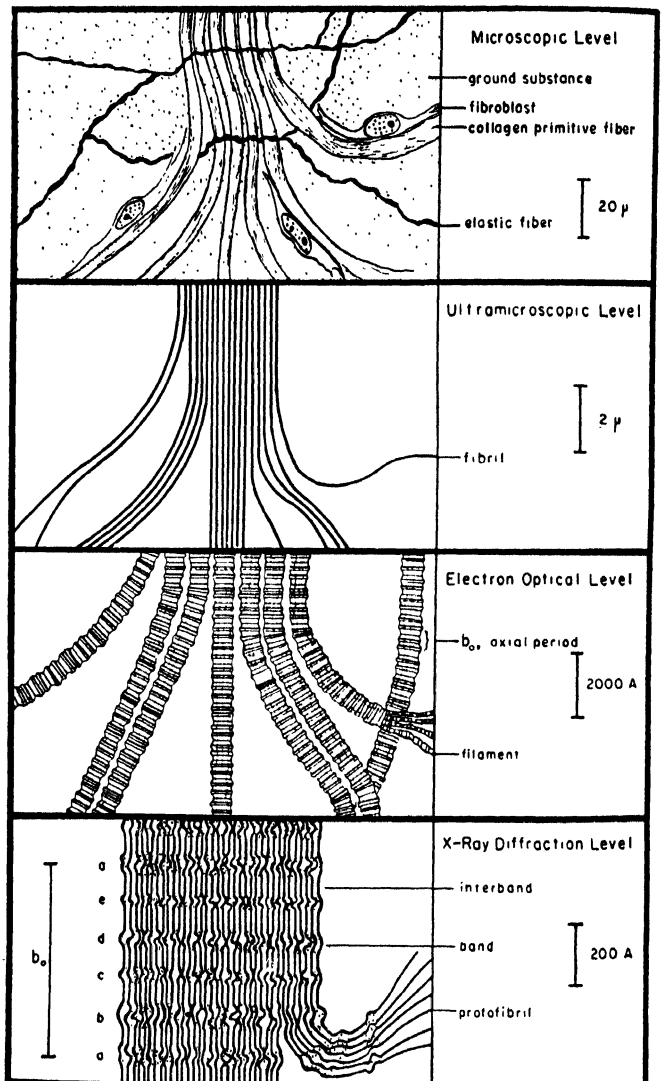

Fig. 144. The submicroscopic structure of connective tissue. (After R. S. Bear, *Advances Protein Chem.*, 7 (1952) 72.)

macromolecules are only slightly oriented on stretching. Amino acids with non-polar side chains predominate in the structure. The material exhibits rubber-like elasticity, and it probably has a constitution similar to rubber, i.e., the macromolecules are twisted in a highly irregular fashion and interlinked to a slight degree by cross-links[8]. The walls of large blood vessels are very rich in this elastic tissue.

The ground substance is the cementing matrix of the connective tissue. It can best be described as a firm gel with a very fine meshwork of linear biocolloids. Two chemically distinct types of macromolecules form the framework of the tissue: linear proteins resembling collagen, and acid polysaccharides, chiefly *hyaluronic acid* (see Chap. 24). The polysaccharide component seems to be important in determining the texture and pore size of the gel, since hydrolytic cleavage of the fibrous macromolecules results in a spectacular increase in permeability of the tissue[9]. This is important for many practical problems, such as facilitating the spread of injected drugs, the spread and growth of tumors, and the fertilization of egg cells. An enzyme—*hyaluronidase*—has been found and isolated in a nearly pure state from various sources, and administration of this enzyme results in a pronounced increase in the permeability of the connective tissue[10]. It was also demonstrated that this increase in permeability is due to the hydrolytic decomposition of the large fibrous molecules of the hyaluronic acid by the hyaluronidase. It is interesting that the enzyme is found in spermatozoids, bacteria, and snake and bee venoms, where its obvious function is to facilitate permeability. The lack of hyaluronidase may be one of the causes of sterility. The mammalian egg cell is surrounded by a layer composed of a framework of fibrous macromolecules resembling those of the ground substance. Clinical applications of the hyaluronidase preparations include enhancement of the effects of local anesthetics. Greater effectiveness and widespreading of the local anesthetic is produced with simultaneous administration of the enzyme. Dispersion of fluid collections (edema) also is facilitated by injection of the enzyme[11].

Hyaluronidase, like all other enzymes, is a protein. The interaction between the enzyme and hyaluronic acid substrate, although involving chemical reactions of hydrolytic decomposition, is at the same time a colloid-colloid reaction. Important is the fact that the enzymatic action may be reversibly or irreversibly inhibited by a number of substances, such as heparin, some chondroitin sulfates,

chemical derivatives of the hyaluronates, and anionic and cationic detergents. Blood serum also contains an inhibitor, probably a mucoprotein. It seems likely that the mentioned biocolloids as well as their derivatives act as competitors for the enzyme, as they show some similarity with the hyaluronic acid and its salts. The isoelectric point[12] of the enzyme is 5.7. It has free carboxyl and amino groups, and thus interacts with both negative and positive polyelectrolytes. The inhibition by other colloids thus is a colloid-colloid reaction, with binding of the enzyme to other particles. It is obvious that the amount of the physiological effect from the spreading factor will be affected not only by the amount of hyaluronidase present in the agent, but also by the presence and amount of various inhibitors. If the inhibiting competitor itself participates in the formation of the framework of the tissue (such may be some of the chondroitin sulfates), and, if the enzyme is able to hydrolyse the competitor, there may be no apparent inhibition in the *in vivo* experiments. Usually, however, the specifically acting enzyme does not attack the competitor to which it is bound.

Noteworthy is the observation of Seifter and Baeder[13] that partly depolymerized hyaluronic acid acquired the enzymatic activity of hyaluronidase. Streptococcal hyaluronic acid was partly cleaved by incubating it with testicular hyaluronidase for a short time, and significant enhancement of spreading in rabbit tissues was found by administering of the partly degraded polymer. Further degradation gave a product with little or no spreading action. These observations seem to indicate that the enzyme is related to the hyaluronic acid, and it contains some macromolecular carbohydrate component joined with a protein moiety, and that it probably may be formed from the hyaluronic acid-protein complex in tissues.

The formation of the biocolloids of the connective tissues in the body has also been studied extensively, chiefly by introducing radioactive isotopes. Sulfur ^{35}S, when introduced as radio-sulfate, is incorporated into various connective tissues, into the ground substance, cartilage, bone and dentine[14]. The incorporation can be visualized by autoradiographic techniques, and the method thus is able to give direct and unequivocal evidence about the pathways of the labeled atoms. Radioactive chondroitin sulfuric acid has been isolated[15], thus the autoradiography demonstrates the presence and hence, the formation of this polysaccharide. Chondroitin sulfate in young suckling rats is first formed in the cartilage of long bones,

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and later also in the ground substance. The ^{35}S labelled substance was rapidly hydrolyzed by hyaluronidase[16], and it was resistant to acids and heat, as is chondroitin sulfuric acid. Although these experiments show the location and development of the colloidal polysaccharides which contain sulfur, nothing is known about the mechanism of their formation. Good reviews about the recent literature on connective tissue are available[17].

3. Permeability Problems in Living Tissues

The facilitation of spreading by partial cleavage of the connective tissue framework is a very drastic treatment, and the process is relatively simple. Much more complex problems arise by considering the passage of substances through membranes and surface layers of cells. Even the cells and cell ingredients which do not possess distinct membranes have a surface layer which seems to differ from the interior. Considerable selectivity in the permeability of various micromolecular and colloidal substances is displayed by living membranes. Certain ions may be preferentially allowed to enter the cell, while others are left outside. It has been long recognized that living membranes do not act like sieves or filters, even though the pore size is of importance[1].

As mentioned in Section 1 of this chapter, the nucleus of some cells has a membrane or membrane-like surface, probably consisting of two layers. These membranes, however, have holes. The cytoplasmic particles called mitochondria also seem to be covered with a "skin" or membrane. Claude has described spectacular swelling phenomena of these particles[18]. When placed in water, the originally rod-shaped particles increase in size and become spherical. The swelling seems to be due to osmosis, since it occurs only in solutions of very low salt concentrations. In water the size of the particle may increase to the size of mammalian red cells, at which size the highly swollen particles finally disintegrate. Claude points out that under physiological conditions, when osmotic variations are slight and localized, the selective membrane must play an important part in the exchange of water and solutes between the mitochondrion and the surrounding medium. The chemical composition of the mitochondria is very complex; apart from various enzymes, the particles contain large amounts (about 25%) of lipids, especially of the phospholipid type, as well as ribonucleic acid. The particle has the consistency of a gel, the consistency may

reversibly change as can the whole protoplasm. Hence, the links in the framework must be relatively weak, although some of the ionic bonds between the positively charged amino groups and the negatively charged carboxyl or phosphate groups may play a certain role in the stability of the framework. The macro-ions of the biocolloids also influence the distribution of water and micromolecular ions of the medium (Donnan effects). The mitochondria are now considered as the "power plants" of the cell through which scores of substances are processed along an "assembly line" of enzymes, therefore the membranes of such particles must certainly be highly organised and adaptable. The present knowledge about the structure of these objects is scanty. Electron microscopic observations have not revealed much, and the original *in vivo* organisation is probably somewhat distorted during the preparation of the specimens. New methods in which undamaged, living objects can be directly observed, such as phase contrast microscopy, but with greater resolving power, would be very desirable.

More information is available about the membrane of the red blood cells (erythrocytes). The red cells differ considerably from the ordinary cells in that they do not contain a nucleus, and they can not reduplicate but are produced in certain tissues (the cells of the red bone marrow). The erythrocyte is a biconcave disc about 0.007 mm in diameter. It behaves as an osmometer by swelling considerably in media of very low ionic strength, similar to the mitochondria described above. Striking is the fact that the concentration of some micromolecular ions, such as potassium, is much higher in the cell than in the surrounding plasma, whereas the sodium content of the plasma is higher than that inside the erythrocyte. This example well illustrates the high specificity of ionic permeability and binding.

When suspended in water or a very diluted solution, the erythrocytes swell and finally burst releasing into the solution their contents which are chiefly hemoglobin. This process, called hemolysis, can be brought about also by means of surface active agents such as soaps and other detergents. It is obvious that hemolysis in water is due to osmosis, whereas hemolysis in isotonic solution (same osmotic pressure due to equal number of particles inside and outside the cell) by detergents indicates a specific damage to the membrane. Extensive studies have been conducted on the structure of these membranes which can be separated by centrifugation as an insoluble residue after hemolysis. Such an individual

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membrane is called the "ghost" of the erythrocyte. The membrane is about 250–300 A thick, and it is composed chiefly of lipids and proteins. The latter, called stromatin (from the word "stroma" assigned to the membrane material), has been slightly investigated. It is a fibrous protein capable of interlinking and thus forms a framework. The lipid components are much better known. The ratio of lipid to protein by weight in the bovine red cell membranes is 1 to 1.76, and the phospholipids lecithin and cephalin comprise the major part of this material. These molecules possess the peculiar structure of a highly polar "head", with the negatively charged phosphate ion and the positively charged tertiary amine ion, and a non-polar "tail". Such molecules, as indicated in Chapter 5, have the tendency to aggregate into oriented micelles. Moreover, an important lipid component is the less polar cholesterol. According to Winkler and Bungenberg de Jong[19], cholesterol has the role of a stabilizer which cements more firmly the phospholipid micelles.

The views about the structure and permeability of the erythrocyte membranes have undergone considerable change, and the all prevailing concepts now admit the presence of a complex mosaic structure. Colloid chemical considerations on the properties of gels (linear macromolecules) and micellar colloids have also been helpful in establishing these concepts. Being unable to present a detailed account of the present theories, we shall mention the conclusions reached by Parpart and Ballentine[20]. Their views are partially based on extensive studies on the binding of various lipids in the framework of the membrane. Cholesterol and half of the cephalins appear to be bound weakly, whereas all the lecithin, part of the cephalins and all the sphingomyelin is bound strongly. According to these authors, the membrane is composed of a highly porous framework of the protein (stromatin) in which appear patches of the lipids (Fig. 145). The polar heads of the lipids are turned toward the interface where part of them are strongly bound to the protein of the framework. The more easily extractable cholesterol and part of the cephalins are within the lipid patches. Water and hydrophilic substances enter the cell through the polar sections of the membrane, whereas lipophilic molecules are dissolved in the isles of lipids. The phospholipids and cholesterol probably form partially oriented micelles thus confering a certain stability to the lipid structure[21].

These models, however, explain only a small part of the complex

problem connected with permeability. Other important problems such as the effect of charges on the framework should be considered (see Chap. 15). They have been considered in quantitative theories[22], although only for relatively simple cases.

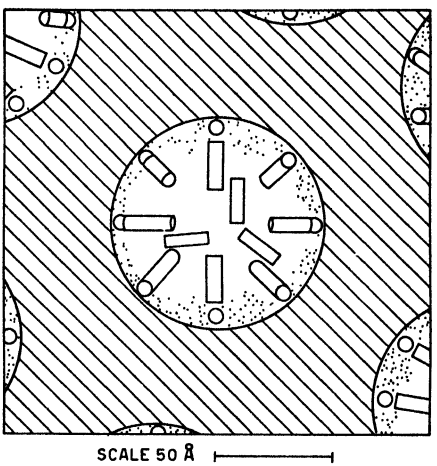

Fig. 145. Submicroscopic anatomy of the red cell membrane in a section tangential to the surface (according to A. K. Parpart and R. Ballentine). Hydrated protein is designated by cross lines; water by stippling; non-aqueous phase, clear; phospholipid by rectangles with circular (polar) heads, and cholesterol by rectangles. (From *Modern Trends in Physiology and Biochemistry*, Academic Press, New York, 1952, p. 144.)

4. Some Colloid-chemical Aspects of Muscular Contraction

The muscle tissue is composed of fibrous and globular proteins. The fibrous proteins have been reviewed in Chap. 27. Among the easily extractable globular proteins are the myogens some of which are important glycolytic enzymes. Relatively smaller amounts of lipids, glycogen, and inorganic and organic micromolecular compounds can also be extracted from muscle. The energy needed for muscular work is released by complicated enzymatic reactions. Glycogen, lactic acid, and adenosine triphosphate (ATP) are some of the important participants in these reactions. Hundreds of publications have appeared in the last two decades about the biochemistry of muscle, including work on isolated muscle proteins,

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and the mechanism of the reversible contraction[23]. Only a few of the more remarkable achievements can be mentioned in this brief review. One of the most interesting demonstrations was the finding that actomyosin threads contract on addition of adenosine triphosphate like live muscular fibers. It has been established that complicated enzymatic reactions occur and that the energy of contraction is released by enzymatic degradation of the ATP, and the contractile actomyosin itself acts as an enzyme in this exothermic cleavage of the energy-rich phosphate bonds. It has also been established that the presence of potassium, magnesium and other inorganic ions is essential in the contraction. Another important discovery is that the same adenosine triphosphate which releases energy is an active participant in the conversion of the G-actin into the fibrous F-actin. It has been calculated that for each molecule of globular actin which lines up in the F-actin filament one molecule of ATP is converted into adenosine diphosphate. One molecule of inorganic phosphate, electrostatically balanced with potassium ions, thereby is released, and simultaneously one sulfhydryl group disappears from the protein. These reactions are extremely rapid in the actomyosin fibers and in the cross-striated voluntary muscle, but they are slower in heart and in the smooth muscle.

Although the primary event in contraction as well as the precise mechanism is unknown, certain points are well established as a basis for speculation. The most probable assumption is that the neural stimulus causes some change in permeability of the muscular tissue system, and that a small change in the concentration of ions initiates the chain of reactions. Perhaps the most essential step in this chain is the linear association of the actin. The F-actin rods could be thought like spirals pulling together the extended myosin fiber. The net charge on the myosin fiber also may be important, and it is probably reduced on contraction and increased on relaxation. The adenosine phosphates moreover participate in the morphological changes of the biocolloids acting not only energetically but also as 'plasticising' agents. One of the chief functions of the 'plasticiser' may be to influence the charge equilibrium both at the myosin fiber and at the ends of the actin molecules, as well as to secure suitable stereochemical conditions for the reversible changes. The spatial conditions are important, because in solutions ATP causes dissociation of the actomyosin complex, whereas in fibers it causes association and contraction. The adenosine phosphates are

highly polar molecules, as are the macromolecules of the proteins participating in the muscular processes. The idea has been expressed that, by the influence of its polar groups, ATP facilitates the disruption of bonds which stiffen the helices of the macromolecules participating in these configurational changes, thus acting like a plasticiser.

5. Submicroscopic Structures and Biocolloids of Nerve Tissue

Significant progress has been made in elucidating the submicroscopic structure and organisation of nerve fibers[24]. Equally important discoveries have been made on the biochemical processes[25], as well as about the electrical processes connected with the transmittance of the nerve impulse[26]. In this section only the structural aspects will be briefly mentioned. The greatest advances in this field are due to biophysical, chiefly optical methods, whereas the

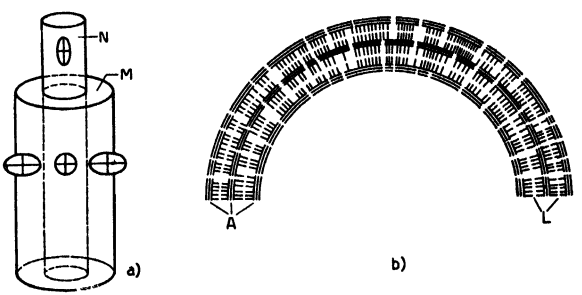

Fig. 146. Fine structure of nerves. (a) N–neurofibrillar string; M–myelin sheath. (b) Submicroscopic structure of the sheath (after Schmidt, 1937); A – lamellae of protein; L – bimolecular lipid layer.

chemistry of the biocolloids participating in the structures is still rather primitive. The first important discoveries on the structure of nerve fibers were made by microscopic studies with polarized light[27], as the fibers were found to be birefringent. Further significant discoveries were made by the use of X-ray diffraction techniques[28], and by electron microscopy[29]. Summarizing the recent experimental evidence, it can be said that the various nerve fibers are composed of the following major parts: (1) an outer membrane, (2) a myelin sheath, and (3) a central core or axon (Figs. 146, 147). The outer membrane is a meshwork of collagen fibers and other

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fibrous biocolloids. The myelin sheath has a laminated structure, and is composed of a series of thin-walled tubes fitted tightly into each other. Each layer is about 180 A thick and contains lipids, chiefly of the polar-non-polar phospholipid type, the molecules being oriented radially to the fiber axis. They probably are associated in liquid crystalline micelles of laminar shape with the non-polar hydrocarbon 'tails' turned inward, and the polar 'heads' directed toward the water and protein. Neurokeratin is the chief protein constituent of this section of the fiber. This is somewhat surprising, as keratinous proteins are usually found on the surface layers of higher organisms. Ingenious experimental work has been done in separating and studying the various layers of the myelin

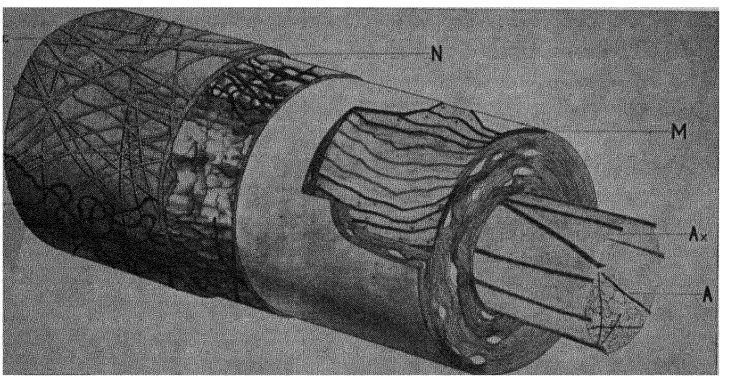

Fig. 147. Submicroscopic structure of a segment of nerve fiber, according to Fernández-Morán. M–myelin sheath, A–axon, C–collagen fibrils, E–dark smooth fibrils.

sheath, even in separating the individual membranes of the concentric cylinders of the fiber.

The presence of long fibers in the central core (axon) was ascertained by the classical birefringence studies[27]. The electron microscope has revealed in the core long, smooth, unbranched filaments which are 100–200 A wide. Some evidence has been presented that this part of the nerve is composed of linear nucleoproteins[30].

The layer structure of the myelin sheath with the oriented polar-non-polar lipid micelles seems to be of particular significance for ionic permeability, for the generation of bioelectric potentials, and for the transmittance of electrical impulses[31]. The mechanism

of these processes, as well as the roles of the various submicroscopic structural elements, are unsolved problems.

6. The Colloid Chemistry of Tanning[32]

This last section of the chapter is concerned with the structure of mammalian skin in relation to the industrially important process of tanning. Skin or hide is composed of three layers: the keratinous epidermis, the skin substance (corium), and the subcutaneous tissue. The upper layer of epidermis is composed of epidermin, a fibrous protein belonging to the keratins (Chap. 27). The medium layer (corium) is a meshwork of collagen fibers which in the subcutaneous layer become rich in the ground substance. From a colloid-chemical point of view, skin thus appears to be a complex gel possessing a framework of collagen and other fibrous proteins.

Tanning of hide is a very important practical process which was discovered at an early stage of civilization, probably independently, in several places and several times. The chief purpose of this process is to make the hide resistant to water and putrefaction, to preserve its flexibility, and improve its mechanical properties and appearance. This can be done by a sequence of treatments, the chief step being the soaking of the hide with a solution of some inorganic or organic substances. Salts of three-valent chromium are the most important inorganic tanning agents, and certain extracts from plants containing tannic acid are the most important organic reagents in this processing of skin. An important preliminary step in tanning is the removal of all keratinous material from the outer side, and also the subcutaneous layer. The removal of keratin is accomplished by immersing the hide in a solution of calcium hydroxide containing sulfides. On a prolonged treatment with such an alkaline reducing agent the keratins are partially dissolved and can be removed easily. It is established that this is partly due to the cleavage of the disulfide bridges, and partly due to the swelling effects. The cleaned swollen hide is composed largely of long interwoven fibers of collagen. The tanning process results in the diffusion of the tanning agent into the swollen gel (hide), and its combination with the fibers thereby interlinking them into a tighter network. Thus, tanning is a process of chain interlinking similar to vulcanisation. The amino groups of the collagen fibers are essential in the interlinking. The mechanism of interlinking is, however, little investigated. The binding of chromium and tannins seems to be chiefly

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by secondary linkages, since the binding with these agents is reversible, especially when the tanning agent is only shortly in contact with the collagen. Formaldehyde, on the other hand, 'hardens' hide by means of interlinking the amino groups of two chains through primary bonds. It may be also mentioned that the vegetable tanning agents are chiefly hexose esters and phenol-carboxylic acids of rather high molecular weight of 1500–5000. Tanning is followed by several other treatments for leather finishing. Complex dispersions containing polymers and dyes are used for this purpose. This finishing, which involves elaborate processing on special machinery, not only produces products appearing pleasant to the eye, but improves also the mechanical properties, such as flexibility and smoothness. The polymers introduced into the framework of the tanned leather act as plasticizers.

The reaction of collagen or gelatin with the tanning reagents can be demonstrated easily in a test tube. When a solution of tannic acid is added to a solution of collagen or gelatin, the mixture flocculates. Gelatin is rendered insoluble also by means of trivalent chromium, this fact being of great practical importance in the typographical work of preparing line cuts.

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CHAPTER 30

Blood as a Complex Colloidal System

1. Corpuscles and Plasma[1]

Blood is an example of a most complex disperse system containing a variety of entities of different sizes and shapes. The blood *corpuscles* represent the coarse entities, whereas the proteins are colloidal; moreover, micromolecular ions and organic molecules are present in the solution. When blood is drawn into a syringe containing an anticoagulant, such as heparin or oxalate, the fluid can be separated into two layers. The yellowish upper layer is called *plasma*, and the dark-red bottom layer is a concentrated dispersion of the corpuscles. The volume of the upper layer normally comprises 55% of the total volume of the blood. When no anticoagulant is present, and the blood is allowed to clot, the corpuscles are agglomerated into a gel which also encompasses some of the plasma proteins. The framework of the gel is made chiefly of *fibrin* which was formed from the plasma fibrinogen upon clotting. The liquid separating from this gel of clotted corpuscles is called *serum*. It has almost the same composition as plasma, with the exception of fibrinogen, which is absent in serum.

Three major types of coarse corpuscles are found in blood: (1) the *red cells* or *erythrocytes*, (2) the *white cells* or *leukocytes*, and (3) the *platelets* or *thrombocytes*. The red cells are the carriers of hemoglobin, which transports oxygen to the body tissues. As mentioned in the previous chapter, a red cell is a complex microscopic body covered with a rather rigid membrane. The interior of the erythrocyte is a colloidal solution of hemoglobin and other

proteins. A steady flow of substances occurs through the membrane of the corpuscle during the life of the erythrocyte. One cubic millimeter of human male blood contains 4.6–6.2 million red cells. The count in female blood is somewhat lower, being between 4.2 and 5.4 million. The number of red cells in other mammals is generally higher than that in humans, e.g., in mouse blood it is 7.7–12.5 million per mm^3. The number of white cells is smaller than that of red cells. There are 4,000–15,000 white cells per mm^3 in the blood of humans, 6,000–15,000 in the blood of rabbits, and 8,000–20,000 in dogs. The normal amount of platelets is 270,000–550,000 per mm^3 of human blood. The white cells and platelets are even more complex than the red cells, and have been investigated to a lesser extent, especially with respect to their submicroscopic constitution. This is due to the fact that appropriate methods for their study have been lacking. The functions of these bodies are known better than their structure. The white cells, which do not maintain a definite shape, are defense forces of the body, as they attack microbes and other foreign bodies which happen to enter the blood stream. The platelets are oval, round or biconcave discs about 0.003 mm in diameter. The chief function of the platelets is to participate in blood clotting.

Blood plasma is a viscous colloidal solution composed of about 90% water and 10% colloidal and micromolecular solutes. 7% of the solutes are proteins, 0.9% represent inorganic salts, and the rest are numerous organic substances, like carbohydrates, amino acids, urea, lactic acid, creatine, and many others.

2. Blood Groups and Agglutination Phenomena[a]

It was mentioned in Chap. 24 (Sect. 5) that human blood contains certain specific mucopolysaccharides which are important with respect to compatibility in blood transfusion. Both of the major group specific substances A and B are molecular colloids, the macromolecules being composed of carbohydrate residues with amino acids as minor constituents. The group-specific substances are bound to the membrane of the red cells.

The typing of blood is based on the fact that on mixing blood taken from different individuals flocculation or agglomeration may or may not occur. The agglomeration depends on the presence or absence of the group specific A and B substances, as well as on the presence of the so-called agglutinins or isoagglutinins which

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are present in the plasma. The latter are proteins of about the same molecular weight as the A and B group mucopolysaccharides. Depending on the specific flocculating or agglomerating capacity, an α-agglutinin and a β-agglutinin are distinguished. The plasma or serum which contains an α-agglutinin (or anti-A-factor) is able to agglutinate the red cells containing the A-substance. The serum containing the β-agglutinin (anti-B-agglutinin) produces flocculation of the B-type erythrocytes.

According to Landsteiner and others, four major blood groups can be distinguished: the O, A, B, and AB types. The determination of type requires the sera only of groups A and B. A drop of serum is placed on a microscope slide and a drop of the blood (containing anticoagulant) to be examined is added. The mixtures are then observed in order to see whether agglomeration of the red cells has occurred or not. If the erythrocytes of the unknown blood are not clumped by either the group A or B serum, the unknown blood belongs to the O-type. If agglutination occurs with the A-type serum, the blood is B-type, and vice versa. Finally, if it reacts with both A- and B-sera, it is classified as the AB-type.

The importance of these tests is obvious, since transfusion of a blood which causes agglomeration of red cells may result in clogging of the fine blood vessels. It should be pointed out that the problem of classification and determination of individual blood types is not yet completely solved. Transfusion of blood which appeared to be compatible in agglutination tests has been fatal in many cases in medical practice. Although a variety of unknown factors may be involved in these cases, the possibility of individual differences within the same group must be considered. Physico-chemical evidence also indicates that the A-group- (or B-group-) characteristic substances differ considerably depending on the source. For example, Gibbons and Morgan have isolated two B-group substances from the same source (ovarian cyst), and they found that the substances differ immunochemically, as well as in their ability to influence agglutination[3]. Moreover, they differ also in their particle size, the more active component possessing much larger particles (mol. weight 1.8 million) than the less active component (mol. weight 460,000). Unfortunately, it has not been completely established that the mucopolysaccharides of ovarian cysts and red cells are identical substances. All the information acquired in the last decade on the immense variability and microheterogeneity of proteins and polysaccharides throws doubt on such identity. Very

little work has been done on the group-active colloid which is bound to the red cell, or on the α- and β-agglutinins of serum, since the fractionation and isolation is difficult and the yields are very low. It is, however, very likely that a variety of similar but not identical macromolecules will be discovered within the same preparation of A- or B-group substance.

The physical differences found between preparations of the purified A- and B-substances are quite considerable, but the chemical differences in composition are small. The molecular weight of the A-substance has been found to be 260,000 by sedimentation velocity and diffusion[4], a value lower than that of the B-substance[3]. Furthermore, solutions of A were less viscous than those of B. The electrophoretic mobility was found to be approximately the same, being anodic at pH 4 and also at pH 8 for both the A- and B-substances. Most significant, however, seems to be the difference found in the optical rotation: the group specific A-substance was found to be dextrorotatory (+15° in water with green light), whereas one of the B-components, the very high molecular B′, is levorotatory (—20° in water). The data provide a solid basis for speculation on the factors involved in the specificity and mechanism of these agglutinations. Although a number of biochemical factors may be involved, the fundamental process is the colloid-chemical process of flocculation. At least two components participate in each of these agglomerations: the red cells and the agglutinins. The specificity is determined by the *configuration* of the A- or B-group substances attached to the surface of the erythrocytes, as well as by the configuration of the agglutinins. The forces which govern the clumping, however, are non-specific. Probably both electrostatic attraction and secondary linking are involved. The isoelectric point of the mucopolysaccharides is somewhat below pH 4, since the anodic mobility of the colloids is slow at this pH, but much faster at pH 8. The isoelectric point of the agglutinins, however, is near neutrality. Consequently, electrostatic attraction between the components is assured. A tendency to combine must prevail, according to these considerations, in all possible configurations, but agglomeration can occur only when spatial factors are favorable. Under such configurationally favorable conditions, then, the colloidal particles of the agglutinins react with the group specific substances, thereby linking the cells together, i.e., causing agglutination.

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3. The Immunochemical Reactions[5]

The important ability of the organism to become immune against a variety of disease-causing microbes and toxins involves a number of diverse colloid chemical reactions. Generally speaking, the phenomena are experimentally simple and well explored but little understood. A very elaborate terminology is used for the description of these complex colloid-chemical phenomena. Only a few of the most important terms will be mentioned here.

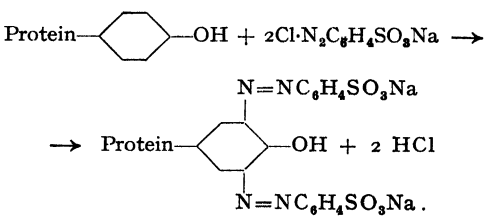
Antigen is any substance which, when introduced into blood or tissues, causes the formation of certain globulins called *antibodies*. Antigen reacts with antibody in a visible way, causing flocculation. For example, the virus which causes measles is an antigen, and incites the production of certain antibodies. These antibodies react with the virus, prevent its reproduction, and facilitate its destruction. After convalescence, the body still contains a surplus of antibodies, and it is now immune against the disease. Important is the fact that any antigen, e.g., a virus or a specific albumin, initiates the production of only one specific antibody. Hence, if an organism is immune against measles, it may not be immune against smallpox. The *specificity* of immunological reactions is very remarkable.

The first step in the immunochemical reaction involves the formation of antibodies. Despite considerable efforts directed toward the solution of this problem, very little is known about the mechanism of this antibody formation. Even the site of formation is not well established. Hypotheses have been advanced that antibodies are produced by interaction of the antigen with the normal γ-globulin components of the plasma. This seems to be substantiated by the fact that most of the antibodies have been isolated from the γ-globulin fraction of the plasma. However, it is unlikely that these reactions of antibody formation occur in plasma. It was demonstrated by using antigens tagged with radioactive isotopes that the agent goes primarily to bone marrow, liver, spleen or lung, which would indicate that these organs are the sites of antibody formation. According to Haurowitz[6], antibodies can be formed in all cells in which proteins can be synthesized, provided the cells are able to bind the injected antigen. The problem thus would be reduced to the general problem of protein synthesis, although in the case of antibody formation this synthesis may involve only minor alterations in the constitution of a starting material, such as γ-globulin. According to Pauling, this alteration involves a partial unfolding

of the γ-globulin molecule under the influence of the antigen; after the modified γ-globulin has been in contact with the antigen for a certain time, it is released, and the partially unfolded macromolecule refolds in a somewhat different configuration[7].

Not all proteins, when injected into the blood stream, act as antigens. In the first place, the proteins or blood corpuscles of the same organism, of the same species, or even of closely related species, are inactive. Human serum albumin is non-antigenic in humans but it is antigenic in rabbits. Even more antigenic are the plant proteins in both humans and rabbits. Of importance also is the fact that the property of antigenicity is conditioned by the molecular size and shape of the proteins. The relatively low molecular linear proteins, such as the protamines, are non-antigenic. Gelatin is another example of a non-antigenic protein. These facts indicate that a certain rigidity of configuration of the macromolecule is an important condition, since the fibrous macromolecules of gelatin are flexible and do not possess a definite, stable configuration. This is substantiated by the fact that partial denaturation results in a partial decrease in antigenicity, as found for example by Maurer and Heidelberger[8] with deaminated and denatured egg albumin. Removal of about one-third of the free amino groups by deamination, if denaturation is carefully avoided, did not influence the serological activity and specificity of egg albumin.

Extensive studies have been carried out on the immunochemical reactions of diazotized antigens. Diazo derivatives of proteins can be prepared easily without causing denaturation, as the reaction occurs at a low temperature of 0° to +10° at pH 7–9. The reaction occurs chiefly with the tyrosine residues of the protein, e.g., as follows:



It was demonstrated that the antigenic specificity of such modified proteins can be varied depending on the chemical structure of the introduced modifying group (haptene). The *polarity* and the *config-*

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uration of the modifying residue were found to be particularly important. Specificity with respect to antibody formation could be demonstrated even between the optical isomers of tartaric acid, which was coupled with the antigen through diazotartranilic acid. This shows that the spatial arrangement of the atomic groups on the surface of the molecules of the antigen is of paramount importance. Different antibodies have also been obtained with the *ortho*, *meta*, and *para* isomers of substituted diazo residues. This is the same kind of specificity as mentioned in the previous section with respect to blood group substances.

Neither antibodies nor antigens are homogeneous. Even crystallized protein specimens (e.g., egg albumin, serum albumin, pepsin) are known to be composed of several closely related components, hence, if used as antigens, they will produce a whole "family" of antibodies. Even more complex is the situation when bacteria or other parasitic microorganisms are used as antigens, since they release many types of proteins. Despite this heterogeneity of some antigens, the antibodies represent globulins which generally have the electrophoretic mobilities and solubility properties of the γ-globulins. These globulins are heterogeneous. No homogeneous antibodies either of ordinary γ-globulins or of those isolated from the blood of animals immunized with certain antigens could be found. All fractions, even those obtained by the most ingenious methods of fractionation (such as electrophoresis convection) proved to be heterogeneous[9]. The mean molecular weights of the antibody fractions have been determined, chiefly by sedimentation velocity and diffusion measurements. Some data are presented in Table 94.

TABLE 94

MOLECULAR WEIGHTS AND FRICTIONAL RATIOS (f/f_0) FOR SEVERAL ANTIBODIES

Horse pneumococcus	M = 920,000	f/f_0 = 2.0
Horse diphtheria antitoxin	184,000	1.4
Cow pneumococcus	910,000	2.0
Rabbit pneumococcus	157,000	1.4
Rabbit egg albumin antibody	165,000	1.6
Human pneumococcus	195,000	1.5

Calculations of the molecular dimensions, by assuming that the particles are anhydrous prolate ellipsoids, have shown that the

macromolecules may be about 25–47 A thick and approximately 200–900 A long[10]. Since a variety of macromolecules are present in each preparation, these figures have little significance. However, it is fairly well established that in most cases the antibodies obtained from sera immunized with certain antigens have molecular weights in the range 140,000–190,000. These antibodies do not differ chemically from the γ-globulin fractions which do not possess antibody activity. It should also be mentioned that the antibodies produced by means of antigens containing the residues of diazo-derivatives, arsenic, iodine or any other active group do not contain these groups. Generally speaking, the antigen acts as a kind of template impressing a specific pattern on the molecules it modifies.

Most important and relatively well investigated are the *reactions between antigens and antibodies*. The reactions occurring in living organisms are very complex, as often many other substances beside the antigens and antibodies are involved. Although the major reaction involves a colloid-colloid reaction, i.e., combination of the two proteins, other less specific components may participate. A macromolecule of an antibody may combine with one or more molecules of a toxin, thus inactivating it. Another possibility is that the large particle of a foreign microbe or virus may be covered with many antibody molecules, which case would represent a sensitization of coarse particles by means of colloidal particles. The reactions are studied mostly *in vitro*. The immune serum taken from some animal is mixed with a solution of the antigen used for immunization, the components being mixed in a series of tubes in different proportions. When the reaction is positive, the mixtures become turbid in some of the tubes. This indicates that large aggregates have been formed between molecules of the antigen and the antibody. The experiments with single antigens and antibody fractions of serum are more elucidating and easier to interpret. Depending on the amounts of the reacting colloidal components and their surface properties, macroscopically invisible combination or flocculation may occur.

Goldberg and Campbell[11] have studied the increase in the intensity of light scattered upon combination of bovine serum albumin (as antigen) and purified rabbit anti-bovine serum albumin antibody. With certain proportions of the components there was a strong increase in turbidity with time, indicating aggregation. The aggregation zone was broad, especially with excess antibody. A narrow-

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er zone of precipitation was found in the cases of diphtheria toxin and antitoxin (antibody) and with human serum albumin and its antibody from horse serum[12]. Maximum flocculation was found with equal numbers of macromolecules of the reacting components, and the turbidity decreased strongly when one of the components was in excess. This is a typical protein-protein reaction, since an excess of one component acts as a stabilizing agent (Chap. 14). Recent sedimentation studies have indicated that egg albumin (as antigen) reacts with its rabbit antibodies (used as the γ-globulin fraction of rabbit serum) according to the equations[13]:

$$\text{Antib.} + \text{Antigen} = (\text{Antib.}) \cdot (\text{Antigen})$$

$$\text{and} \quad (\text{Antib.}) \cdot (\text{Antigen}) + \text{Antigen} \rightleftarrows (\text{Antigen}) \cdot (\text{Antib.}) \cdot (\text{Antigen}).$$

The second reaction is reversible at pH 8.5 at an ionic strength (veronal–sodium chloride buffer) of 0.3 at 0° C. The first reaction between the molecules of antigen and antibody is reversible in acid solutions of pH 3.0–4.5. Equilibrium constants and thermochemical data were calculated, and it was concluded that only one ionized carboxyl group is involved in each antigen-antibody bond in this system[14].

Details of the mechanism of the combining of the colloidal particles of the antigens and antibodies, however, are unknown. As mentioned before, polar groups and molecular configuration are both important in determining specificity. Even if only one ionic bond may be involved in some cases, many secondary linkages certainly are participating in the formation of the associates. The specificity may depend on a definite, specific distribution of polar groups at the active sites of the antibody which is such as to secure maximum interaction. The stereochemical conditioning may involve spatial fitting of both components.

4. The White Cells

The purpose of white cells is defense. When a foreign body, for example, a microbe appears in the blood, the white cells "attack" it; the white cell sticks to the foreign particle, engulfs it, and slowly digests it. The process is called *phagocytosis*, it can be observed clearly under the phase contrast microscope, and beautiful microcinematographic demonstrations are possible by modern techniques. Although the process appears to be a definitely teleologic

one, the combination of the particles is a colloid-chemical process, and thus will be described here briefly.

Not all white cells or leukocytes found in the blood are alike. Several types can be distinguished, the lymphocytes and the neutrophils comprising the major portion. The white cells differ from red cells not only in their color but also in their shape and cellular content. One of the most important features of the white cell is that it does not possess a definite shape but is able to change it steadily. The cell, when alive, does not assume the smallest volume of a sphere but it assumes various asymmetric shapes. Limb-like pseudopods often extend from the body, especially if a foreign particle is present in the vicinity. The pseudopods then tend to embrace the particle and to engulf it. The membrane of the white cell is remarkably distensible, in contrast to the rather stiff membrane of the red corpuscle. It should be mentioned also that the white cell is preferentially wetted by water and hydrophilic liquids, whereas the red cell is wetted by oily liquids. The surface layer of the white cell is a negatively charged protein. This negative charge may serve to explain the fact that the white cells do not agglomerate, even when highly concentrated. However, the net charge of the particles is not a sufficient reason for explaining the selective combination with all of the invaders and simultaneous indifference to the normal components of the blood. The process of phagocytosis seems to be very complex and it is far from being wholly understood.

Inside a white cell are found various particles resembling the cellular mitochondria. These particles are rich in all kinds of enzymes, especially the proteolytic enzymes which catalyze the degradation of proteins. Noteworthy is also the fact that the white cells carry considerable quantities of antibodies. It has even been demonstrated that antibodies can be produced *in vitro* in the primitive cell precursors of white cells[15]. The antibodies carried in the white cells certainly play an important part in reacting with the toxic substances any foreign micro-organisms produce. Consequently, the white cells have a double purpose in the defense of the organism: they inactivate foreign bodies by macroscopic engulfing, and also act as carriers of antibodies and enzymes which take care of the liquidation of the invader.

Important progress has been made in the Harvard University Physical Chemistry Laboratory on the separation and preservation of leukocytes[16]. The optimal temperature for preservation was

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found to be +4° C. Temperatures below freezing were found to do irreparable damage to the cells. It was also found that the lifetime of isolated leukocytes can be prolonged by suspending them in a gelatin solution. A one per cent gelatin sets to a gel at +4° C, and such leukocytes suspended in a gelatin gel may stay alive for weeks. Certain additives (electrolytes and others) to the medium also improved the preservation.

5. The Clotting of Blood[17]

The clotting of blood is part of the complex defense system of the body. Fibrinogen is the chief starting material in the formation of the clot, but many other proteins and micromolecular substances are involved. An enzyme—*thrombin*—serves as a catalyst in the conversion of fibrinogen into fibrin (see Chap. 27); moreover, the calcium ions, a number of plasma globulins and a lipoprotein of the platelets all participate in the chain of reactions. The importance of all these components is ascertained by elimination. For example, if calcium ions are removed, the blood does not clot. Coagulation also does not occur if the platelets are removed even though the other components are present.

Thrombin does not exist in the activated state in the blood, but a passive precursor of it—*prothrombin*—is present. Calcium and the macromolecular components mentioned above are needed specifically for this activation of prothrombin or for its conversion into thrombin. Prothrombin and thrombin now have been isolated in a reasonably pure state, and it has been found that they are proteins containing a small amount of carbohydrate[18]. The sedimentation constant of prothrombin is 4.8 *S*, its intrinsic viscosity was found to be 0.041, and the diffusion constant $6.2 \cdot 10^{-7}$. The molecular weight was calculated as 62,700, and the macromolecules seem to be slightly asymmetric[19]. According to these data, the macromolecules seem to be of the same size and shape as those of serum albumin. The chemical composition of prothrombin, however, differs considerably from that of serum albumin[20]. The amino acid composition of human and bovine serum albumin is given in Table 80 (p. 447), which shows that the major amino acids in these proteins are glutamic acid (17%), lysine (12%) and leucine (12%). The major amino acids of prothrombin, however, are: glutamic acid (12%), aspartic acid (10%) and arginine (8%). Due to the fact that there are many carboxyl groups on the polypeptide helices,

prothrombin is an acidic protein, its isoelectric point[18] being estimated as 4.8. This is not much different from the isoelectric point of serum albumin. The example of albumin-prothrombin is a very instructive example to demonstrate that the same colloid-chemical properties of size, shape and charge do not necessarily imply similarity in other properties. Even disregarding the specific enzymatic activity of prothrombin, the solubility of the two proteins is different. In contrast to the very soluble albumin, prothrombin belongs to the relatively insoluble components of plasma, and it is precipitated together with the globulins. Also of interest is the specific adsorption of prothrombin on alkaline earth salts. Moreover, the adsorbed prothrombin can be eluted by means of concentrated citrate. The latter forms complex compounds with prothrombin and is even able to convert prothrombin into thrombin[21], a conversion which requires a number of components in blood[22]. The specific configuration and distribution of polar and non-polar groups on the surface of the macromolecules of prothrombin seem to be responsible for all this. A more thorough study of these problems indeed could elucidate the fundamental problems of the dependence of solubility, adsorbability and biochemical activity on the structure, configuration and surface pattern of the large molecules of proteins. An obstacle, however, is the low concentration of the prothrombin in plasma. It has been estimated that there are only 70 mg of prothrombin in a liter of plasma.

Apart from these intermediate steps in the clotting mechanism, most challenging and extremely interesting from a colloid-chemical point of view is the *initiation* of clotting. What prevents the blood from clotting in the veins? What elicits the chain of reactions resulting in clotting, when the blood drains out of the vein? Definite answers to these questions cannot be given as yet. However, the primary cause of clotting may be due to *surface effects* which produce some changes in some of the very labile components of the blood. This is substantiated by the fact that clotting time varies depending on the kind of surfaces with which the blood comes in contact after drawing. For example, clotting occurs faster when the blood is in contact with a plain glass surface than with silicone coated glass. It is possible that a kind of *surface denaturation* of the very labile components occurs at the blood-air or blood-glass interfaces. Which of the many components are affected first is not known; they may be the thromboplastin components of the platelets, the labile and versatile molecules of the prothrombin, or even something else.

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Extensive studies have been made on isolated fibrinogen solutions *in vitro*. When a small amount of thrombin is added to a dilute solution of fibrinogen, the solution remains clear for a certain time. A weak opalescence then appears, and the increase in the intensity of scattered light indicates that aggregation has occurred in the solution. At a specific moment the viscosity of the solution increases sharply, and gelation occurs. The gel becomes more and more rigid, and the opacity increases up to a certain limit. It can be shown that the aggregation and gelation of fibrinogen is also associated with chemical reactions incited by the thrombin. Some peptide bonds of the fibrinogen are opened, and fragments of peptides are split off. The NH_2-terminal residues in bovine fibrinogen are glutamic acid and tyrosine, while those in fibrin are glycine and tyrosine[23]. Hence, thrombin acts as a very specific proteolytic enzyme which activates the macromolecules of fibrinogen in a very limited and specific way. The activated molecules aggregate linearly as well as laterally, encompassing thereby large amounts of water. The framework of the gel may have a different colloid chemical stability with respect to maintaining its structure and water content. The very opaque gels are composed of a coarse framework, and they synerize easily, whereas the relatively clear gels have a fine and stable framework[24] (see Chap. 15). The chemical properties of the framework also may differ, as the gel or fibrin clot may be either soluble in concentrated urea solutions or it may be insoluble. In the urea-insoluble fibrin —S—S— bonds are assumed to be responsible for interlinking the network. Such insoluble clots are formed with fibrinogen which contains some of the serum factors other than thrombin participating in the clotting. However, if reagents are added which combine with the active sulfhydryl groups —SH, the clot is soluble in concentrated urea[25]. This supports the concept that no primary links are involved in building the junctions of the framework, if the active sulfhydryls are eliminated. Pure fibrinogen does not contain any free —SH groups, but they seem to be made accessible under the influence of the activating agents. The mechanism of the fibrinogen-thrombin interaction is unknown.

It is important not only to secure immediate clotting of blood in the case of injury, but also to prevent accidental unnecessary clotting. Failure of normal clotting ability is due to the absence of some factors involved in the activation of prothrombin, and is very dangerous because of possible large losses of blood on injury.

Unnecessary premature coagulation of blood in the fine blood vessels may be even more dangerous. The clogging of blood vessels owing to the formation of clots is often fatal. The importance of these problems is self evident, and extensive studies have been made on finding out the causes for accidental clot formation (thrombosis). Another promising approach to this problem is to try to find means by which to remove the clot. Since fibrin is digested by proteolytic enzymes, the latter are used for this treatment, especially trypsin, due to its activity in nearly neutral solutions. It has also been found that normal blood plasma contains a protein *plasminogen* which can be converted into a proteolytic enzyme—*plasmin*—, the latter then being able to digest the unwanted fibrin clots which occasionally are formed. The activation of plasminogen may be a complex chain of reactions, like that of activation of prothrombin, although it has been found that activation *in vitro* may be accomplished by means of a bacterial enzyme called streptokinase[26]. The process of activation of plasminogen *in vivo*, however, is unknown. Plasminogen itself is a globulin of low solubility, and it has been isolated and even crystallized[27]. A remarkable property of this protein is its stability toward denaturing agents, such as acids and alkalies. Unactivated plasminogen does not possess anti-clotting capacities since it is unable to digest clots.

Heparin is a relatively much better investigated anti-clotting agent of normal blood. It is a strongly acid polysaccharide (Chap. 24). Heparin acts both *in vivo* and *in vitro* in that it prolongs the clotting time of blood. It has been shown that heparin interferes with the complex mechanism of prothrombin conversion into thrombin. Heparin in blood is probably bound to a protein, and this complex probably regulates the clotting mechanism in that it prevents unnecessary conversion of prothrombin into thrombin[28].

Finally, the surgical applications of artificial fibrin clots are worthy of mention. The opaque fibrin gels release most of the water when placed under slight pressure[29]. A strong, rubbery fibrin sheet results which can be worked also into threads. These fibrin films and threads are occasionally used in surgery, e.g., in brain operations; they can be left in the body, as they are gradually absorbed without any harmful reaction.

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CHAPTER 31

Colloid-chemical Characterization of Biocolloids in Disease

1. Plasma Proteins in Disease[1]

It has been known for a long time that diseases affect the pattern of the blood plasma proteins. This is true not only in the case of infectious diseases in which certain antigens and toxins incite an increase in specific globulins which act as antibodies, but changes in plasma proteins occur also in noninfectious diseases. A very common change is a diminution in serum albumin. The changes caused by a disease may be of either a quantitative or a qualitative nature. Present efforts have been and are directed chiefly toward the determination of the *quantitative* differences, i.e., the albumin/globulin ratio or the per cent variations in the ordinary protein components, such as albumin, the α_1-, α_2-, β- and γ-globulins, and fibrinogen. However, quite unusual, *qualitatively* different proteins occasionally appear in the plasmas of sick individuals. Moreover, it is strongly suspected, although not yet definitely proven, that in disease the ordinary components, such as albumin, may differ qualitatively from normal albumin. The problem, of course, is of tremendous practical importance. It has been established that differences between plasmas from normal, healthy and sick persons indeed exist, although relatively little is known about the relation of the protein abnormalities to the diseases. Intrinsic difficulties in correlating the protein pattern with the specificity of the disease are due to three major reasons: (1) variations in the groups called "normals", (2) variations within the group of individuals with a disease, and (3) insufficient knowledge of the proteins involved in these studies.

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Many colloid-chemical methods have been and are used for the characterization of plasma proteins in diseases. Three major approaches will be discussed in this section: (1) flocculation methods, (2) sedimentation in the ultracentrifuge, and (3) electrophoresis. The *flocculation* methods are the oldest, but they still have sufficient diagnostic significance to be worth mentioning. Whole plasma or serum is tested, although occasionally some fractionation is performed and the isolated protein fractions are then investigated. Since such fractionation is laborious and time-consuming, and moreover, requires special experience, testing of isolated fractions has not acquired clinical significance. The various practically important clinical tests are performed with whole plasma or serum. The flocculation methods then show the *colloid-chemical stability* of the plasma proteins. It can be shown that the stability of the plasma proteins toward certain flocculating agents is different in various cases, and that it depends on disease. Certain correlations have been established between the colloid stability and the diseases.

A great variety of flocculation tests are known, and most of them have proved to be of little specificity and useless for diagnosis. However, about a half dozen procedures are extensively used with some degree of success. We shall discuss here some of the most popular plasma flocculation tests. The *Takata-test,* for instance, involves flocculation of mercuric oxide colloid in a plasma medium[2]. To plasma diluted to various degrees is added a sodium carbonate solution, mercuric chloride, and a dilute solution of fuchsin. Flocculation of the plasma protein and mercuric oxide, which is formed in the alkaline solution, is then observed. Since mercuric oxide is an unstable lyophobic sol, it flocculates easily, and the plasma proteins act as protective lyophilic colloids (see Chap. 13 and 14). Albumin has a greater protective power than the globulins, hence the stability of the mixture depends chiefly on the amount of albumin present in the plasma. The flocculation, however, depends also on the amount and properties of the globulins, so a clear cut relation between the concentration of the albumin and flocculation does not exist.

Another popular flocculation test is the *heat coagulation* of plasma or serum in the presence of calcium chloride. This test was introduced by Weltmann, and it has acquired great popularity in Central Europe[3]. Equal amounts of serum are mixed with various amounts of calcium chloride in a series of test tubes. The tubes then are placed in boiling water, and the heat coagulation is observed. Ten

tubes often are used, and the concentration of calcium chloride is varied from 0.005–0.05%. Low concentrations of calcium chloride promote heat coagulation, whereas high concentrations retard it and stabilize the proteins. Hence, in the case of normal serum or plasma there is a turbidity maximum in the series of tubes which contain very little calcium chloride (Fig. 148). In the sera of patients, however, this turbidity maximum is shifted to different concentrations of the calcium salt. The height and position of this turbidity maximum in the series can be correlated with certain illnesses. Studies on the mechanism of this heat flocculation have indicated that serum *globulins* are flocculated. The turbidity maxima depend

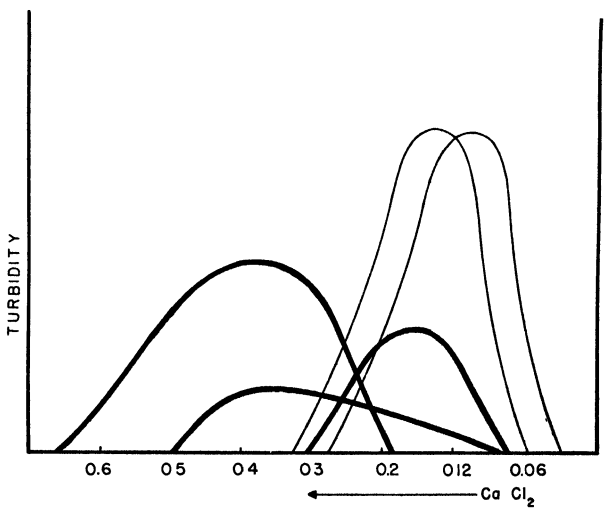

Fig. 148. The turbidity series with normal and pathological sera (Weltmann reaction). On the abscissa are plotted decreasing concentrations of calcium chloride, on the ordinate the relative turbidity. The three heavy curves represent three pathological cases. (After Wuhrmann and Wunderly.)

on the amount and kind of globulins. Albumin also is involved, having the role of a stabilizer. The chief process involved in this Weltmann reaction is *denaturation* of the plasma proteins by heat. The unique configuration of the polypeptide helices of the macromolecules is distorted by the molecular impacts, and some uncoiling occurs. The molecular asymmetry and the molecular volume and reactivity increase, and the denatured macromolecules stick together

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more readily than undenatured macromolecules. Since the globulins are less stable than albumin to heat coagulation (see Chap. 26), they become denatured and flocculate first. Very low concentrations of calcium chloride facilitate the flocculation, as the proteins have a negative net charge, and the calcium ions have a high flocculating power on particles which are negatively charged. High concentrations of calcium chloride have an opposite effect, as the excess of calcium ions will become adsorbed at the surfaces of the macromolecules and confer a positive net charge. Inasmuch as albumin has a higher adsorptive power than the globulins, it is stabilized more readily than the latter, and most of it stays in solution. Also interesting in this connection is the finding of the protein chemists at Stanford University (California) that serum albumin is protected from heat coagulation by the addition of caprylates[4] and similar fatty acid salts. Only albumin thereby is protected but the globulins become denatured and flocculate completely[5]. This again is due to the high adsorptive capacity of albumin, the caprylate increasing the charge as well as covering the helices, whereas no such effects occur on the surfaces of the globulin particles.

Many other flocculation tests have been proposed. For example, Wunderly and Wuhrmann have advocated flocculation by means of cadmium sulfate without heating[6]. Even gradually varied concentrations of simple flocculating agents, such as ammonium sulfate, can be used with some success[7]. The discontinuities in the curves showing the precipitation of the plasma components as a function of the ammonium sulfate concentration demonstrate in this case the solubility differences of the various proteins in concentrated ammonium sulfate solutions. Organic substances, such as alcohols, acetone, etc., can also be used as flocculating agents. In England a saturated solution of thymol is frequently used as a flocculating agent[8]. In the United States a colloid-colloid reaction introduced by Hanger is popular[9]. This is a very interesting one since it involves mutual flocculation between the proteins and micellar colloids of cephalin and cholesterol. The reagent is prepared by extracting 100 mg cephalin and 300 mg cholesterol with 8 ml of ether, and pouring the ether extract in 280 ml of water. The solution of these lipids in ether is micromolecular, but when this solution is mixed with excess water, the molecules of the lipids associate into micelles (Chap. 5). The colloid is liberated from ether (best under reduced pressure), and flocculation is tested by adding 1 ml of the reagent

and 4 ml of physiological saline to 0.2 ml of serum. The flocculation of the colloidal mixture is observed after 24 to 48 hours, and differences in the degree of flocculation are indicative of some diseases. It can be shown that flocculation in this case is caused chiefly by the γ-globulins. Cholesterol colloid alone, without cephalin, gives similar results[10]. The mechanism of these flocculations is not quite clear, and deserves further study.

According to Wuhrmann and Wunderly[1], the best diagnostic picture can be arrived at by applying several of the tests, especially the Weltmann heat flocculation, the thymol flocculation, the precipitation with cadmium sulfate, and the cephalin-cholesterol test. The coordination of these flocculation results with the electrophoresis data, which will be mentioned later, is of even greater value. Wuhrmann and Wunderly present in their book[1] quite convincing evidence that these coordinated tests have a definite diagnostic value. For example, in cases of acute inflammation (e.g., pneumonia), the Takata, thymol, and cephalin-cholesterol precipitation tests are negative, the cadmium flocculation is positive, and the Weltmann turbidity maximum is shifted strongly to the higher concentrations of calcium chloride. In the case of hepatitis (a liver disease) the cephalin-cholesterol, thymol, and cadmium sulfate tests are positive, while in the Weltmann series the turbidity peak tends to shift to the lower concentrations of calcium chloride. In the nephrotic syndrome (kidney disease) all flocculation tests are more or less strongly positive, and the Weltmann peak is very low and shifted strongly to very high concentrations of calcium chloride.

Sedimentation studies by means of the analytical ultracentrifuge aroused great hopes among some clinicians even in the early period of development of sedimentation analysis[11]. The method, however, never attained sufficient popularity because of the intricacy of the method and high cost of the instruments. Moreover, the method appears not to be as sensitive as expected in revealing chemically different components. Interesting abnormally sedimenting components have been revealed in many cases of malignant diseases[12]. The diagnostic value of these findings, however, is limited, as many plasmas from patients with different kinds of neoplastic growth show a normal sedimentation pattern. Quite interesting variations in the sedimentation pictures have been presented by Putnam[13]. The ultracentrifuge diagrams are presented in Fig. 149. On the top is shown the sedimentation pattern of normal serum; the following series of pictures represent the sedimentation of two sera

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(B and C) from two patients with multiple myeloma, which is a malignant disease of bone marrow. The series D, E, and F represent the sedimentation of globulins isolated from sera of patients having the same disease, and on the bottom (G) is a sedimentation diagram of normal γ-globulin. Plasma and sera of many patients with various types of neoplastic diseases have been run in the analytical ultracentrifuge in the Biochemistry Laboratory of this institute, and definitely abnormal patterns have been observed only in about 8% of all cases. Two such cases are presented in Fig. 150. Series 1 and 2

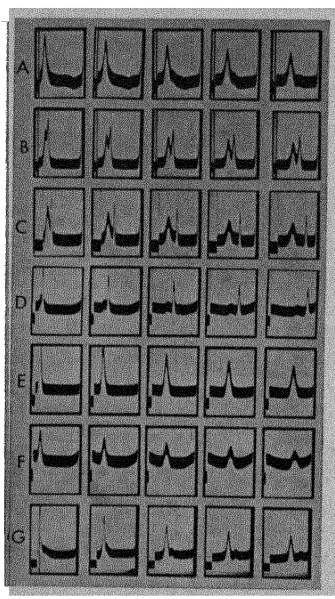

Fig. 149.

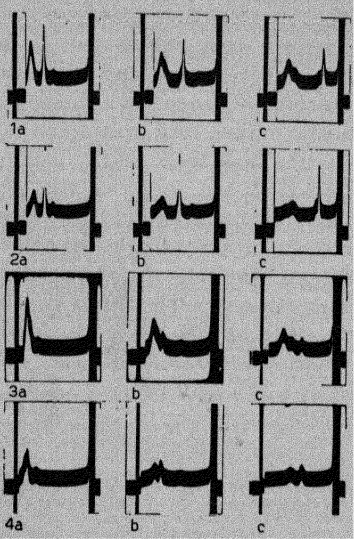

Fig. 150.

Fig. 149. Ultracentrifuge diagrams of multiple myeloma sera and of the isolated proteins. Sedimentation proceeds to the right with all photographs taken at 16 minute intervals. (From F. W. Putnam and B. Udin, *J. Biol. Chem.*, 202 (1953) 734.)
Fig. 150. Sedimentation patterns of abnormal serum globulins. Series 1, serum of a patient with malignant lymphoma; 2, abnormal globulin fraction from the same serum. Series 3, serum from a case of generalized lymphosarcoma; 4, abnormal globulin from the same case.

represent the sedimentation of two sera, and series 2 and 4 of the abnormal globulins isolated from these sera[14]. The diagnosis for case 1 (and globulin 2) was malignant lymphoma, and for case 3 (and the globulin fraction shown in series 4) it was generalized lymphosarcoma.

The third colloid-chemical approach—determination of the *electrophoretic mobility* (Chap. 10)—is the most efficient and promising method of testing plasma proteins in disease states. Microelectrophoresis on paper is now established as a simple and clinically feasible method, although the classical free boundary electrophoresis also is used extensively[15]. Both methods have been compared and satisfactory agreement has been found in most instances[16]. The subject is thoroughly treated in the book by Wuhrmann and Wunderly[1], who correlate the electrophoresis diagrams with other

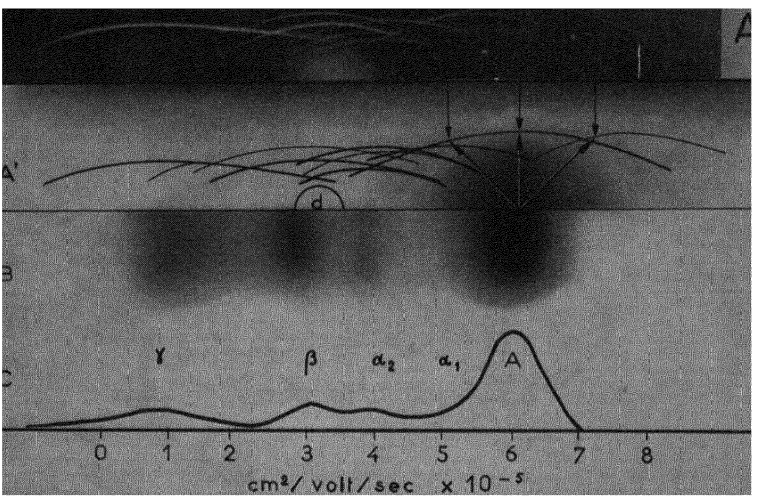

Fig. 151. The immunoelectrophoresis of Grabar. The white arcs in the top section (A) illustrate the zones of precipitation. The dark arcs in section A' show the zones more distinctly. Section B illustrates the separation in paper electrophoresis, and C the same in free boundary electrophoresis (for comparison). (From P. Grabar, C. A. Williams and J. Courcon, *Biochim. Biophys. Acta*, 17 (1955) 67.)

clinical data, such as the various protein flocculation tests and the sedimentation rate of red cells. It must be emphasized that an electrophoresis diagram alone is of little diagnostic value, since in many cases the electrophoretic patterns of sick individuals appear essentially normal. Moreover, similar variations in the electrophoretic mobility diagram often are produced by several different diseases. In clinical testing the mobilities of the various plasma or

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serum protein components are seldom calculated, but only the amounts of the components are compared. The most common changes in the patterns in the case of illness are lower albumin peak and higher α- and β-globulin peaks. The appearance of high spikes in the region of the β- or γ-globulins is less often observed. It is customary to make the runs in sodium barbiturate (veronal) buffer at pH 8.6, although components with abnormal mobilities may sometimes show up in acid solution[17].

Grabar and Williams[18] have proposed an interesting method in which the electrophoretic mobility test is combined with immunochemical precipitation. The proteins of serum are first resolved by electrophoresis in a layer of water-rich agar jelly. Subsequently antibodies in the serum of an animal immunized with human antigens are allowed to diffuse from channels parallel to the direction of electrophoretic migration. When the diffusing antibody meets its specifically combining antigen, the components flocculate, forming a white line. Since the electrophoretically separated antigen is located in a discrete zone, and since it diffuses radially to all directions, the lines of precipitate have the shape of arcs. This is illustrated in Fig. 151. Up to 18 components could be detected in human serum by the use of horse anti-human serum. At least two α_1-globulins, five α_2-components, and four β_1-globulins could be separated.

2. Abnormal Hemoglobins[19]

Hemoglobin, the oxygen carrier of the red cells, is composed of the small colored hem and the globin component (Chap. 26, Sect. 7), and this protein has been more completely investigated than have the very complex plasma proteins. Significant colloid-chemical differences have been found between adult and foetal hemoglobin, especially the observation that the latter shows a higher resistance to denaturation by alkali. Hemoglobin isolated from the blood of human embryo spreads on surfaces more slowly than does normal adult hemoglobin (Chap. 6). An important finding is that the alkali resistant hemoglobin may occur also in adult blood in some diseases, such as some types of anemias and leukemias[20].

Very interesting from a colloid-chemical viewpoint are the abnormal hemoglobins discovered in inherited anemias. It was found that in some cases of anemias the blood of the patients contained strangely shaped red cells resembling a sickle. Electrophoretic

analysis of the hemoglobin showed that the hemoglobin of the sickle-shaped corpuscles has a somewhat different mobility and different isoelectric point from that of normal hemoglobin[21]. Even more striking, however, were the differences in solubility between reduced normal and reduced sickle-cell hemoglobin. The sickle-cell hemoglobin appeared to be highly insoluble, and gels were formed readily in concentrated solutions. The cells themselves showed birefringence which indicates the presence of highly asymmetric oriented particles. Chemical differences were found in amino acid composition and in end groups, as well as in the amount of sulfhydryl groups. It was found that normal hemoglobin has two —SH groups per molecule (mol. wt. 68,000), whereas sickle-cell hemoglobin has three such groups[22].

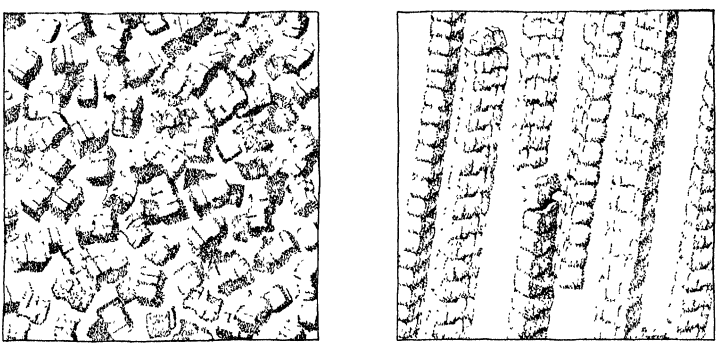

Fig. 152. Molecules of normal hemoglobin (on the left), and of oxygenated sickle-cell anemia hemoglobin (on the right), On the right, long rods of molecules of the sickle-cell anemia hemoglobin are shown, assuming parallel orientation. (From L. Pauling, *Proc Amer. Philos. Soc.*, 96 (1952) 564.)

An interesting observation is the formation of oriented particles and gelation of sickle-cell hemoglobins. Harris found[23] that concentrated solutions of sickle-cell hemoglobin became very viscous upon exposure to low oxygen pressures. When these solutions were observed under the phase contrast microscope, relatively large oriented particles (tactoids) resembling the sickled cells were visible. Harris concluded from these observations that the peculiar shaping of the red cells in inherited anemias is due to tactoid formation, i.e., orientation of long particles. Singer and colleagues[24] could not find definite tactoids under the microscope but they substantiated the fact that the solutions of sickle-cell hemoglobin set,

if they are exposed long enough to carbon dioxide in the absence of oxygen. The structural problems of these processes have been discussed by Pauling[25]. The mechanism of these aggregations, and the problem as to why normal hemoglobin does not form linear aggregates but sickle-cell pigment does, is still obscure. No differences are suspected in the hem-component, but differences indeed have been found in the globin moiety. The fashion in which the two components are united may also differ. The difference between concentrated solutions of normal and sickle-cell hemoglobin is illustrated in Fig. 152.

Three more abnormal hemoglobins have been discovered recently in the blood of patients with inherited anemias, and the proteins are designated as hemoglobins C, D, and E[19]. Hemoglobin-C, for example, differs in electrophoretic mobility from normal hemoglobin even more than does sickle-cell hemoglobin. These abnormal hemoglobins, however, appear very seldom.

3. The Urinary Bence-Jones Proteins

In addition to the very small amounts of proteins which normally appear in urine, abnormal proteins may appear in small or high concentrations in some neoplastic diseases, especially in multiple myeloma. It was found by Bence-Jones about one hundred years ago that these abnormal proteins coagulated on heating urine to 50–60° C, and that they redissolved on boiling; on cooling the boiling solution they precipitated again. This redissolving of a heat-coagulated protein is a quite unique property, and the test was considered to be of great diagnostic value. Many biochemists and colloid chemists have been engaged in the study of this abnormal protein. It was found to possess a high solubility comparable to that of albumin. Bence-Jones protein is soluble in water, and very high concentrations of ammonium sulfate (40–50% saturation) are required to salt it out. The isoelectric point was found to be near pH 5, although the values found by various investigators differed considerably (Gutman[1]). Other differences in properties among various specimens soon became apparent. Even the classical test of redissolving on boiling appeared to be inconsistent. All Bence-Jones protein specimens coagulate when urine or an aqueous solution is heated to about 50° C, but many specimens do not redissolve on boiling[26]. Differences were found also in electrophoretic mobility, in salting out[27], and in amino acid composition. The electrophoretic

mobilities fall in the range of the β- and γ-globulins, but the variations indicate the existence of a variety of Bence-Jones proteins[1]. Moreover, the protein found in the urine of one patient could be fractionated by either salting out or gradual heat coagulation into several components[27,28].

A recent thorough study on urinary Bence-Jones proteins from 18 cases of multiple myeloma indicates that there seem to be as many Bence-Jones proteins as there are individual cases of the disease. Certain groups, however, can be distinguished. For example, with respect to homogeneity in sedimentation, there seem to be reasonably homogeneous and very inhomogeneous polymolecular Bence-Jones proteins[29]. In this study of 18 cases the reasonably homogeneous group comprised 10 cases. The sedimentation constants of these proteins varied within the limits of 3.1–3.6 Svedberg units. The isoelectric point and stability of these proteins varied considerably. Four of the specimens were immunologically related, and their molecular weights were estimated to be 43,000. The molecules were split on acidifying to pH 3–4 without heating, or on heating a neutral solution. The remaining 8 specimens were very inhomogeneous, with relatively low average molecular weights.

The denaturation, flocculation and solubility of pure, isolated specimens of Bence-Jones proteins has been studied in the Biochemistry Laboratory of the M. D. Anderson Hospital and Tumor Institute. It was found that Bence-Jones proteins are readily denatured by propyl alcohol, and that the denatured protein is flocculated by 15–20 vol.-% propanol at pH 4.5–6.5. However, in water-propanol mixtures containing 30–50% propanol the denatured protein is more soluble than in solutions containing less propanol[30]. If flocculation is carried out in a series of test tubes with constant amounts of protein and at a constant pH of 4.5–6.5 with gradually increasing concentrations of propanol, turbidity peaks appear in time (Fig. 153). The variations in the flocculating and solvating capacity of the solvent of different propanol-water proportions must be considered. The suspended particles of the Bence-Jones protein precipitating in the mixtures with 15–20% propanol are compact, whereas in the media containing 40 or even 80% propanol they are translucent microgels. This gelation may be due to the formation of fibrous aggregates which seem to be highly solvated. Bence-Jones protein is solvated more by 40% propanol than by 20% propanol, an observation which was also directly demonstrated by solubility experiments[30]. It seems likely

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that the solubility in water-alcohol mixtures of high alcohol content is due to the high hydroxyamino acid content of the Bence-Jones proteins[31].

Many authors have tried to correlate the appearance of Bence-Jones proteins in the urine with the presence of abnormal globulins in the blood serum[29, 32]. It was found that the abnormal components of the serum have much larger particles than do the urinary proteins[13, 32]. The amino acid composition varies considerably, but similarities between the urinary Bence-Jones proteins and the serum globulins (especially the γ-globulins) sometimes are apparent. It could then be assumed that the urinary Bence-Jones proteins are

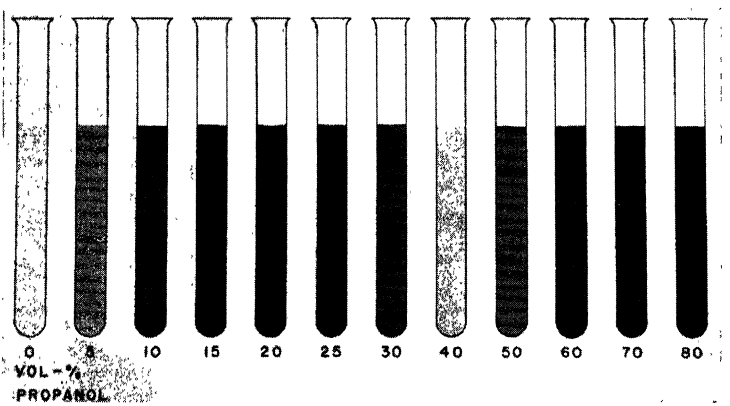

Fig. 153. Variation of turbidity in the flocculation of Bence-Jones protein with various concentrations of propyl alcohol. Darker shading means stronger turbidity.

products of cleavage of the larger globulin particles, and that the relatively smaller macromolecules are simply filtered out by the kidneys.

It is interesting to note that Landua succeeded in isolating a serum protein[28] which showed the positive heat coagulation and redissolving on boiling. This was a well established case of multiple myeloma in which the patient produced large amounts of Bence-Jones protein in the urine, and abnormal globulins also appeared in the blood. It is noteworthy that abnormal globulins are sometimes found in human blood serum which precipitate on cooling and redissolve on warming the serum to room temperature[1, 14, 33].

The serial flocculation of urine specimens with various concentra-

tions of propanol has been tried as a diagnostic aid in this hospital. Characteristic turbidity maxima were observed with 15–20% propanol (at pH 4.5–6.0) and 37° C in all of the cases in which some abnormal protein appeared in the urine. The turbidity in the mixtures containing 30–90% alcohol differed strongly from case to case. High turbidities were measured with 50–90% propanol when albumin was also present in the urine, whereas with pure Bence-Jones protein the turbidity values with these high concentrations of propanol were much lower. Unfortunately, the turbidity peak of the mixtures with 15–20% propanol is not specific for the Bence-Jones protein, as some of the α- and β-globulins which appear in urine in some kidney diseases (together with albumin) give similar peaks.

4. Abnormal Tissue Proteins in Disease

Abnormal proteins and other biocolloids have been found in various tissues. For example, Abrams and coworkers isolated a cryoglobulin from the lymph nodes of a patient suffering from lymphosarcoma[34]. Most interesting is the fact that the same cryoglobulin was also isolated by these authors from the blood serum of the same patient. The globulin had the unusual property of precipitating when the serum or extract of the lymph nodes was cooled to 4° C and the precipitate redissolved when the serum was warmed up to room temperature[33]. The cryoglobulin had the electrophoretic mobility of the β_1-globulins, and it appeared reasonably homogeneous on electrophoresis. Sedimentation in the ultracentrifuge revealed one major component, and small amounts of one or two other components. The diffusion constants have also been determined, and the molecular weight was calculated to be 600,000. The frictional ratio f/f_0 was found to be 1.45, and the axial ratio of the macromolecules was estimated to be 9:1, assuming zero hydration. The abnormal globulin was found in the lymph nodes in large amounts. The cause of its formation in the nodes is obscure, though it was suspected that it may be formed in an immunological reaction. The abnormal globulins are thought to be antibodies whose formation was incited by the presence of a very active antigen[35], for example, a virus. However, there is no experimental evidence to support this hypothesis.

Interesting studies on histones isolated from tissues have been carried out recently by Stedman and associates[36] at the University

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of Edinburgh, Scotland. In the first place, it was found that the amount of histones which could be isolated from neoplastic tissues is smaller than the amount isolated from normal tissues. Extensive studies in this field, however, indicated that these quantitative differences are not consistent. More consistent are the *qualitative differences* found between the histones isolated from normal and from neoplastic tissues[36]. According to Stedman, histones are cell specific, and qualitative differences in histones produce definite qualitative differences in the life of the cell. Transformation of a normal into a malignant cell thus would necessarily be associated with changes in the histones of these cells. Indeed, the Scottish

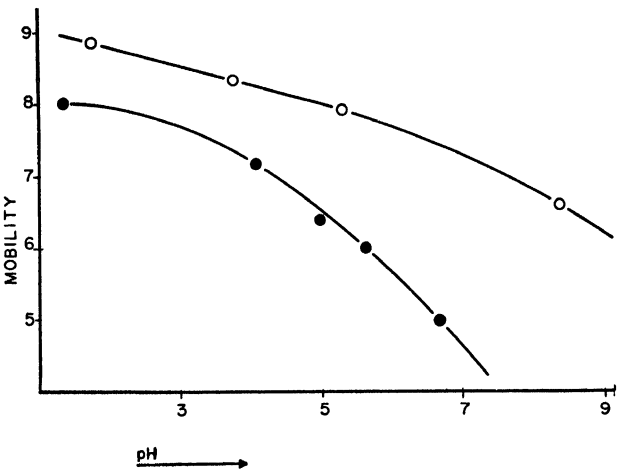

Fig. 154. The electrophoretic mobility of normal (upper curve) and rat hepatoma histone (lower curve) at different pH. (From Cruft, Mauritzen and Stedman, *Nature*, 174 (1954) 584.)

biochemists could show that all specimens of histones which were isolated from malignant tissues differed from normal histones in two respects. They proved to be less soluble, and to have smaller electrophoretic mobilities than normal histones. The isoelectric point of normal histones is known to be at pH 9.5–11, whereas the histones from malignant cells were isoelectric at a pH of approximately 7. Precipitation of malignant histones occurred upon dialysis of the solutions against a weakly acid buffer at a pH of 5–7, whereas normal histones never flocculate at this pH. Differences in electrophoretic mobility are shown in Fig. 154. It could even be shown

that the mobilities of the histones decrease as the tumors (experimental hepatomas in rats) grow. The differences in mobility seem to be caused by the same factors as the differences in solubility, namely by a smaller positive net charge of the malignant histones. Histones have a surplus of free amino groups over free carboxyls, hence there is a positive net charge in acid and neutral solutions. The greater this surplus, the greater the mobility and the solubility, if all other parameters remain constant. Hence, the histones from malignant tissues are expected to contain less of the basic amino acids than the normal histones, a fact which was confirmed by amino acid analyses.

5. Colloidal Components Resembling Viruses in Milk and Tissues[37]

Extensive studies on the incidence of breast cancer in mice have shown that the appearance of tumors is determined by three factors: (1) hereditary factors, (2) hormonal influences, and (3) on the presence of a virus-like colloid in milk. The influence of hereditary factors was ascertained by extensive breeding experiments in which certain strains with very high breast tumor incidence were made available. Apart from these genetic factors, the incidence of tumors can be increased or suppressed by the application of hormones, especially the estrogens. Finally, as was pointed out by Bittner[38], the incidence of tumors appeared to depend on some factor which is present in milk. Tumors could be induced even in male mice by treating them with both estrogens and milk which contains the tumor-inducing colloid. Although the active colloidal agent was also found in tumors and other tissues, the richest source is milk of the strains of mice showing high breast tumor incidence. Efforts directed toward isolation and characterization of the factor have been only partly successful. It could be shown that the active factor is not thrown down in the ordinary centrifuge, and that it runs through dense bacterial filters. The supernatant of milk from which the lipoproteins and casein have been separated still showed high activity for cancer induction. Observations under the electron microscope revealed the presence of spherical particles about 300 A in diameter[39] and smaller (Fig. 155). The material sedimented in the ultracentrifuge, and when no particles of several hundred angstrom size were visible in the supernatant, the latter lost its activity. Some investigators have assumed that the milk factor is a virus, as it seems to be reproduced from genera-

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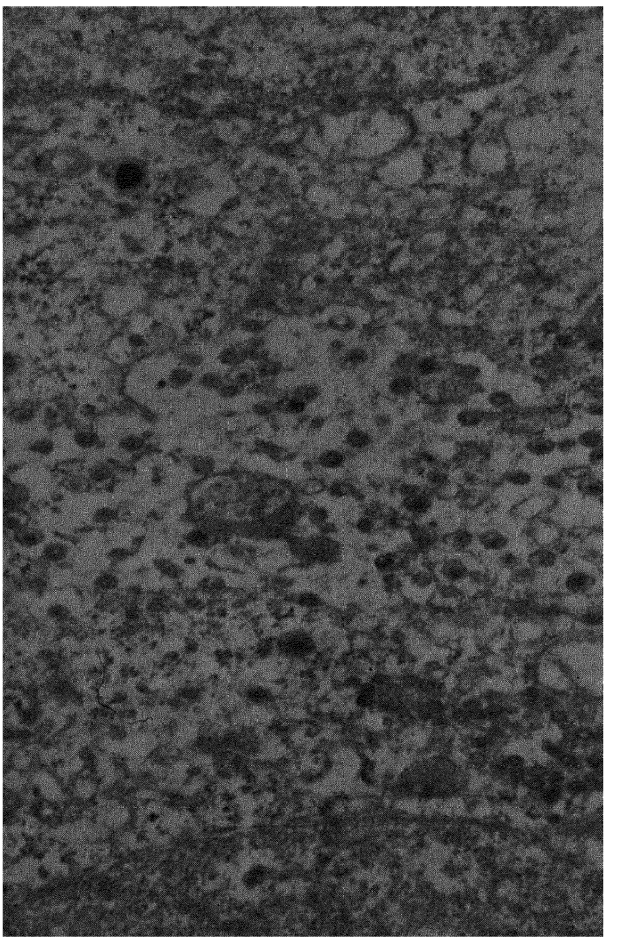

Fig. 155. Electron micrograph of the milk factor particles. This section is from a mammary tumor of mouse. The internal structure of the oval bodies is discernible. Magnification 30,400 ×. (Courtesy Dr. L. Dmochowski, Houston, Tex.)

tion to generation. The particle size, although not yet fully ascertained, would also indicate a material of virus-like nature.

The chemical composition of the active particles has not been well investigated. The particles contain nucleic acids and protein. Freeze drying and extraction of lipids does not affect the activity, but heating to 60° C denatures the factor, and it loses its activity for promoting tumor induction. The substance is relatively stable toward proteolytic enzymes. Most interesting is the fact that immunologically the milk factor is not active in mice, i.e., it does not produce definite antibodies and thus behaves as a normal—not foreign—constituent of the body. Hence, if this factor is to be classified as a virus, it indeed represents a quite specific type of virus. It is also noteworthy that lactating mammary glands were found to be richer in this factor than mammary tumor tissue.

Fractional centrifugation of various normal and neoplastic tissues has shown that the "milk factor" activity is associated with the microsomal fraction of the cytoplasm (Chap. 29). The tumor-inducing particles are of the same size as the microsomes. The idea has been put forward that this type of "virus" is a somewhat modified microsome. This explanation then would be compatible also with the immunological results, i.e., inability to produce definite antibodies. Claude[40] has pointed out the important fact that not only nuclear chromosomes but also the mitochondria and microsomes of the cytoplasm are particles capable of multiplication. If they are able to reproduce themselves upon division of cells, they probably may also mutate, and some of such mutations may lead to tumor-inducing agents.

It is possible that similar considerations may be valid not only for the induction of breast cancer in mice, but also with respect to breast cancer in humans, and even with respect to other types of human tumors.

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CHAPTER 32

Milk as a Colloid

Milk is an example of a disperse system which, like blood, contains particles of various sizes. There are coarse, microscopic particles of milk fat, occasional microbes, colloidal particles of milk proteins, and finally, a great variety of micromolecular substances. The latter include salts, lactose (milk sugar), and vitamins. Milk in the mammary glands is devoid of microbes, and milk fat can be considered liquid at body temperature. On standing in the air in a container, milk is invaded by various micro-organisms which find it an excellent medium to live in and multiply rapidly. This growth of undesired invaders is suppressed by heating milk for a certain time at a temperature of 60–70° C, and sealing the container.

The milk of various mammals differs somewhat in composition. Cow's milk contains 87% water, 3–4% protein, 3–5% lipid, 4.5–5% sugar, and 0.7% ash. Human milk contains less protein (1.0–1.5%) and less ash, but more sugar (7–7.5%) than cow's milk. Milk is not only a full value food for the new-born, but it also contains a considerable amount of antibodies. This is especially true with respect to the milk produced during the first days after birth. In this time, human milk contains more proteins than later, the excess proteins being immune globulins. The colostral milk of the cow contains up to 20% protein, most of these proteins being immune antibodies destined for the protection of the new-born. Gelation of the colostrum on heating is due to denaturation and coagulation of these globulins. On boiling ordinary milk only a small part of the proteins coagulate; the skin formed on the top of boiled milk is composed of this coagulated protein lifted to the top by the light

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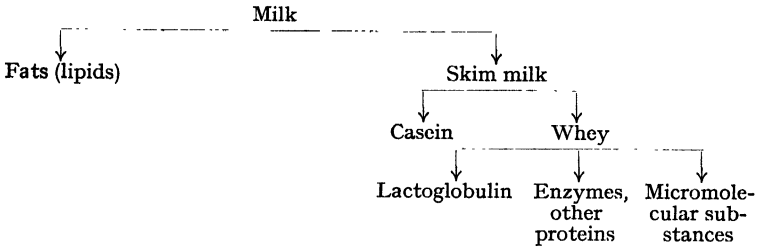
droplets of lipids. A partial separation of the lipids (milk fat) can be achieved by spontaneous creaming on standing.

Cow's milk is not only an important food product but also a starting material for other food products and materials important in industry. The food product technology includes manufacturing of butter, cheese and condensed milk; moreover, casein is used as starting material for the manufacture of glues, casein plastics and artificial protein fibers. The first step in many of these procedures is the removal of the fat component. This is accomplished by means of centrifugation, the fat being thrown to the top as the lighter component. The heavier large portion of the liquid is called *skim milk*. This is a very polydisperse colloid which contains all the ingredients of milk, except most of the lipids. Casein is separated next by increasing the acidity to a pH of approximately 4.7. Casein precipitates at this pH and is removed by either filtration or centrifugation. The residual colloid is called *whey*. It contains a number of proteins, chiefly β-lactoglobulin, some enzymes, and all of the micromolecular salts, sugar and water-soluble vitamins. The course of separation can be illustrated as follows:



Ordinary untreated cow's milk, if left at room temperature, becomes acidic in time. This is due to the activity of microbial enzymes which convert milk sugar (lactose) into lactic acid. When the pH drops to 4.7, the milk curdles. This curdling is due to the coagulation and gelation of the casein.

1. The Caseins of Cow's Milk[1]

Approximately 80% of the proteins of cow's milk is casein. It is separated from skim milk by precipitating with acid. Residual lipids are extracted from the precipitate with ether, and the casein is purified by redissolving in alkali and reprecipitating. This proce-

dure is repeated many times, avoiding surplus alkali and higher temperatures in order to evade denaturation. Casein is an acid protein containing a surplus of free carboxyl groups and chemically bound phosphoric acid. Calcium ions are the chief counter-ions in the colloidal casein of milk.

Casein is a heterogeneous protein. The solutions are polydisperse on sedimentation tests in the ultracentrifuge. They are heterogeneous also in electrophoresis. Moreover, at least three chemically different components can be separated by fractional precipitation. According to recent determinations, casein is composed of 75% α-casein, 22% β-casein and 3% γ-casein[2]. The α-casein has the highest phosphorus content, and also the highest electrophoretic mobility. γ-Casein contains but traces of phosphorus, and it moves very slowly on electrophoresis. The separation of the chief components (α- and β-caseins) is accomplished best by the method of Warner[3]. Casein is dissolved in dilute sodium hydroxide solution to give a 1% solution of pH 6.5. The solution is chilled, and acidified with excess hydrochloric acid to pH 3.5. The protein remains in solution, the particles now possessing a positive net charge. The solution is diluted with water, and the precipitation of the α-casein fraction is accomplished by adding 0.01 N sodium hydroxide until pH 4.2 is reached. The protein fraction then is purified by redissolving and reprecipitating it at the same pH. The β-casein fraction remains in the supernatant.

The amino acid composition of the α- and β-caseins is different, as shown in Table 95. The composition of the β-lactoglobulin of whey also is shown[4].

The average molecular weight of the caseins depends on various factors, and the casein particles are associates of many macromolecules. Disaggregation into single macromolecules occurs in alkaline solutions. The relative particle weight varies from 15,000 to 400,000, depending on pH and other factors. According to recent studies of von Hippel and Waugh[5], complete and reversible dissociation of the casein particles occurs at pH 12, and this is when α-casein has a molecular weight of 13,000–15,000, and β-casein 15,000–25,000. Sedimentation, diffusion and viscosity studies indicated that the macromolecules are asymmetric, with the axial ratio of 12:1. Aggregation of the macromolecules occurs on neutralizing some of the excessive hydroxyl ions, and a new peak of heavy sedimenting particles occurs in the sedimentation diagrams when the pH is decreased to 10.8. It appears that only the α-casein

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TABLE 95

AMINO ACID COMPOSITION OF MILK PROTEINS
(g amino acid in 100 g protein)

	α-Casein	*β-Casein*	*β-Lactoglobulin*
Alanine	3.81	1.99	7.07
Glycine	2.26	1.56	1.50
Valine	6.3	10.2	5.67
Leucine	7.9	11.6	15.48
Isoleucine	6.4	5.5	5.88
Proline	7.57	15.1	5.27
Phenylalanine	4.6	5.8	3.86
Tyrosine	8.1	3.2	3.69
Tryptophan	1.6	0.65	1.92
Serine	6.3	6.8	4.07
Threonine	4.9	5.1	5.15
Cystine/2	0.43	0	2.29
Cysteine	0	0	1.10
Methionine	2.5	3.4	3.21
Arginine	4.3	3 4	2.88
Histidine	2.9	3.1	1.60
Lysine	8.9	6.5	11.30
Aspartic acid	8.4	4.9	11.46
Glutamic acid	22.5	23.2	19.10
Phosphorus	0.98	0.61	

thereby aggregates, whereas the β-casein remains monomeric. The aggregation of the α-casein continues up to pH 7, and the β-casein does not change, if the solutions are kept cold. At higher temperatures of 32° aggregation of the β-casein also occurs. It must, however, be emphasized that the solutions of casein do not flocculate on heating. The aggregation and flocculation phenomena are generally very complex, especially if salts are also present in the solutions.

The solutions of the caseins, which are alkaline with respect to the isoelectric point of the caseins, contain negatively charged particles. These colloids flocculate if salts with bi- or polyvalent cations are added to the solution. The acid solutions of the caseins, on the contrary, contain positively charged particles which are most readily flocculated by polyvalent anions. Flocculation can be accomplished also by alcohols and similar protein precipitants, especially if small amounts of salts also are present. In the absence

of salts, however, flocculation of casein with ethyl-, and especially with propyl alcohol, is incomplete or else does not occur at all, even at a pH close to the isoelectric point. Water-propanol mixtures containing about 50% propanol are able to peptize casein, a phenomenon which can be observed with several other proteins (see Sect. 3, Chap. 31). Even greater peptizing or dissolving capacity is shown by such mixed solvents which contain a concentrated salt solution instead of water[6]. Large amounts of casein can be directly dissolved in a mixture of propanol-water (in the proportion 1:1) containing, for example, 0.2–0.3 *M*/1 calcium chloride. Mixtures of water with pyridine, dioxane, and several other solvents have a similar peptizing capacity for casein. The dissolution is facilitated by warming, and probably is associated with denaturation.

Streaming birefringence and viscosity of casein solutions has been studied extensively by Nitschmann and associates[7]. The casein colloids become weakly birefringent on streaming, and the solutions have a moderate viscosity. The streaming birefringence and viscosity data, according to Nitschmann, indicate an axial ratio of about 8:1. The intrinsic viscosity depends on the pH of the solutions. The viscosity is reduced upon the addition of salts, which in this case, seems to be due to lateral aggregation of the rod-shaped particles. The diminution of the net charge, brought about by the oppositely charged ions, also may contribute to these effects[8].

Deamination of casein is accomplished easily by means of nitrous acid in diluted, weakly acid solutions of casein in acetic acid. Nitrogen is evolved thereby, and a yellowish desaminocasein precipitates. The chief chemical change in this reaction is the replacement of the free amino groups of the lysine residues with alcoholic hydroxyl groups. The resulting desaminocasein is soluble in alkalies, and the solutions are very viscous[9]. Denaturation probably occurs in this treatment, associated with the uncoiling of the peptide chains.

Extensive studies on the electrophoretic mobility (with various buffer solutions), sedimentation and diffusion of casein have been continued recently in various laboratories. Sullivan and associates[10] found from sedimentation and diffusion data that the molecular weight of β-casein is 24,000, if the temperature of the nearly neutral solutions is below 15° C. For the α-casein a higher value of 121,800 was found. It was also found that the aggregation of the α-casein is dependent more upon ionic strength than on temperature. Electrophoretic analyses of solutions containing urea showed increased

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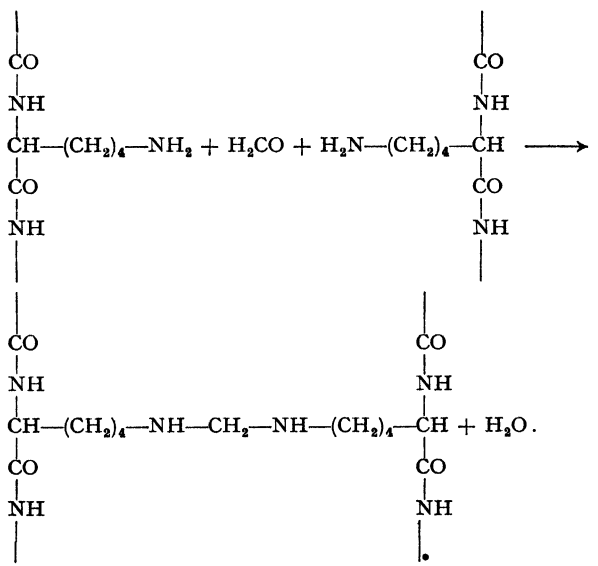
amounts of β-casein, even up to 33%. The authors conclude that complex particles between the α- and β-caseins are formed in solutions.

Light scattering studies of the solutions of α- and β-casein have revealed a different behavior of the components to the flocculating effect of potassium chloride[11]. The α-casein aggregated more rapidly than β-casein. Moreover, the aggregation of the α-casein was reversible which was not the case with β-casein. Halwer came to the conclusion that casein behaves like a denatured protein[11]. Owing to the ease with which the aggregation effects occur, the entities in solutions can not be regarded as macromolecules, but rather as molecular associates without any definite molecular weight.

The clotting of milk or casein solutions by the enzyme rennet is of great practical importance in the manufacturing of cheese. The colloid-chemical changes occurring in this process have been observed by means of the electron microscope[12]. Particles with a diameter of 500–1000 A were observed before the addition of the enzyme, and larger round particles were present when calcium chloride was added. After the coagulation by rennet, a reticular web instead of round particles could be seen in the electron microscope. The transformation continues even after the coagulation, in that the rounded particles in the aggregates are replaced by irregular filaments. The chemical changes produced by the enzyme have not been fully clarified as yet. It was assumed that rennet acts as a weak proteolytic enzyme. Recent studies with pure, crystallized rennet preparations have revealed that a peptide is split off of the α-casein component by the enzyme[13]. This distortion of the particles then incites aggregation which is chiefly linear. A fibrous meshwork thereby is formed, in which further changes occur, depending on a variety of factors, such as the presence of enzymes (in the case of manufacturing cheese), pH, and temperature. The process of clotting by rennet has been interpreted also as depriving the complex casein particles of a stabilizing component which could be thought of as a protective colloid. Whatever the mechanism of rennet coagulation is, the final product is a linear colloid. Elastic threads can be drawn from heated cheese. These threads, as well as the microfibrils observed in the electron microscope, are not composed of extended linear molecular chains, but they are linear aggregates of the denatured, partly disorganized (but not fully uncoiled) macromolecules of casein. The submicroscopic structure and mechanical properties of the products (cheeses) obtained by the coagulation of

casein by rennet depend greatly on the procedure as well as on the composition of the starting material. Cultivated strains of bacteria are added in order to accomplish enzymatic processes leading to certain changes in flavor and other properties.

The commercial importance of casein adhesives, plastics, and fibers is still quite considerable[14], especially in countries with extensive dairying. Since butter is the most demanded milk product, large quantities of skim milk remain as a by-product, hence a cheap source for the manufacturing of casein. Artificial horn (galalith) is one of the most important casein plastics. It is made by kneading dry casein with water, squeezing the hot dough through a screw press, shaping in sheets, and hardening the sheet-shaped plastic in dilute formaldehyde solution. Colored material and other ingredients are added before final shaping and hardening. Formaldehyde reacts with the free amino groups of the casein (chiefly the free amino groups of the lysine) forming methylene bridges between the chains of the macromolecules:



Casein horn is hard, tough, and sufficiently elastic. It is an insoluble polymer of high chemical resistance. Its chief disadvantage is absorption of moisture which is associated with deterioration of the material.

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Artificial casein fibers are manufactured by dissolving casein in sodium hydroxide and spinning the colloid into a solution of sulfuric acid containing salts. The threads are hardened by treatment with formaldehyde. The tensile strength of the casein fiber is relatively low. The fiber is inferior to wool and rayon, and it is used blended with wool and other fibers. Improvement of the mechanical properties is achieved by introducing acetyl groups, as well as by adding carbon disulfide to the alkaline spinning solution[15].

2. The Whey Proteins[16]

Whey is a colloidal solution containing numerous components, as can be demonstrated by sedimentation runs in the ultracentrifuge or by electrophoresis. The major protein component of whey is *β-lactoglobulin* which can be prepared from whey as a crystalline product as follows[17]. The whey is adjusted to pH 6 by means of ammonium hydroxide, and the least soluble globulins are removed by precipitation with ammonium sulfate of 2.3 *M* per liter of the mixture. The protein precipitate is removed, and the concentration of the ammonium sulfate in the solution is increased to 3.3 *M*/l by adding solid sulfate. At this high salt concentration the whole "albumin fraction" of the whey is precipitated, including the β-lactoglobulin. The salted out protein mixture is dissolved in water and dialyzed. When the dialysis is complete, the pH is adjusted to 5.2. The β-lactoglobulin then crystallizes out, and can be purified by recrystallization.

β-Lactoglobulin is one of the best investigated proteins, and excellent up to date reviews are available[16]. The molecular weight of the protein is 35,000, the same values being obtained by osmotic pressure, by sedimentation-diffusion, and by light scattering[18]. The protein is practically insoluble in water and in salt solutions, but it dissolves in dilute acids and alkalies. Although quantitative solubility determinations seemed to indicate homogeneity, electrophoresis of the ordinary preparations at pH 4.8 and 6.5 revealed the presence of three components[19]. Further fractionation of the crystalline preparations was attempted, for example, by alcohol fractionation at low temperatures[20]. The amino acid composition of β-lactoglobulin is shown in Table 95. All essential amino acids are present in this protein, the high percentage of lysine being conspicuous. One molecule of β-lactoglobulin contains 31 free ε-amino groups of the lysine residues, but only 19 of them react with

fluorodinitrobenzene (Sanger's reagent, see Chap. 26). Three N-terminal leucyl groups per molecule have been found by Sanger's method[21]. This indicates that there are three polypeptide chains in the macromolecule. It could also be demonstrated that only two of the four histidyl residues are reactive, and that all four become reactive after denaturation of the protein. In spite of the high content of lysine, the macromolecules of β-lactoglobulin have an excess of free carboxyl groups, hence the macromolecule is weakly acid. The isoelectric point of the protein is at pH 5.2. The denaturation of β-lactoglobulin has been studied by several authors[22].

There are several other interesting proteins in whey. A crystallized α-lactalbumin was isolated from the supernatant from which β-lactoglobulin had been precipitated[23]. This α-lactalbumin is unusual in that it is practically insoluble in water and diluted salt solutions. It dissolves in dilute acids and alkalies. This protein has the molecular weight of 16,000. Remarkable is the high tryptophan content (7%) of this protein[17]. α-Lactalbumin appears homogeneous with respect to sedimentation. A single boundary was observed also on electrophoresis in glycine, phosphate and barbiturate buffers at various pH values, whereas two boundaries were observed in lactate buffer at pH 3.3[17].

Some proteins of the whey resemble serum proteins. Bovine serum albumin has been isolated from whey by fractionation with ammonium sulfate and alcohol[24]. This protein comprises about 5% of the total of whey proteins. It has the same electrophoretic mobility as bovine serum albumin, and it is immunologically equivalent to it[25]. Other proteins resembling the serum γ-globulins are found in the globulin fraction of whey which is precipitated with 2.3 M ammonium sulfate (see p. 588). According to the method of Smith[26], these globulins can be fractionated as follows. The precipitate obtained by half-saturation with ammonium sulfate is dissolved, and ammonium sulfate is added, first, only to 25% saturation at pH 4.6. This precipitate, which contains some casein, is discarded, and the globulins are salted out with ammonium sulfate at 40% saturation at pH 6.0. The precipitated globulins are redissolved and fractionally precipitated at 30% and 40% saturation. It could be shown that these globulins contain antibodies.

Extremely rich in the immune globulins is colostrum. It could be shown that the γ-globulin fraction of the colostrum is transferred directly into the blood of the new-born calf after his feeding upon colostrum. Electrophoretic analysis has shown that the blood serum

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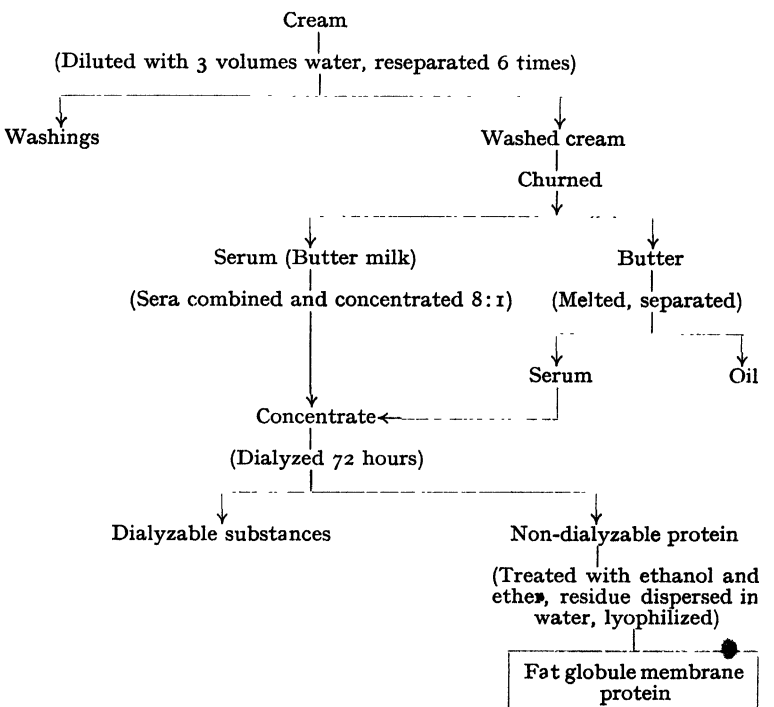
of a new-born calf is practically devoid of γ-globulin, and that the γ-globulin component is increased strikingly after feeding the animal with colostrum[27]. (The transfer of immune globulins in the case of human new-borns, however, seems to be chiefly through the placenta.) The colostral immune globulins have been fractionated and studied by Smith and colleagues[28].

3. Homogenization and the Stability of Fat Globules of Cream

Since the density of milk fat is smaller than that of the skim milk, the fat particles ascend to the top forming there a layer of cream. This natural creaming is accelerated by centrifugation. Microscopic examination shows that the fat globules in milk or cream are of various sizes. Very large particles can be formed by agglomeration of the smaller ones. This agglomeration of the small fat globules of cream depends on various factors, such as pH, temperature, and stirring. Weakly acid reaction and moderate stirring facilitates the agglomeration, which in this case is called churning. The macroscopic lumps of fat then can be separated easily from the "serum". Butter represents a semisolid dispersion of the aqueous phase in the fat.

From the colloid-chemical point of view, the *stability* of the fat globules in milk and cream is interesting. When the practical aim is butter making, the conditions are sought which will favor agglomeration of the globules. When, on the contrary, cream or homogenized stable milk is desired, agglomeration of the fat globules must be prevented. Stabilization against creaming of the fat droplets is achieved by *homogenization*. Warm milk is forced through small clearances in machines which work on the principle of colloid mills (Chap. 5). The large globules thereby are disintegrated into smaller ones, whereas the very small globules are not affected. Consequently, the fat dispersion (or emulsion) of the milk becomes more homodisperse than before. The average particle size is considerably decreased by the homogenization. Owing to the intense Brownian motion of the small particles, the tendency to cream is greatly hampered. The very small droplets are more liable to undergo the chaotic motion, caused by uneven molecular impacts, than the large globules on which the irregular impacts mutually neutralize. Many new water-fat interfaces are formed on homogenization, and, as the vigorously moving small globules collide, there certainly must be a tendency to agglomerate. Why then is a homogenized milk or cream so stable?

It was assumed long ago that the stability of the fat globules of milk and cream is due to a *protective film of protein or phospholipid* adsorbed on the surface of the fat globules[29]. The electrically charged atomic groups of the proteins and phospholipids confer a certain net charge to the surface, thus securing repulsion of the colliding surfaces. Moreover, the adsorbed protein forms an elastic membrane around the droplets thus mechanically protecting them from coalescence. The proteins of the stabilizing film have been separated recently by the following method[30]. Cream was diluted with water and reseparated six times. The washings which contain all of the proteins and micromolecular solutes not bound to the fat globules were discarded. The washed cream then was churned, the butter was melted, and the oil separated from the water phase ("butter serum"). The proteins of the sera then were isolated by dialyzing the solutions and lyophilizing them, as illustrated in the chart below:



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The fat-adsorbed proteins were isolated from ordinary and from homogenized milk, and considerable differences were found in amino acid composition, in electrophoresis, and in sedimentation of the proteins. It was found that homogenization changes the nature of the fat-membrane protein. The fat-membrane protein of homogenized milk contained less cystine, glycine, and glutamic acid, and more lysine than non-homogenized milk[30]. The electrophoresis patterns also were different[31]. The sedimentation diagrams revealed one principal fairly homogeneous protein component ($S_{20} = 7.3$) in the fat-membrane protein preparation of non-homogenized milk, whereas three components ($S_{20} = 2.2$, 8.2, and 4.4) and possibly one of lower concentration and mobility were detected in the fat-membrane proteins of homogenized milk[32]. The fat-membrane protein in the ordinary non-homogenized milk seems to be a globulin of the type of the immune globulins. The fat-adsorbed proteins of the homogenized milk include casein, β-lactoglobulin and α-lactalbumin in addition to the larger globulins. These changes upon homogenization are comprehensible when one considers the large increase of fat surface on the splitting of the globules. The freshly formed surfaces tend to adsorb everything which comes in contact with them. It is conceivable that all the proteins of the milk then are adsorbed to a greater or lesser degree. In ordinary milk the proteins which have a large amount of lipophilic groups on the surface will be preferentially adsorbed. Such are the globulins of the immune globulin type which may even contain a bound lipid component. The highly dispersed globules of homogenized milk also adsorb the other proteins possessing less of such lipophilic surface groups than the γ-type globulins.

4. Whipped Cream and other Complex Disperse Systems

Vigorous whipping of cream transforms the liquid into a consistent foam—whipped cream. This is a complex disperse system composed of milk fat, an aqueous phase, and air. The air bubbles are trapped in the coherent phase which is composed of the fat globules stabilized by a small amount of adsorbed protein. The mechanism of the formation of the coherent framework is not clear. It is, however, conceivable that the clue to this transformation is to be found in the protein component. The mechanical treatment of whipping probably causes a surface denaturation of the proteins which stabilize the fat globules. The denatured proteins tend to

flocculate, thus facilitating the agglomeration. The partly agglomerated, but still protein stabilized, fat particles orientate around the whipped-in air bubbles, thus forming a honey-comb-like framework.

The acidity of cream increases on standing. This is due to enzymatic processes, chiefly to the conversion of the small amounts of milk sugar of the water phase into lactic acid. The viscosity thereby increases considerably, as sour cream assumes the consistency of a paste. The acid seems to have the same denaturing influence on the fat-stabilizing globulins as repeated exposure to air surfaces (whipping). The partially denatured globulins of the membranes tend to flocculate. When the system is stirred, large, relatively compact lumps of butter fat are formed (churning); if, however, the acid cream is left alone, the globules agglomerate slowly into loose, voluminous flakes encompassing the water phase.

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CHAPTER 33

Emulsions and Micellar Lipids

Stable emulsions are composed of three components: two immiscible liquids and a stabilizing agent. Many more components may be present in the system, as we have seen in the example of milk. In most natural and practically important emulsions the dispersed phase contains several chemically different substances; for example, the droplets of milk fat contain a variety of glycerin (glycerol) esters of fatty acids and other lipids. The continuous phase usually is water containing several solutes, although emulsions with an oil as the continuous phase are not uncommon. Butter and margarine are examples of such emulsions. Natural petroleum often appears as a complex emulsion of water in oil. Various creams and ointments used in medicine and cosmetics are emulsions of either oil-in-water or water-in-oil type.

No strict borderline can be drawn between emulsions and dispersions, as there are intermediate states between liquid and solid consistency. This is obvious in the case of milk fat, which is semisolid at ordinary room temperatures. Only molten butter is a true emulsion. The rubber particles in natural rubber milk (latex), are also semisolid as are the particles in several dispersions of synthetic polymers, and the particles of the many different lipids found in cells and tissues. The consistency of the whole disperse system also may be very different; it depends not only on the relative amounts, consistency, and degree of dispersion, but also on the interactions between the components. The increase of consistency of cream with rising acidity was mentioned in the preceding chapter.

Micellar colloids, such as soaps and phospholipids, differ from

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emulsions in one important property: they can be prepared without the aid of a third component (stabilizer). Micelles can be formed spontaneously, owing to the presence of both hydrophilic and lipophilic atomic groups in the dispersed molecules (see Chap. 5). However, the molecular size of the dispersed substance is also important; micelles are formed of molecules which are built of a relatively large non-polar part and a small polar group. The consistency and degree of orientation of these molecules within a micelle may be very different, although micelles ordinarily are more solid than droplets of an emulsion.

1. Particle Size and Stability of Emulsions

Extensive studies have been conducted in the branch of the colloid chemistry of emulsions[1]. This is due chiefly to the great importance the emulsions have in various industries, pharmacy, food research and other branches.

As already mentioned, stable emulsions can be prepared only with the aid of *emulsifying agents*. The best emulsifying agents are substances which are capable of forming micelles, such as soaps and synthetic detergents. Proteins and other macromolecules possessing both polar and non-polar atomic groups also act as stabilizers. Finally, droplets of a liquid which is insoluble in a particular solvent can be stabilized by finely ground powders.

All emulsions differ from most other colloidal systems in the relatively *large size of the dispersed particles*. The droplets of emulsions have diameters of approximately 0.001–0.01 mm, although homogenized emulsions may contain smaller particles of even 0.0001 mm. The droplet size in ordinary emulsions can be measured by means of a microscope.

Emulsions differ from many other colloidal systems not only in the liquid consistency of the dispersed particles and their large size but also in the *large volume concentration of the dispersed phase*. In solutions of molecular colloids, as well as in micellar dispersions, the dispersed phase comprises only a few per cent of the total volume. The amount of the dispersed phase in emulsions is often 10–30%, or even more. For example, there is about 15–25% fat in cream. Geometrical calculations indicate that the maximum volume of spherical droplets of equal size which can be dispersed in another liquid is 74% of the total. Emulsions in which the partial volume of the dispersed liquid is greater than the partial

volume of the continuous phase (dispersion medium) are quite common. It is obvious that in such cases the question may arise as to which of the two immiscible liquids is the continuous phase and which the dispersed liquid. *Two types of emulsions* are distinguished in this respect: (1) the *oil-in-water* (O/W) emulsions and (2) the *water-in-oil* (W/O) type systems. The continuous phase can be revealed then by the following three methods: (1) the electrical conductivity of emulsions with a continuous oil phase is much lower than of an emulsion with a continuous water phase; (2) an emulsion can be diluted with a liquid which represents the continuous phase, and (3) if water is the dispersion medium the emulsion can be dyed with a water-soluble dye.

The ease of emulsification, i.e., dispersion of one liquid into another (in which the first liquid is insoluble) depends on the *interfacial tension* of both liquids. The lower the interfacial tension the more easily droplets are formed. Surface active substances, such as ether, lower the interfacial tension and hence facilitate droplet formation, for instance of oil in water. The emulsion thus obtained, however, will not be stable, since the droplets coalesce rapidly. This is caused by the tendency to decrease the free surface energy which increases with increased degree of dispersion.

The emulsifying ability of a soap or a synthetic detergent is also due to its adsorption at the oil-water interface, and decrease in the interfacial tension. However, these agents not only facilitate the formation of small droplets but also *stabilize* them. The mechanism of this stabilization is now fully understood, chiefly on the basis of the classical studies of Langmuir, Harkins, Rideal, Schulman and others, on monomolecular films (Chap. 6)[2]. The soap molecules are oriented in the oil-water interface: the ionic, polar 'heads' of the molecules are turned to water, and the lipophilic hydrocarbon 'tails' are immersed into the oil droplet. Hence, an electrically charged membrane of the ionized soap molecules is formed around each oil droplet in oil-in-water (O/W) emulsion. This membrane protects the droplets from coalescence.

The mechanism of stabilization is somewhat different in the water-in-oil (W/O) emulsions. The calcium, magnesium, and heavy metal salts of the fatty acids (water-insoluble soaps) are known as the best stabilizing agents for these emulsions. The polar "heads" in this instance are turned inward, to the water droplet, and the lipophilic hydrocarbon "tails" of the stabilizer are immersed in the continuous oil phase. Hence, in the case of W/O emulsion the droplets are not electrically charged.

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Stabilization of droplets by finely ground powders is explained on the basis of preferential wetting of the powder by either oil or water. If the powder is wetted by water, the larger part of the solid particle will be turned toward the water at the oil-water interface. Hence, the relatively large oil droplet will be surrounded by a thick crust of a solid layer protecting the droplet from coalescence. The solids which are wetted preferentially by oils can be used as stabilizing agents for W/O type emulsions. The stabilization of water droplets in oil by means of calcium oleate and similar water-insoluble soaps has been interpreted on the basis of preferential wetting by the oil of the insoluble, relatively large soap particles. The larger lipophilic portion of the solid is immersed in oil, and a small part of the particle is in contact with the water droplet.

According to the data mentioned above, the electrical charge of the stabilizing agent may not be essential in many cases. This has been ascertained not only for the W/O emulsions, but also for some O/W systems[3]. Oil droplets in water can be stabilized not only by soap, protein, and similar electrically charged stabilizers, but also by some unionized polymers, such as polyvinyl alcohol. Moreover, it was found that the stabilizing effect of serum albumin does not depend much on pH. Nearly isoelectric albumin which possesses a negligible net charge stabilized oil droplets as well as did albumin with a relatively high net charge[3]. The combined effects of albumin and detergent as stabilizers have also been studied[4]. The *saponins* represent another group of surface active emulsifying agents which are not ionized. The oil droplets, however, are highly protected from coalescence by the adsorbed saponin layer. Saponins are glucosidic derivatives of steroids, the molecules being composed of lipophilic hydrocarbon ring systems and hydrophilic polar groups. It was concluded that the main factor determining stability is the resistance to wetting of certain segments of the adsorbed film by the droplets. The stabilizing film can be regarded as a protecting barrier. The molecules comprising it have a partial affinity toward both the water and oil phases. It is obvious that the affinity of the agent for the droplet must be a limited one, since otherwise the particles will be pulled inside the droplet. The affinity for the continuous phase can be interpreted also as solvation, which definitely contributes to the stability.

The British researchers mentioned, especially Schulman and Cockbain[2, 3], have also studied thoroughly the effect of the molecular structure of the stabilizing agent on the stability of emulsions.

The effects exhibited by two stabilizers possessing various constitution and solubility properties were also studied. It was found for example, that cholesterol or sodium cetyl sulfate alone are poor emulsifying agents, but a mixture of the two stabilizes oil droplets in water very well. Cholesterol is very soluble in oil and practically insoluble in water. Sodium cetyl sulfate, on the contrary, is relatively insoluble in oil. When both are present, a complex film of cholesterol and cetyl sulfate is formed on the surface of the droplets. Secondary cohesive forces probably hold the molecules of both stabilizers together in a tight layer or membrane. It is also interesting that the kinked *cis* isomers have a weaker stabilizing effect than do the elongated *trans* isomers. The stability of an emulsion with a mixture of cholesterol and sodium oleate (*cis* isomer) was poorer than the stability in the presence of cholesterol and sodium elaidate (*trans* isomer). The stability is even greater, if, instead of the relatively flat cholesterol molecules, cetyl or elaidyl alcohol is used. Very stable emulsions could be obtained, e.g., by using combined stabilizers containing cetyl alcohol and sodium cetyl sulfate. This is explained on the basis of the geometry of the molecules involved. The straight hydrocarbon 'tails' of the cetyl derivatives can be packed more tightly vertically to the droplet surface than the bulky condensed-ring residues of cholesterol or the crooked chains of the *cis* isomers. Hence, a tight protecting film can be formed best by such polar-non-polar molecules which are geometrically fit for the formation of such a film.

The inversion of phases is a very interesting phenomenon studied chiefly by Clowes. If some calcium chloride is added to a concentrated emulsion of oil in water, which is stabilized by a soap, the oil becomes the continuous phase instead of water. The calcium ions combine with the fatty acid anions; the calcium salts of the fatty acids thus formed are insoluble in water, and they are thus unable to stabilize the oil droplets which coalesce. However, the particles of the fatty acid calcium salt have preferential affinity for the oil, so that on stirring the mixture they surround the water droplets, thus forming a W/O emulsion. The change of phases is illustrated in Fig. 156. Not only calcium salts but also barium, magnesium and many other bi- and polyvalent cations have the same effect. The O/W emulsions which are stabilized not with soaps or anionic detergents, but with long chain cationic detergents, such as hexadecyltrimethylammonium bromide, can be inverted by means of sulfates and other bi- or polyvalent anions.

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McBain has pointed out[5] that the stabilizing agent at the oil-water interface is not necessarily the same physically as well as chemically as the agent added to the bulk liquids. Deutsch has demonstrated[6] that many dyes and indicators, when shaken with two immiscible liquids, change color, and that the original color reappears when the phases separate.This indicates that structural and other changes may occur at the interface. McBain mentioned the fact that the soap used as a stabilizing agent is partly hydrolyzed, and that the fatty acids thus formed are more soluble in the oil than are the unhydrolyzed molecules; acid soap is adsorbed on the surface of the oil droplets, i.e., the stabilizing layer is composed not only of fully dissociated anions of the fatty acids, but also of the undissociated molecules of the fatty acids[5]. Albumins and other proteins, if vigorously shaken with a mixture of water and

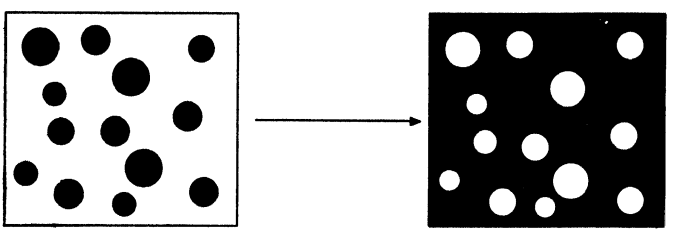

Fig. 156. The change of phases of a concentrated emulsion.

a water immiscible liquid, are denatured on the surfaces of the droplets. Relatively thick skins of the denatured protein are formed around the droplets as protective membranes.

Quite striking and of practical importance is the emulsification of fats and fatty oils (triglycerides) by means of alkalies. For example, stable emulsions of olive oil, cottonseed oil, or cod liver oil can be prepared by shaking them with a dilute solution of sodium hydroxide. No emulsion will be formed when pure water, saline, or dilute acid is used instead of hydroxide, although a solution of sodium carbonate is effective. Neither sodium hydroxide nor carbonate directly stabilizes the oil droplets, but it is the soap which is formed in the reaction of sodium hydroxide with the fatty acids of the oils. (At a higher concentration of the hydroxide and a sufficiently high temperature, a large portion of the glycerol esters of the oils will be saponified by the alkali.)

The breaking of emulsions stabilized with ordinary soaps can be

accomplished by adding an acid or a small amount of a salt with a bi- or polyvalent cation. Acid suppresses the dissociation of the soap in the stabilizing layer. Coalescence of the droplets then occurs, partly due to the diminution of the electrical charge, although the destruction of the stabilizing layer is also important, because of the solubility of the fatty acids in the oil. Sodium chloride and similar salts often are poor flocculating or breaking agents for emulsions, which indicates that changes in the electrical properties on the surface may not be decisive. The breaking of W/O emulsions is accomplished by adding agents which facilitate the formation of the opposite type (O/W) emulsions. Surface active substances which are soluble in both water and oil, such as butyric acid, may also be effective. It is conceivable that such agents will disturb the adsorption equilibrium at the interfaces. Physical agents, such as high voltage electrical discharge or ultrasonic waves of relatively low frequency, are sometimes used for breaking emulsions[1]. Emulsions of the O/W type are frequently encountered in the petroleum industry, and breaking of these emulsions is an important technological problem. The water droplets are stabilized by a layer of bituminous substances which are preferentially wetted by the oil.

2. Practical Applications of Emulsions[1, 5]

Many applications have already been mentioned, especially milk products (Chap. 32) and the importance of monomer emulsions in emulsion polymerisation (Chap. 4 and 18). The final dispersions obtained in emulsion polymerisation, however, are not emulsions but dispersions of aggregated macromolecules, although these systems are often wrongly called emulsions. The same pertains to rubber latex, which is a dispersion of soft aggregates of the macromolecules of rubber, the particles being stabilized by a layer of proteins. No inversion of phases or similar phenomena characteristic of emulsions can be observed with rubber milk, as rubber is not liquid. There is a great difference between cream and rubber latex in that the fat droplets in cream are composed of *small* molecules (fat is *micro*molecular), whereas the particles in latex are composed of *large* molecules (rubber is *macro*molecular). This difference is not sufficiently emphasized even in some relatively recent texts.

There are, however, plenty of true emulsions of practical importance. These include oil emulsions for leather treatment, dry-cleaning emulsions, textile lubricants, pharmaceutical emulsions of oils,

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lotions, creams, mayonnaise and similar food products. The 'fat liquoring' of leather is an interesting procedure. The emulsions of fatty oils are stabilized by soaps or sulfonated castor oil and introduced into the leather. The negatively charged droplets probably are bound to the positively charged amino-groups of the protein in the pores of the leather, thus making it more pliable and water-repellent.

Many of the hand lotions and creams are emulsions of the O/W type containing lanolin and hydrocarbons (mineral oil, vaseline) which are emulsified by means of soaps and other detergents, sometimes also by proteins and polysaccharides. The semisolid ointments and creams may be either of the O/W type or W/O type. Lanolin, cholesterol, lecithin, the monoglycerides and long carbon chain alcohols are included as stabilizing agents. The 'vanishing creams' are of the O/W type, whereas the more greasy 'night creams' are emulsions and dispersions of the W/O class. Fat-soluble vitamins, hormones and other physiologically active ingredients sometimes are added in small amounts to cosmetic creams, although their absorption through the skin is very limited. Beneficial effects have been reported with relatively large amounts of estrogenic hormones, as well as with the highly unsaturated fatty acids present in linseed oil, and with A and D vitamins[7].

The colloid chemistry of emulsions is important in the modern oleomargarine industry. The chief starting materials are vegetable fatty oils, such as peanut, soya bean, and cotton seed oils which are emulsified in water by means of lecithin and monoglycerides. (The oils are occasionally hydrogenated in order to increase the consistency, and they may be deodorized; fat-soluble vitamins are added to the oils, salt and preservatives to the water phase.) The oil which contains the emulsifying agent is churned with cultured sour skim milk, one part of it being mixed with four parts of oil. Part of the water phase thereby is emulsified into the oil medium, the W/O emulsion becoming coherent on cooling. The water droplets of the margarine can be seen in a microscope. The droplets are stabilized by a layer of monoglycerides and lecithin, the polar 'heads' of the stabilizers being turned inside the droplet, the surface of which is uncharged. The margarine oil when separated from water, however, contains very little lecithin; this indicates that a considerable affinity exists between lecithin and water, and that the greater part of the lecithin is within the stabilized droplets. It is interesting to note that not lecithin, but the mono- and digly-

cerides are the chief water-retaining agents in margarine. In the absence of the monoglycerides, the product 'weeps', a process resembling syneresis of gels. Hence, the chief stabilizing agent of the water droplets is not lecithin but the monoglyceride, although lecithin has other beneficial influences on the properties of the product[8]. It seems likely that in the first phase of churning an O/W emulsion is formed in which lecithin is the stabilizer, and that an inversion of phases follows in which the lecithin component is pulled inside the water droplets.

3. The Resorption of Fats

The resorption of fats is an important physiological process, the colloid-chemical aspects of which have been emphasized by Frazer[9] and Schulman[10]. The physiologists have well established the fact that the resorption of fat occurs in the small intestine. Two important organs participate in this resorption: the gallbladder and the pancreas. The first produces emulsifying agents which re-emulsify the rather coarse dispersion of the fat which has passed the acid medium of the stomach. The pancreas produces juices which contain fat-cleaving enzymes. The major emulsifying agents of the bile are sodium glycocholate and sodium taurocholate, which are derivatives of steroids containing the same condensed four-ring system as cholesterol. Three OH groups are attached to the ring system. Moreover, a carbon side chain is present. The parent substance of the two emulsifiers is cholic acid, which contains a free carboxyl group at the end of the side chain. In glycocholic acid this side chain is coupled with glycine, in taurocholic acid taurine is present instead of the glycine residue:

$C_{23}H_{36}(OH)_3—CO—NH—CH_2—COONa$ sodium glycocholate

$C_{23}H_{36}(OH)_3—CO—NH—CH_2—CH_2—SO_3Na$ sodium taurocholate.

As illustrated by the formulas, the emulsifying agents are strongly polar, although they also have some affinity for lipids. Both agents have a strong ability to stabilize O/W emulsions. The enzymes of the pancreas (pancreatin) probably catalyze only a limited cleavage of the triglycerides, although much controversy prevails on this point. According to Frazer and Schulman[9,10], the emulsified fat can be resorbed directly through the intestinal wall, without con-

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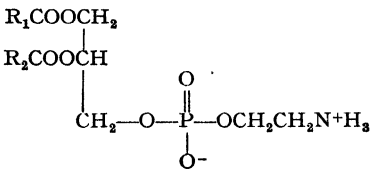
siderable enzymatic hydrolysis. Frazer has shown[9] that: (1) complete inhibition of pancreatin did not impair the absorption of olive oil, (2) that the pancreatic enzyme does not produce fatty acids and glycerol, but mono- and diglycerides with free fatty acids, and (3) that even finely emulsified paraffin oil (diameter of droplets 0.0005 mm) can be absorbed equally as well as olive oil, if negatively charged emulsifying agents are used. Schulman[10] who studied thoroughly emulsification by the various agents possibly involved in intestinal emulsification, came to the conclusion that not only the cholates but other emulsifying agents participate. These include the products of the enzymatic cleavage of the fats: chiefly oleic acid and the mono and diglycerides. It was found that even spontaneous emulsification of oils occurred if oleate, bile salt and monostearate all were used together for the stabilization. With such combined emulsifiers, small droplets of 0.0003–0.0005 mm diameter were formed not only in weakly alkaline solution at pH 8.5 but even at pH 6–7. The following combinations of emulsifiers also had a strong emulsifying capacity for fat: (1) oleate/monostearate, and (2) oleate/cholesterol/monoglyceride. In weakly alkaline solutions of pH 8.0–8.5 effective emulsifying activity was also shown by oleate alone, as well as by bile salt together with the oleate, oleate/cholesterol, and bile salt/oleate/cholesterol.

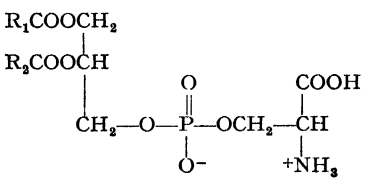
Transport through the intestinal wall, according to Schulman[10], is due to the membrane potential of the intestinal membrane. Sodium taurocholate is the substance which is responsible for the creation of this membrane potential. Owing to the large difference in size and mobility of its small sodium ion and large taurocholate ion, the sodium cations in the intestinal wall move ahead of the slower taurocholate anions, thus producing a membrane potential. This membrane potential is sufficient to pull the negatively charged fat emulsion particles through the wall. That fat emulsion can indeed penetrate a membrane of sufficiently large pore size was shown in *in vitro* experiments. While without the bile salt a fine emulsion could hardly pass the membrane, addition of bile salt noticeably facilitated the passage. Hence, the bile salts have a double role: they participate in emulsification, and they create the membrane potential which pulls the emulsified droplets through the intestinal wall.

4. Micellar Phospholipids and Similar Colloids

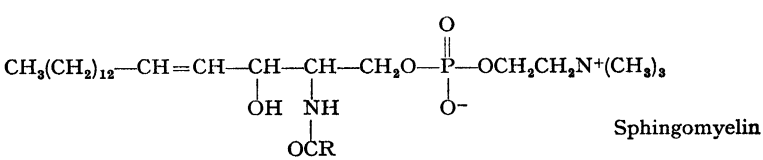
Phospholipids are micromolecular substances of nearly the same molecular weight as fats (triglycerides). In contrast to fats, which are esters of fatty acids and glycerol, and do not contain any nitrogen or phosphorus, the phospholipids contain both nitrogen and phosphorus[11]. Three major groups of phospholipids have been distinguished: the *lecithins*, the *cephalins* and the *sphingomyelins*. Abbreviated structural formulas of some of these phospholipids are presented below:



Cephalin, phosphatidyl ethanolamine



Cephalin, phosphatidyl serine



Sphingomyelin

On hydrolysis the lecithins yield fatty acids (R_1COOH and R_2COOH), glycerol, phosphoric acid, and choline, $(CH_3)_3N(OH)—CH_2CH_2OH$, the latter being a strong quaternary base. The molecules of all phospholipids are dipolar ions (zwitterions). The lecithins and sphingomyelins are nearly neutral because of intramolecular neutralization. The ethanolamine cephalins are weakly acid, since the primary amino group has a lesser tendency to dissociate than the quaternary amine. The phosphatidyl serine cephalin is even more acidic, because of the additional carboxyl group. The serine cephalin yields serine on hydrolysis, whereas ethanolamine is ob-

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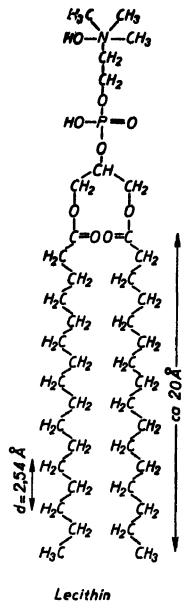
tained in the case of the ethanolamine cephalins. Noteworthy also is the molecular shape: the molecules of all phospholipids are branched. In the solid state the molecules resemble forks (Fig. 157). The dipolar phosphate-ethanolamine (or phosphate-choline, or phosphatidyl serine) residue represents the 'handle' of the fork, and the rather long hydrocarbon chains of the fatty acid residues (R, R_1, R_2) represent the branches.



Lecithin

Fig. 157. Molecular structure of lecithin.

The fatty acids of the lecithins and cephalins usually contain 18–22 carbon atoms, e.g., R_1 may be $C_{19}H_{39}$. The fatty acids of the sphingomyelins contain 18–24 carbon atoms.

The phospholipids are important constituents of cells and tissues, as has already been mentioned in previous sections. The colloid-chemical behavior of these substances can be predicted in part from the chemical composition and molecular constitution. Important in this connection is the fact that the molecules of all phospholipids are composed of a large lipophilic part ('branches' of the 'fork') and a strongly polar group. Phospholipids are soluble in fats. However, they also have an affinity for water: they swell in water, and can be dispersed easily as aqueous micellar colloids. Unlike the fats, these substances can be dispersed without a stabilizer, and thus resemble the soaps and detergents. Although the particles in the dispersions of phospholipids at higher temperatures are liquid, the systems should not be called emulsions, but micellar colloids. In bulk, these substances are soft solids or they have the consistency of greases.

The consistency of some phospholipids depends on the moisture content. Soya bean lecithin with less than ½% of water is a viscous liquid. On standing it absorbs moisture and changes into a more consistent semisolid mass. The maximum consistency of a waxy solid is attained when the water content is raised to 1½–2%. Commercial soya bean lecithin is composed of 2/3 lecithin and 1/3 of a fatty oil, and the consistency depends on mutual solubility of the components; the solubility of the lecithin in the oil decreases upon hydration, and the solid aggregates of the hydrated lecithin form a consistent meshwork. This solidification is accompanied by

an appearance of opacity which is caused by this aggregation of lecithin.

Reviews of the older colloid-chemical literature on phospholipids are available[12]. Since ill-defined preparations of the phospholipids have been often used, the findings were not always concordant, and the interpretations of such results have little value. Thorough studies with highly purified, well-defined materials would be desirable by means of the modern methods of colloid science.

The chief sources of lecithin are soya bean oil and egg yolk. Cephalins and sphingomyelins are found in nervous tissue. The lipids can be extracted by means of alcohols or ether, and the purification is quite complex. Aqueous sols can be prepared most readily by pouring an alcoholic solution of the lipid into a large amount of water. The sols are opalescent and polydisperse. The stability of these sols varies greatly, depending on the starting material, impurities, and the method of preparation. Since the micelles are amphoteric, the stability depends on the pH of the solution. Interesting studies on the stability, electrical charge, and mobility of the micelles of purified lecithin have been made by Bungenberg de Jong and his associates[13]. Reversal of charge could be accomplished not only with ordinary salts but also with alkaloids. It was found that the larger the organic cation the more effective it was in reversing the originally negative charge of the lecithin particles to a positive charge. This indicates that the reversal of charge is due to the adsorption of the organic cations onto the surface of the micelles. The results are presented in Fig. 158[12].

X-ray diffraction studies on hydrated lecithin and cephalin have been made by Schmitt and Palmer[14]. Hydrated lipids showed larger spacings than does dry material. This difference is due to a water layer adsorbed between the polar groups of the oriented molecules. Studies on monomolecular films by the surface balance techniques have indicated that the orientation of the molecules on the water-air interface is the same as that of soaps (Chap. 6), namely, that the polar 'handle' of the molecule is immersed in water, while the hydrophobic 'fork' is pushed out in the air[15].

Chargaff and colleagues have done interesting research on the reactions of colloidal phospholipids with proteins[16]. Noteworthy is the finding that basic proteins, such as histones and protamines, were the most effective precipitants for lecithin and cephalin. An important observation is that the cephalin forms salt-like compounds

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with salmine (a protamine, see Chap. 25) over a wide range of pH between 2 and 11, whereas lecithin produces such compounds only in narrow pH limits of 10–11. Thymus histone reacted with cephalin between pH 2–7, but with lecithin only at pH 7–8. These findings are easily explicable considering the electrochemical difference between lecithin and cephalin, namely, the latter is more electronegative than the former. The micelles of the cephalins are negatively charged over a wide pH range, and thus react with the positively charged basic proteins easily, whereas the lecithin micelles carry a negative net charge only in strongly alkaline solutions. The structure of the lipid-protein compounds was studied also by means of X-ray diffraction[14]; oriented structures in which a monomolecular layer of protein is included between two layers of lipid have been

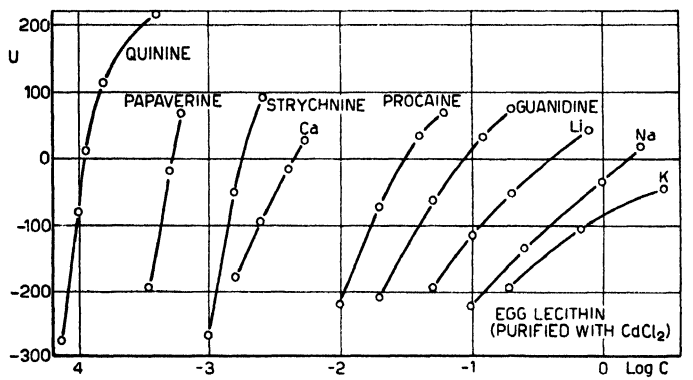

Fig. 158. Reversal of charge of lecithin with some alkaloid cations and with some inorganic cations for comparison. On the abscissa is plotted the logarithm of salt concentration, on the ordinate the mobility.

observed. The polar 'handles' of the phospholipids are turned toward the protein layer[17].

Numerous other polar-nonpolar lipids are known. The cerebrosides represent a lipid group found chiefly in brain tissue and the myelin sheath of nerves. The molecules are similar to those of sphingomyelin, except that the choline residue is replaced by a hexose sugar, mainly D-galactose. Like the phospholipids, the cerebrosides appear as micelles in an aqueous medium. In contrast to the micelles of the phospholipids, these cerebrosides are not electrically charged.

Cholesterol and other steroids also belong to the large and diverse

class of lipids. The cholesterol molecules are composed of a condensed four ring system with a hydrocarbon chain attached. Only one polar OH group, linked to one of the hydroaromatic rings, is present in the molecule. Hence, cholesterol is much less hydrophilic than are the phospholipids. Cholesterol is readily soluble in fats and fat solvents, but it is insoluble in water. Micellar colloids of cholesterol, however, can be prepared easily by dissolving cholesterol in ether or hot alcohol, and pouring the micromolecular solution into a large amount of water. Stable, beautifully opalescent sols of cholesterol can thus be prepared. The particles appear to be negatively charged, which seems to be due chiefly to fatty acid and phospholipid impurities present in the cholesterol. Cholesterol sols behave as lyophobic colloids, and they can be stabilized by adding phospholipids. It has been shown that cholesterol forms molecular compounds (association or complex compounds) with phospholipids and fatty acids.

5. Proteolipids and Lipoproteins

Proteolipids and lipoproteins represent two large groups of biocolloids. Proteolipids are soluble in fat solvents and insoluble in water. The lipoproteins, on the contrary, are soluble in water and insoluble in fat solvents. Both are composed of a macromolecular protein component and a micellar component of the lipids. The structural details are unknown. It is doubtful whether there would be much value in speaking about definite particle sizes and molecular weights, because a micellar component is involved. Most of these complexes of the macromolecular proteins and micellar lipids are unstable, and the lipid can be extracted readily by lipid solvents.

The lipoproteins of blood plasma have been studied by many authors, and good reviews are available[18]. According to their electrophoretic mobility, solubility, and sedimentation characteristics, at least three types of plasma lipoproteins can be distinguished: α_1-, α_2-, and β-lipoproteins. They all are spherocolloids of relatively low density as a result of the high lipid content, which usually amounts to between 40 and 70%. The β_1-lipoprotein was isolated by spinning a concentrate containing 1 *M* sodium chloride, in order to increase the density of the medium; the lipoprotein then rises to the top of the cell, and can be separated easily. It could be shown that the particles have a "molecular weight" of approximately 1.3 million[19]. Most surprising was the fact that the lipid

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component of the particles was larger than the protein component. Phospholipids, cholesterol and its esters, as well as fatty acids, and a number of other fat-soluble compounds are present in the particles. There is not enough protein even for the formation of a surface shell, hence, the phospholipids and other components which have polar groups contribute to the hydrophilic nature of the particles. The blood plasma lipoproteins are the carriers of fats, phospholipids, steroids, carotenoids, and other physiologically important fat-soluble substances.

The proteolipids[20] are exceptionally interesting biocolloids. These substances have been extracted from brain and some other tissues by 2:1 chloroform-methanol mixtures. Some of the proteolipids were birefringent, microcrystalline substances containing 20–75% protein, the rest being cerebrosides and phospholipids. The peculiar solubility behavior may be due to the specific constitution of the particles, in which the protein probably constitutes the core, and the lipophilic cerebrosides are on the surface, the hydrocarbon chains being turned outward. It seems likely that such particles have been formed in an oily medium, whereas the lipoprotein particles originate in aqueous solutions.

Folch and associates isolated from brain tissue another crystalline, compound which, according to its particle weight, is colloidal[21]. This substance, called strandin, is freely soluble in water and in chloroform. Its particle weight is approximately 250,000, and the particles are composed of several types of lipids and carbohydrates. The colloid is practically homogeneous on sedimentation and electrophoresis.

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CHAPTER 34

Micellar Detergents, Dyes, and Pigments

In this chapter are included a few important types of substances which tend to form colloidal micelles. The ordinary soaps, synthetic detergents, a variety of dyes, and natural pigments, such as chlorophyll, comprise this group. Certain practically important procedures, such as washing and dyeing also will be reviewed from a colloid-chemical point of view.

1. Three Types of Detergents[1]

Detergents are surface active cleansing agents capable of emulsifying oils and holding dirt in suspension. The ordinary soaps have been for centuries the only practically important detergents. The early soap manufacturer used to boil fat or some fatty oil with the alkaline aqueous extracts of ash which contain potassium hydroxide and potassium carbonate. The soap thus obtained was soft and sticky. The early inventor learned later that a solid soap can be obtained from the soft potassium soap by salting it out with ordinary salt. Sodium hydroxide, which is now the common starting material for the saponification of fats, became commercially available only toward the end of the 18th century. However, reasonably cheap soap has been offered to the common consumer only since the first decades of the 19th century after a number of improvements and additional inventions were made in this branch of chemical technology. For about one hundred years the common sodium soaps which contain as the active ingredients the salts of oleic, palmitic, stearic, and other acids have been the leading

detergents. The cheaper products usually contained a variety of additives, such as rosin and sodium silicate. The soft or even liquid potassium soaps, manufactured by boiling linseed oil (and similar unsaturated material) with potassium hydroxide, also were used widely.

The chief disadvantage of the ordinary anionic fatty acid soaps is that they are precipitated in hard water. Even relatively low concentrations of calcium or magnesium salts greatly reduce the efficiency of ordinary soap. Moreover, the washing, emulsifying and dispersing activity of ordinary soap is hampered by acids. In the presence of calcium ions, insoluble calcium salts of the fatty acids are formed, while strong acids cause suppression of dissociation and precipitation of the fatty acids. The phenomena can be explained also as flocculation caused by reduction of the charge on the micelles.

At the present time a great variety of synthetic detergents are available, and some of them are efficient also in weakly acid solutions or in solutions containing calcium salts and other electrolytes. All synthetic detergents can be divided into three large groups: (1) *anionic*, (2) *cationic*, and (3) *non-ionic* detergents. They all contain *non-polar and polar* atomic groups, the non-polar part of the molecule must be larger than the polar moiety (see Chap. 5). The ordinary soaps belong to the group of the anionic detergents; chemically modified soaps and fully synthetic detergents comprise another subgroup. The synthetic detergents may be subdivided further into straight hydrocarbon chain aliphatic, aromatic, and branched chain detergents; they may be classified also according to the chemical constitution of the polar group. The cationic and non-ionic detergents are synthetic substances. Some examples are presented in Table 96.

Single, unassociated molecules of the detergents are present only in very diluted aqueous solutions. The hydrocarbon chain has no affinity to water, hence it coils up in order to avoid any contact with the solvent. Above a certain low concentration (critical concentration) the molecules aggregate into micelles (Chap. 5) in which the hydrocarbon tails are turned inward, and the polar heads are in contact with the water molecules. It must be emphasized that the hydrocarbon chains are flexible, and they never are quite straight. Even in solid, crystalline state the carbon atoms form a zig-zag line. The *cis*-isomers, such as oleic acid, are even less straight. The longer and more straight the hydrocarbon 'tails' the

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TABLE 96

EXAMPLES OF ANIONIC, CATIONIC, AND NON-IONIC DETERGENTS

1. *Ordinary soaps:*

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO^-Na^+$

Sodium palmitate

$CH{\cdot}CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$
$\|$
$CH{\cdot}CH_2CH_2CH_2CH_2CH_2CH_2CH_2COO^-K^+$

Potassium oleate

2. *Chemically modified soaps:*

$CH_3CH_2CH_2CH_2CH_2....CH_2COOCH_2CH(OH)CH_2OSO_3^-Na^+$

Fatty acid monoglyceride sulfate (Vel)

$CH_3CH_2CH_2....CH_2COOC_2H_4SO_3^-Na^+$

Fatty acid esters of oxyethane sulfonates (Igepon T)

3. *Fully synthetic anionic detergents:* (from petroleum and aromatic hydrocarbons)

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2{\cdot}SO_3^-Na^+$

Sodium dodecyl sulfonate

$CH_3CH_2CH_2CH_2CH_2....CH_2$— —$SO_3^-Na^+$

(may be branched) Sodium alkyl-aryl sulfonates (Nacconol, Santomerse)

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2OOC-CH—SO_3^-Na^+$
$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2OOC—CH_2$

Sodium dioctyl sulfosuccinate (Aerosol)

4. *Cationic detergents:*

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2{\cdot}N^+(CH_3)_3Br^-$

Hexadecyl-trimethyl-ammonium bromide

5. *Non-ionic detergents:*

$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOCH_2CH_2OCH_2CH_2O(CH_2CH_2O)_6$—
—CH_2CH_2OH

Polyethylene glycol monolaurate.

stronger is the tendency to associate into micelles in an aqueous solution. The cations of the cationic detergents associate as easily as the anions of the anionic detergents. Even the molecules of the non-ionic detergents associate in water. Relatively stable, uncharged micelles are formed, the stability in this instance being determined by the hydration of the polar 'heads' of the molecules. In the non-ionic polyethylene glycol monolaurate about five $-CH_2CH_2-O-$ groups with the alcoholic OH at the end have approximately the same affinity to water as one negatively charged sulfate group[2].

Sedimentation, diffusion, and viscosity studies have indicated that the micellar size and shape depend chiefly on the following factors: (1) length of the hydrocarbon 'tail' of the detergent molecule, (2) concentration of the detergent, (3) temperature, and (4) concentration of the salts present[3]. Micellar weights between 12,000 and many millions have been reported for various anionic and cationic detergents. Extensive studies about the sizes and shapes of the colloidal particles of various detergents have been done recently by means of the light scattering method[4, 5, 6, 7]. The 'molecular weight' of the smallest micelles of the detergents was found in the absence of salts, and values of 11,000–25,000 were reported. In the presence of salts larger micelles of relative weights of hundreds of thousands appear. Although some controversy about the particle shape prevails, it seems likely that the rod-like shapes dominate in diluted and fairly concentrated solutions. In highly diluted solutions the rods are short, whereas in more concentrated solutions they become longer. In highly concentrated solutions large, leaflet-shaped micelles also may appear[8]. Such flat particles tend to orientate into layers, and the system may become birefringent, and show other optical effects resulting from such orientation. Liquid crystals of various degrees of orientation have been observed in concentrated soap solutions[9]. Separation into two liquid phases (coacervation) takes place at certain high temperatures in the presence of high salt concentrations[10]. The colloidal solutions of detergents in water are hydrophilic systems, and simple salts, like sodium chloride, do not affect the systems' stability, except when added in large amounts, in such an instance the soap is 'salted out'. It must be mentioned that some of the synthetic anionic detergents are more sensitive to the flocculating influence of sodium chloride than ordinary soap, although the synthetic detergents are very insensitive to acids.

An important reaction occurring in aqueous soap solutions is

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hydrolysis. The amount of hydrolytic decomposition depends chiefly on the strength of the acid or base formed. The fatty acids are weaker acids than those containing the —SO_3H group, hence the hydrolysis in ordinary soap solutions is much more pronounced than in the aqueous solutions of sulfate and sulfonate detergents. While the ordinary soap solutions are weakly alkaline due to hydrolysis, the solutions of the synthetic detergents are either neutral or weakly acid. Addition of acid to ordinary soap solutions can be interpreted as promotion of the hydrolysis which is accompanied by the formation of large quantities of free fatty acids. As the long chain fatty acids are practically insoluble in water, flocculation takes place. In a pure aqueous solution of the soap the amount of free fatty acid formed in hydrolysis is very small, and the molecules of the free fatty acids are incorporated into the micelles of the soap. The amount of the free fatty acid may vary in certain limits depending on the pH of the solution which can be regulated by adding free acid or buffers. Acid soap of the composition An^-Na^+ (AnH), i.e., containing approximately equal proportions of soap and fatty acid, can be prepared; such acid soaps have peculiar solubility properties, namely, they are soluble in benzene and similar lipophilic solvents in which ordinary sodium or potassium soaps are insoluble[11].

2. The Colloid-chemical Principles of Detergency

Detergency or removal of dirt adsorbed on fabrics and other surfaces, according to McBain[11], can be formulated as follows:

Fabric.Dirt + Soap = Fabric.Soap + Dirt.Soap,

or more generally:

Surface.Dirt + Detergent = Surface.Detergent + Detergent.Dirt.

Thus, detergent first *liberates* the contaminant from the surface; secondly, it *keeps it suspended* in the water, and, thirdly, it replaces the dirt on the surface. The first two steps, namely liberation and dispersion of the dirt, are the most important and universally occurring processes in detergency. The third step, i.e., the adsorption of a part of the detergent on the cleaned surface may or may not occur depending chiefly on the properties of the surface and the detergent used.

The very important first step in the detergency—the partition

of the adsorbed dirt from the surface—depends mainly on the *surface activity* of the detergent. The aid of detergent is needed especially for the removal of greasy, *non-polar* surface contaminants, as adsorbed polar particles can be washed off by water alone. The soaps and all other detergents are surface active substances, i.e., they accumulate on any surface. Being highly surface active, they disturb the adsorption equilibrium between the surface and the dirt, become adsorbed at both the surface and the dirt, and thus part the dirt from the surface. The detergent which is adsorbed on the surface (replacing the dirt) is usually bound very weakly, and can be removed by subsequent rinsing with pure water.

All detergents are surface active, although not all surface active substances are good detergents. Surface activity alone is insufficient in detergency because of several reasons. First, very tightly adhering dirt may not be removed from the surface because the surface active agent can not penetrate between the tightly sticking contaminant and the surface. In such instances the removal of dirt is accomplished by *encompassing it into the micelles*. This important process, called *solubilization* is an essential feature of detergency. Solubilization (see Chap. 12) is important also in the second phase of cleansing: holding the removed dirt in dispersed form. If the dirt were not held by the micelles in dispersed or solubilized state, it might redeposit on the surface.

Much attention has been paid lately to the problems of solubilization[12]. The mechanism of encompassing and binding of water-insoluble or sparingly soluble substances in or at the surface of detergent micelles depends on both the substance to be solubilized and the micelle. It is obvious that large micelles will be able to encompass more and larger lipopnilic particles of grease and dirt than small micelles. Assuming a certain detergent of constant micellar size, *three types of solubilization* can be distinguished: (1) *occlusion* in the interior of the micelle, (2) *inclusion into the surface* among the detergent molecules, and (3) *binding at the surface* of the micelle. What kind of binding occurs, depends on the *polarity* of the solubilized substance. Non-polar oily dirt will be occluded and held in the interior lipophilic part of the micelles. Substances which are partly polar and partly non-polar, such as oleic acid, will go into the surface between the detergent molecules, Finally, the strongly polar particles, such as some proteins, will be held at the surface.

Simultaneous solubilization of several sparingly soluble substances by the same detergent has been studied extensively, al-

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though the field can hardly be fully covered because of the unlimited possible combinations of components. Some general rules, however, have emerged from the studies thus far, as already mentioned. It is established that solubilization of polar compounds occurs at different loci from those of non-polar substances. It is also found that the amount of solubilized substance increases with increasing micelle size, and that the micelles expand on solubilization. When several substances are solubilized simultaneously the term *cosolubilization* is used. For example, the effect of cosolubilized octyl alcohol on the solubilization of *m*-dinitrobenzene, *α*-naphthol, and naphthalene has been determined[13]. It was found that, e.g., the solubilizing power of potassium laurate colloid for azobenzene and naphthalene is increased by increasing amounts of cosolubilized amyl alcohol[13].

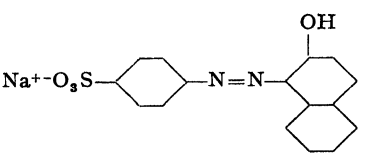
3. The Colloidal Behavior of Dyes

The property of color which depends on selective absorption of a certain wave length of light is not directly dependent upon molecular size. The macromolecular polysaccharides are as colorless as the micromolecular sugars, and pure polystyrene is as colorless as the monomeric styrene. The color of the micromolecular soap does not change upon association into micelles. The appearance of color depends on the presence of certain atomic groups with unsaturated bonds, such as —N=N—, conjugated carbon double bonds, —N=O and others. The molecular size of dyes may be very different, as can be the chemical constitution. When the molecules are composed of a relatively large non-polar part and a small polar moiety, the micromolecular dye may form colloidal micelles, whereby the color may change somewhat. Synthetic organic chemistry has provided thousands of artificial colored substances[14]. Many of them associate into micelles in suitable solvents. Although the soluble dyes are micromolecular substances, the insoluble colored matter formed directly on the fiber in some methods of dyeing is colloidal.

Most of the synthetic dyes are aromatic or heterocyclic compounds. The molecules of the dyes contain several hydrocarbon rings which are hydrophobic, and several polar and hydrophilic groups such as —SO_3Na, —OH, and others. The molecular weight of such dyes is in the range of several hundred, and they usually are soluble in water, alcohol, acetone, and many other solvents. No micelle formation occurs in the cases of the relatively small

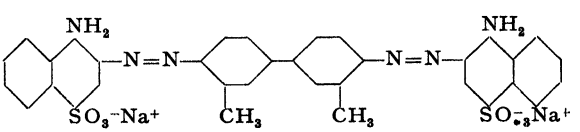
molecules, such as methyl orange, or β-naphthol orange, which are composed of a few rings and contain the strongly polar —SO_3Na and OH groups. Association, however, occurs with dyes whose molecules contain large hydrophobic groups, as in cases of many polymethine (cyanine) dyes. Some of them do not associate in alcohol, but do so in aqueous solutions. The viscosity increases, and changes occur also in the absorption spectrum[15]. The association is reversible, as it is with soap solutions. The correlations between the degree of association and changes in the absorption spectrum are very complex; they vary depending on the dye and solvent, and it is impossible to calculate the micellar size from the absorption[16].

A thorough study on micelle formation of several dyes has been published recently by Alexander and Stacey[17]. Orange II (β-naphthol orange), benzopurpurin, and several others have been investigated by the method of light scattering at various temperatures, and in the presence of various sodium chloride concentrations. It was found that the relatively small molecules of the Orange II do not form stable micelles, although some association and even flocculation



Orange II

occur in time in the presence of sodium chloride. Association into stable micelles of various sizes and shapes has been observed with benzopurpurin. Even long, needle-like particles showing birefringence and tending to form gels can be obtained. The micelle formation depends on the concentration of the dye and sodium chloride



Benzopurpurin 4B

as well as on the temperature. No micelles could be detected in hot solutions at temperatures over 60 °C. In low concentrations of salt, micelles with the 'molecular weight' between 10,000 and 50,000

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were formed at temperatures between 20° and 40 °C. In solutions containing more salt, very large 'giant micelles' of benzopurpurin appeared. Similar aggregations could be detected with polar yellow which gave stable micelles with the relative weight of 110,000 in the presence of 0.05–0.2 *M* sodium chloride at 20–60 °C. Generally, the association of the various micromolecular dyes seems to be governed by the same rules as the association of detergents, phospholipids, bile acids and similar polar-nonpolar molecules. In either case the micelle formation is *reversible*, i.e., they dissociate on elevation of temperature.

Other colloid-chemical processes are involved in the *dyeing of fibers*[18]. Dyeing usually is treated as adsorption, although other ways of binding can also be involved. For example, the binding of dyes by the amphoteric protein fibers is due in part to the formation of salt linkages between the ions of the protein macromolecules and the oppositely charged ions of the dye. Hydrogen bonding and Van der Waals attraction, however, seems to be dominant in most cases, especially with cellulose and rayon fibers[19]. It is established that the molecules of the dye are linked to the free hydroxyl groups of the cellulose. The adsorption of the dye is secured by the polar —OH, =CO, —SO_3, —NH_2 and other groups. It must be borne in mind that the dye molecules are extremely small in comparison with the microfibrils of a substrate, so that the dye may even penetrate inside the microfibril between the fibrous macromolecules. This would be possible at least in the amorphous sections of the fibers. The dyeing of some synthetic fibers such as the polyethylene terephthalate (Dacron polyester) has posed difficult problems. It was found in the Du Pont Laboratories that this fiber can be dyed with certain types of dyes at high temperatures, when the relatively small molecules of the dye diffuse inside the polymer. In this case the dyeing can be described essentially as dissolution of the dye in the fiber. The chemical composition, the polarity, and the molecular size and shape proved to be important in achieving good results. Especially interesting is the fact that the long narrow molecules of the azo dyes proved to dye Dacron more easily than the angular bulky molecules of the anthraquinone derivatives. This indicates that the binding of the dye to the macromolecules of the fiber, along with other considerations, is important. The geometry of the molecules undergoing such association is an important factor, as was mentioned before in treating various other phenomena of interaction. First, a monomolecular

layer of the dye is fixed on the surface of the yarn or fabric, then further amounts of the dyestuff may be precipitated upon that layer. The surface layer may be composed of micelles or even small microcrystals of the dye. It has been shown that in some cases changes in the absorption spectrum of the dye are exhibited upon fixing onto the fiber, and that these changes are similar to those observed on association of the dye in solutions. Some dyes which can not be directly fixed on the fiber can be bound by means of colloidal agents, the so-called *mordants*. Highly dispersed hydroxides and basic acetates of aluminum, chromium, iron and tin possess the ability to bind, e.g., the alizarin dyes to fabrics. The mordant forms complex chelates with the dye, and this complex is readily bound to the surface of the fiber. The chelation is accompanied by certain changes in absorption (color).

Very durable and strongly bound dyes are those which are chemically prepared in the presence of the fiber. For example, a fabric may be impregnated with some soluble salts of aniline, and then exposed to some oxidizing agent. The very small molecules of the aniline salt penetrate deep into the amorphous regions of the fibers, as do the small molecules of the oxidizing agent. The oxidation then takes place at a high temperature resulting in the formation of colored matter (dark brown, black, and other colors, depending on the conditions of oxidation, starting materials and additives). In this oxidation, polycondensation of the aniline molecules occurs, whereby relatively large, long, strongly absorbing molecules are formed. The following reactions in this formation of the 'aniline black' have been considered as possible[14]:

$$n\mathrm{C_6H_5NH_2} + m\mathrm{O} \longrightarrow$$

$$\mathrm{C_6H_5{-}NH{-}C_6H_4{-}NH{-}C_6H_4{-}N{=}C_6H_4{=}N{-}C_6H_4{-}N{=}C_6H_4{=}N{-}}$$

$$\mathrm{{-}C_6H_4{-}N{=}C_6H_4{=}NH}$$

Nigraniline

$$+ x\mathrm{C_6H_5NH_2} + y\mathrm{O} \longrightarrow \boxed{\text{Aniline black}}$$

(larger molecular size than nigraniline).

4. Colloidal Organic Pigments

Not all colored substances can be used as dyes, as many of them are not firmly enough adsorbed on fibers. Some of the naturally occurring pigments, such as chlorophyll and hemoglobin have im-

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portant biological functions. The strongly absorbing hem component of hemoglobin, as mentioned in Chap. 26, is a relatively small, micromolecular part of the large hemoglobin molecule. Chlorophyll and the carotenoids occur in green plants. The beautiful yellow, orange and red colors of autumn leaves are due to the carotenoids. The isomers of carotene, $C_{40}H_{56}$, and the xanthophylls, $C_{40}H_{56}O_2$, are the chief carotenoids. These highly lipophilic substances can be prepared in the form of colloidal solutions by using appropriate

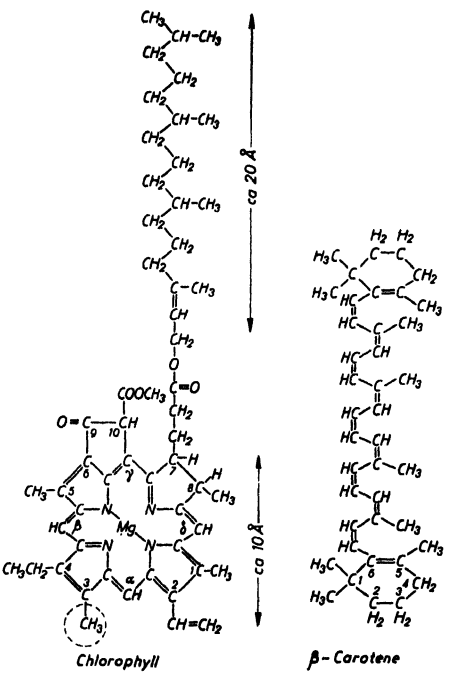

Fig. 159. Molecular structure of chlorophyll and carotene.

solvents; for example, carotene can be dissolved in acetone, and the micromolecular solution poured into water. The stability of the dispersion can be greatly improved by adding a stabilizer, such as a detergent or protein[20].

The structural formulas of carotene and chlorophyll are presented in Fig. 159. The molecular shape of the chlorophyll resembles a tadpole. The bulky 'head' of the molecule is built of symmetrically

arranged pyrrole rings, thus resembling the hem; in the center of the 'head', however, we find a magnesium atom instead of iron. A long hydrocarbon 'tail' is attached to the large head through an ester bridge. Inspection of the structure shows that the chlorophyll molecule contains both non-polar and polar groups, and hence may be expected to form micelles. Chlorophyll dissolves in alcohol or acetone forming micromolecular solutions. When such solutions are mixed with water—a colloidal chlorophyll is obtained. It is interesting that the micromolecular solutions of chlorophyll show red fluorescence when exposed to light, whereas colloidal chlorophyll does not show this optical effect. In green leaves chlorophyll appears bound to a protein and lipid in small granules which are called

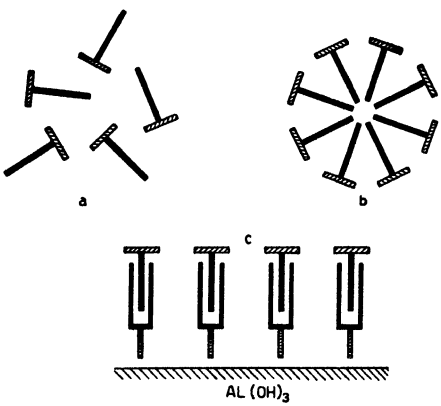

Fig. 160. Schematic illustration of the T-shaped chlorophyll molecules; (a) in micromolecular solution, (b) as micelles in a colloidal solution, and (c) adsorbed on a monomolecular lecithin layer.

chloroplasts. These green chloroplasts are birefringent, and, moreover, they show fluorescence. This indicates that the chloroplasts have a certain oriented structure, and that the chlorophyll appears not in the micelles, but in separate molecules. According to Noack (1927) and Frey-Wyssling[21], chlorophyll is present in chloroplasts in monomolecular layers. It seems likely that these layers are formed not solely by oriented chlorophyll, but that they are mixed films of chlorophyll and lipid. This is ascertained by the fact that the fluorescence is enhanced when chlorophyll is adsorbed on a monomolecular layer of lecithin. According to Hubert (1935) and Frey-Wyssling[21], lecithin makes the chlorophyll molecules even

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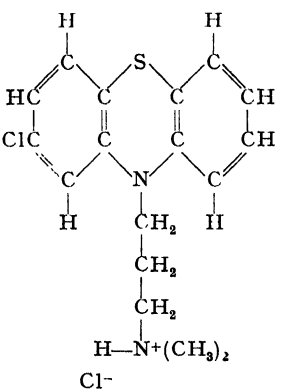
more independent than in a micromolecular solution, because of the peculiar molecular geometry of both substances. Fig. 160 illustrates this. The hydrocarbon tails of the chlorophyll molecules are inserted between the lipophilic forks of the lecithin, thus preventing aggregation of the chlorophyll. The layer structure of the chloroplasts seems to be the most efficient arrangement for the important biochemical reactions of carbon dioxide fixation occurring in green plants. Crystalline, green protein-lipid compounds containing chlorophyll have been isolated from plants[22]. It has been shown recently that chlorophyll is not chemically bound to the protein, but held in the crystals by weak cohesive forces[23].

5. Colloidal Phenothiazines

The phenothiazines are neither detergents nor dyes, but they will be mentioned for two reasons: (1) they are important insecticides, and (2) they have been thoroughly studied lately[24]. The constitution of one of the most important representatives of this class (Megaphen) is shown below.



It was shown that the molecules associate into micelles in aqueous solutions, if the concentration is raised over a certain critical limit. The colloidal solutions have a low surface tension, and they froth strongly. The size of the micelles was determined by sedimentation in the ultracentrifuge, by diffusion, and by light scattering methods, and it was found to be dependent on the presence of salts and on temperature. For example, in the presence of 0.9% sodium chloride

the micellar weight was 17,200 in which case 54 molecules of the phenothiazine have associated. These micelles have a strongly asymmetric shape, as indicated by viscosity measurements. At lower salt concentrations the micelles are smaller and less asymmetric. Dissociation of the micelles is facilitated by the increase in temperature or by dilution of the sol, phenomena which have been observed also with many other micellar colloids, such as dyes and detergents.

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