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INTRODUCTION TO AGRICULTURAL BIOCHEMISTRY

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BY

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To Those Scientists
Who Have Contributed to the Development
of Modern Scientific Agriculture
This Book is Dedicated

PREFACE

THE present volume is an outgrowth of lectures presented to agricultural students over a period of nearly twenty years. The book is designed for those students who have had preliminary training in inorganic and organic chemistry.

We have tried to impress the student with the importance of chemistry in the development of a permanent system of agriculture. Where it was possible to do so, we have endeavored to emphasize the importance of scientific research, for it has been our experience that the interest of students is greatly stimulated by learning how new facts and theories are discovered.

The authors have made no effort to state all things as facts but, on the contrary, have endeavored to leave the reader with the knowledge that agricultural and biological science is in a state of flux, and that the solution of many problems awaits the future investigator.

The book is largely a compilation of material obtained from many sources over a period of many years and, although every effort has been made to give credit to authors for their work and opinions, it is entirely possible that some have been overlooked. The comprehensive nature of the work has made it impossible for the writers to cover all phases of agricultural science in the manner they merit

It is our hope that readers and teachers will feel free to offer constructive criticisms and call our attention to any errors that may be found.

We have devoted considerable space to the chemistry of the proteins, lipids, carbohydrates and other compounds of biological importance, for it has been our experience that most courses in elementary organic chemistry do not have time to stress the importance of these compounds.

We offer the present volume with the hope that it will stimulate interest in the teaching of agricultural biochemistry, and it is our hope that the book may be useful as a general reference book for all readers who are interested in the part that chemistry has played in the development of agricultural science and in the intelligent and economical production of plants and animals.

We wish to acknowledge the generosity and coöperation of many individuals and organizations for illustrative material and permission to use published data. Credit for these is given with the figures and tables.

Special thanks are due Dr. M. W. Lisse, Dr. N. B. Guerrant, Prof. E. H. Rohrbeck and Dr. C. A. Browne for assistance in reading certain portions of the manuscript.

R. ADAMS DUTCHER
DENNIS E. HALEY

Pennsylvania State College, 1931

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INTRODUCTION TO AGRICULTURAL BIOCHEMISTRY

PART I. GENERAL AND INTRODUCTORY

CHAPTER I

THE DEVELOPMENT OF AGRICULTURAL CHEMISTRY

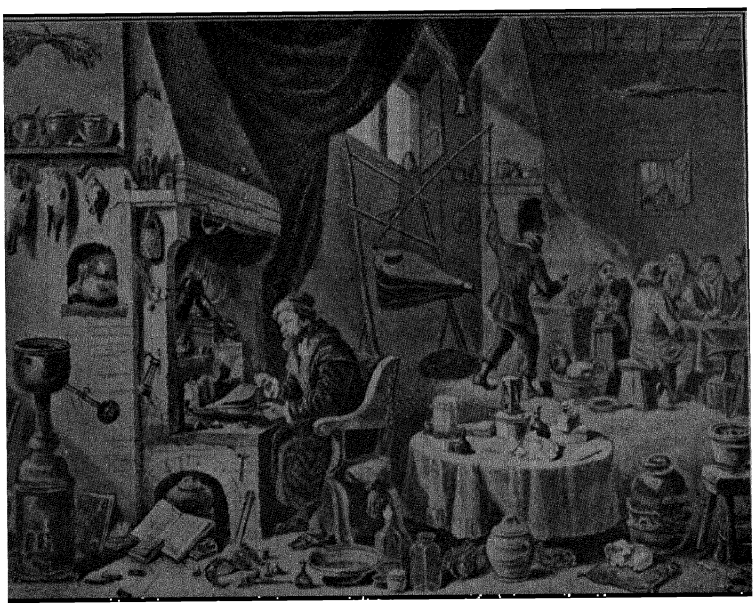
When we attempt to study the factors which have played important rôles in the development of scientific agriculture, we find that chemistry has occupied a most prominent place. The part that chemistry has played in this development has been of such far-reaching importance that a special branch of this science, known as agricultural chemistry, has been a natural outgrowth. It is to this particular phase of chemistry that we wish to direct the reader's attention, for agricultural chemistry, probably more than any other single factor, has been responsible for the development of the quantitative aspects of modern agricultural practice and for the elimination of the old "rule-of-thumb" methods which had been followed for centuries.

The Influence of Alchemy

If, in turn, we wish to trace the historical factors which had to do with the development of the science of chemistry we must go back to the very beginning of chemical effort, viz., to the beginning of alchemy. It has been suggested that the name alchemy is of Arabic origin, probably dating from the eighth century. In the Arabic the prefix *al* means *the*, and *chemia* is the equivalent of *to hide*. Some authorities believe that the word alchemy is derived from the Egyptian word *chemi* meaning black. At least it is clear that the early workers looked upon alchemy as the hidden art or science, and in fact for centuries it was considered the "black art."

The writings of the very early alchemists have been lost, but those of a later date which have been deciphered are replete with mysticism and characterized by obscure terminology, accurately described by a recent writer as "incomprehensible nonsense." It is quite evident that many

of these early alchemists were far from honest. All of them had but three goals toward which they were striving: the first, the transmutation of gold from the baser metals; the second, to find or discover the "philosopher's stone," the possession of which would permit the owner to perform miraculous feats, such as transmutation, the extension of human life, curing of disease, etc.; and the third, the discovery of the



(Courtesy of the Fisher Scientific Company.)

"elixir of life" which would insure perpetual youth. In spite of the fact that alchemy, by even the greatest flight of fancy, could not be termed a science, nevertheless, the science of chemistry as we know it today owes its origin to the labors of the alchemist.

It was stated in the preceding paragraph that many of these workers were not strictly honest. If space permitted, many interesting and humorous tales could be told of the extent to which the alchemist often went to mislead his king and his public. For example, Albertus Magnus, a thirteenth century alchemist, was described by many witnesses as having the power to transform a winter day into one characterized by balmy summer sunshine. A pupil of Albertus Magnus, one Thomas Aquinas, constructed a statue of bronze which his teacher transformed

by means of the "elixir of life" into a very useful domestic servant. It is recorded that the servant tended to be noisy and talkative, which annoyed his master to the point where it became necessary to punish him severely with a hammer in order that the master might pursue his studies without interruption.

Turning to the more serious and constructive side of alchemy, it is clear that Aristotle, in the fourth century B.C., undoubtedly gave impetus to the alchemical concept of the structure of matter which had been the result of philosophical thinking since the fifteenth century B.C. This postulation conceived the universe to be made of the four elements, earth, air, fire and water. As the art of alchemy developed, these terms gradually became somewhat comprehensive in their nature. All vapors, gases, steam and smoke were included under "air," while sparks, lightning and all incandescent objects were described as "fire." Likewise all liquids, such as blood, milk and chemical solutions, became known as "water," and all solids were called "earth." As a result, we have today the term "rare earth metals."

These early workers knew that water could be converted into steam and that steam could be condensed into water. In other words, water could be transmuted into air and air into water. What could be more natural, therefore, than to conceive that metals could be transmuted from one form to another? Out of fairness to the ancient alchemist we must add, however, that it is entirely possible that his hopes of transmutation of metals may not have been so ephemeral after all, for the modern chemist has made the discovery that a number of so-called "elements" are really not elements at all. For example, the element uranium during its turbulent life history has given rise to no less than fifteen different atomic species in addition to eight atoms of helium.

In addition to the term "rare earth," mentioned in a preceding paragraph, we have also retained the alchemical terms alcohol, borax, alkali, and elixir. Many of the alchemical writers refer to Hermes Trismegistus as the Father of Alchemy, and for this reason the "hidden art" has become known as the "hermetic art." It is from this source that we get our modern term "hermetically sealed." Geber, an Arabian physician, attained considerable fame as an alchemist, and to him is ascribed the credit of having written the first chemical textbook, entitled "Summa Perfectionis" (The Summit [or height] of Perfection). Paracelsus, in the sixteenth century, was the first to call attention to the interdependence of chemistry and medicine. The union of chemistry and medicine was a fortunate circumstance for both, but particularly for the science of chemistry, for the reason that it attracted men of culture and education. As a result the new workers in the medical-alchemical field dis-

covered new compounds and paved the way for the development of organic chemistry. Another and equally important situation developed as a result of the interest displayed by the physicians of that time. Prior to this period the alchemist had been striving purely for selfish purposes,

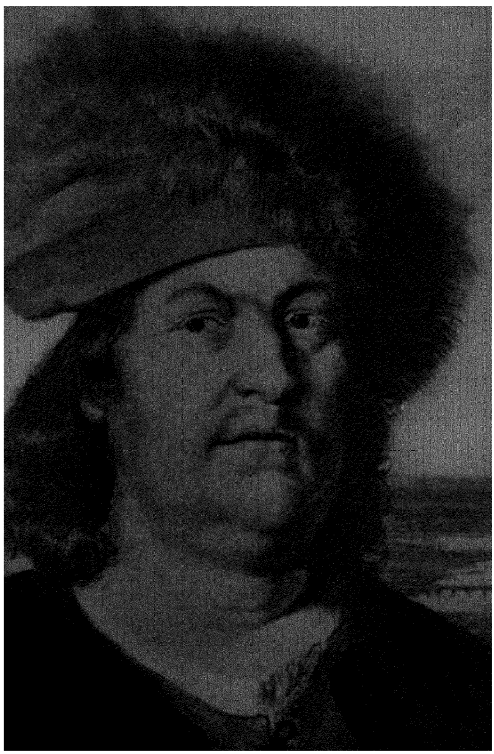


FIG. 2. Paracelsus, famous 16th century alchemist. (From a painting by Rubens, in the Musse Anciens, Brussels.)

for what he could gain personally by obtaining a quick and easy way to riches and perpetual youth. The new workers, like the research workers of today, began to seek for new knowledge, not so much for economic gain, but rather for the general good of mankind and for the intellectual satisfaction derived from constructive, purposeful and unselfish labor.

One of the outstanding figures of the thirteenth century was Roger Bacon. Although little need be said of his chemical accomplishments,

the following paragraph, translated from his "Epistola de Secretis Operibus Artis et Natural de Nullitate Magical," will give the reader some idea of his almost prophetic vision of what was to happen hundreds of years later. Bacon states that "Machines for navigating can be made so that without rowers great ships can be guided by one pilot on river or sea more swiftly than if they were full of oarsmen. Likewise vehicles are possible which, without draught animals, can be propelled with incredible speed, like scythed chariots, as we picture them, in which antiquity fought. Likewise a flying machine is possible, in the middle of which a man may sit using some ingenious device by which artificial wings will beat the air like those of a flying bird. Also machines, small in size, can be constructed to lift and move unlimited weights, than which in an emergency nothing is more useful."

The Beginning of Genuine Chemistry

The alchemical period which we have described so briefly comprised, roughly, the period 300 B.C. to A.D. 1700. In the seventeenth century

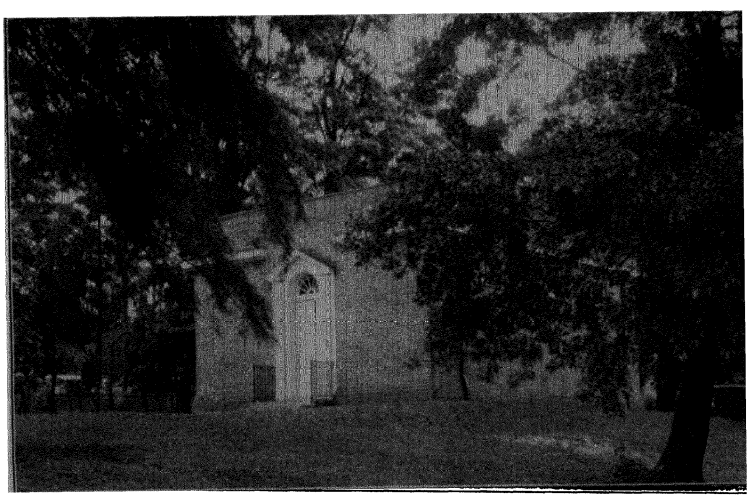


FIG. 3. Priestley Memorial Museum at Northumberland, Penna., established through the efforts of the alumni of the Pennsylvania State College. (Courtesy of Pauline Beery Mack.)

we shall point out but one matter of consequence. It was during this period that the phlogiston theory was advanced; in fact, the seventeenth century might well be called the *phlogiston period* of chemical development. This theory was advanced to explain the phenomenon of com-

bustion. The advocates of this theory contended that a substance to be combustible must contain a substance known as phlogiston. Reasoning in that manner, they concluded that wood or coal, for example, contained large quantities of phlogiston which escaped during combustion, whereas incombustible stones or iron were practically devoid of this important substance. It was not until the discovery of oxygen that the true nature of combustion was demonstrated.

Genuine chemistry had its beginning during the eighteenth century, with the work of Priestley, Cavendish, Scheele and Lavoisier. The work of Priestley is important from a historical standpoint for his isolation of oxygen and the study of oxidation. Cavendish enriched our early knowledge through the use of the electric spark in the formation of nitric acid from the oxygen and nitrogen of the air and water. Scheele, a Swedish chemist, contributed to the chemistry of oxygen and chlorine, and his researches gave us, for the first time, bleaching powder and chloroform.

Up to this time the phlogiston theory was quite generally accepted. It was not until the work of Lavoisier, a French chemist, that the true nature of oxidation was demonstrated. All these workers and many others had their influence on the development of true chemistry and on the development of modern agricultural chemistry.

Search for the "Principle" of Vegetation

When we turn our attention to the early workers who were interested in solving nature's secrets as they relate to agriculture, we find nearly all of them trying to discover "the principle of vegetation." In other words, they were seeking to answer the question, "Why and by what method do plants grow and develop?" Of course, these observers were no less observant than the nature lover of today. As a result, they had noted that plants often grew well in certain soils although the same crops did not yield well in other soils, even under similar climatic conditions.

One of the first theories which aimed to explain the secret of plant growth was that advanced by a Belgian physician and alchemist by the name of Van Helmont. This investigator, working in the latter part of the sixteenth and early part of the seventeenth centuries, was among the first to introduce the use of the balance and interpret data from the quantitative standpoint. It should be remembered that *water* was one of the recognized chemical elements at the time of Van Helmont's work, and as a result of his studies he concluded that *water* must be the "principle" of vegetation, for he cited the following experiment as proof of his theory that water could be transformed into plant tissue. Using Van

Helmont's own words: "I took an earthen vessel in which I put two hundred pounds of soil, dried it in an oven, then I moistened it with rain water, and pressed hard into it a shoot of a willow weighing five pounds. After exactly five years the tree that had grown up weighed one hundred sixty-nine pounds and about three ounces. But the vessel had never received anything but rain water or distilled water, to moisten the soil when this was necessary, and it remained full of soil which was tightly packed, and lest any dust from the outside should get into the soil, it was covered with a sheet of iron coated with tin, but perforated with many holes. I did not take the weight of the leaves that fell in the autumn. In the end I dried the soil once more and got the same two hundred pounds that I started with, less about two ounces Therefore, the one hundred sixty-four pounds of wood, bark and roots arose from the water alone."

This experiment, however, is thoroughly typical of much of the early investigational work in agricultural chemistry, as well as of other sciences. In this, as in other branches of science, it is very easy to fail to consider a vital factor and, as a result, draw, from perfectly good experiments, a conclusion which appears to be correct but which is, in reality, entirely wrong. In the work cited above, Van Helmont failed to take into consideration two most important factors, namely, the rôle played by the constituents of the atmosphere and the two ounces of soil which had disappeared. Hopkins has very truthfully said that "An experiment is a question put to Nature, and Nature always answers every question truthfully, but the question that Nature answers and that the experimentor asks is not always the question that he thinks he asks."

Some years after Van Helmont reported his results, Glauber proposed the hypothesis that *saltpeter* is really the "principle of vegetation." This conclusion was reached by Glauber because he secured such large increases in the yield of crops by applying this material as a fertilizer. In support of his conclusion he also cited the fact that he "obtained saltpeter from the earth cleared out from cattle sheds," and that "It must have come from the urine or droppings of animals and must, therefore, be contained in the animal's food, i.e., in plants." For many years this view of Glauber was widely accepted by the different agricultural writers. The only prominent opponent to this view was Jethro Tull, who believed that the fineness of the soil particles had a beneficial influence on plant growth. According to his view, it "was the very minute particles of soil loosened by the action of moisture, that constituted the proper 'pabulum' of plants. The pressure caused by the swelling of the growing roots forced these particles into the lacteal mouths of roots where they entered the circulatory system. All plants live on these

particles, that is, on the same kind of food." Various other ideas regarding the "principle of vegetation" were proposed. The general view held at the close of this period cannot be better summed up than in Tull's own words: "It is agreed that all the following materials contribute in some manner to the increase of plants, but it is disputed which of them contributes most to that increase of food: nitre, water, air, fire, and earth."

During the latter half of the eighteenth century a considerable interest was manifested in all phases of agriculture. Textbooks were written, experimental work was stimulated, and societies were formed for the promotion of agriculture. In 1755 the Edinburgh Society of England employed a chemist by the name of Francis Home "to try how far chemistry will go in settling the principles of agriculture." Home, believing that the whole system of agriculture was dependent upon plant growth, prosecuted his research along the lines of plant nutrition, finally drawing the conclusion that there were at least six plant food materials: air, water, earth, salts of different kinds, oil and "fire in a fixed state."

FIG. 4. Home of Joseph Priestley, Northumberland, Pennsylvania. (Courtesy of Pauline Beery Mack.)

After the work of Home there was no important advance in agricultural chemistry for forty years. From what has been stated, it is evident that anything like an adequate idea of the growth and composition of plant bodies could not be obtained until certain of the important chemical elements had been discovered and the composition of water and other common substances had been established.

During the period 1770 and 1800 some of this necessary work had been accomplished, but its agricultural importance was not appreciated at the time. This work, the discovery of oxygen by Priestley, and Scheele, the composition of water by Cavendish, and the explanation of combustion by Lavoisier, has already been cited. These discoveries served to overthrow alchemy and opened the way for the development of modern chemistry.

The Beginning of Modern Agricultural Science

After the work of Home, further progress in scientific agriculture was hardly possible until greater use was made of accurate chemical methods of investigation. This type of experimentation soon found expression in the epoch-marking work of the French chemist, Theodore de Saussure. To this investigator is due much of the credit for the quantitative statistical method which has been the basis of all scientific work since that time.

Although Ingen-Housz receives the credit for the discovery of the rôle that carbon dioxide plays in plant economy, it remained for de Saussure to place this discovery on a firm scientific basis by the use of quantitative methods. His book, " Researches upon Vegetation," published in 1804, was really the first scientific work showing the source of the carbon compounds in plants. He established, by means of quantitative experiments, that the increase in the amounts of carbon, hydrogen and oxygen, when plants are exposed to the sunlight, was obtained from the carbon dioxide of the atmosphere and the water of the soil. This early investigator also stated that the mineral elements derived from the soil are essential to plant growth; in proof of this point he gave the results obtained from the analyses of the ash of many different plants. He believed that plants obtain the greater part of their nitrogen from the soil. These views of de Saussure have since been investigated and verified by many different scientists and are substantially those held at the present time regarding the fundamental principles of plant nutrition. In the days of de Saussure, however, this view was not accepted as true, and it was nearly half a century later that Boussingault, Liebig and others repeated the investigations of de Saussure and confirmed his results, which were finally accepted by the scientific world

From the time of de Saussure's work until 1835 there was little active work in progress relating to agricultural science, but the facts which had already been accumulated were given attention and some attempts were made to apply the results to actual practice. Between 1802 and 1812, Sir Humphry Davy delivered lectures annually on agricultural chemistry. These lectures were published in book form in 1813, and entitled

" Essentials of Agricultural Chemistry." This was the first textbook of the modern period, and it treated of the composition of the air, soil, manure and plants, and of the influence of heat and light on plant growth. Although some of the views expressed in this work were very erroneous, it was for the most part a carefully prepared summary of the best accepted knowledge obtained from the results of previous investi-

FIG. 5. Sir Humphry Davy who published the first agricultural chemistry textbook of the modern period (1813). This book, "Essentials of Agricultural Chemistry," went through several American editions and exerted a definite influence on the development of scientific interest in the United States. (Courtesy of the McGraw-Hill Book Company.)

gations. One of Davy's contemporaries, Thaer, also published (1809–10) an important work entitled " Principles of Rational Agriculture." Thaer proposed the so-called *humus theory* of soil fertility. He believed that plants obtain their nourishment from the humus and consequently it is this material which determines the productivity of the soil. This idea of Thaer's, however, was shown later to be inadequate in accounting for the sources of plant food, and as a result it prevented, for a time, the recognition of the actual value of humus as a factor in soil fertility.

Although some of Thaer's conclusions were erroneous, his writings were of a very practical nature and did much to stimulate investigational work.

About 1830 there was a revival of interest in scientific investigations which related to agriculture. At this time Boussingault, a Frenchman, became actively engaged in agricultural research, and began a series of field experiments on his farm in Alsace. He was the first investigator to have a chemical laboratory upon a farm, and to make thoroughly practi-

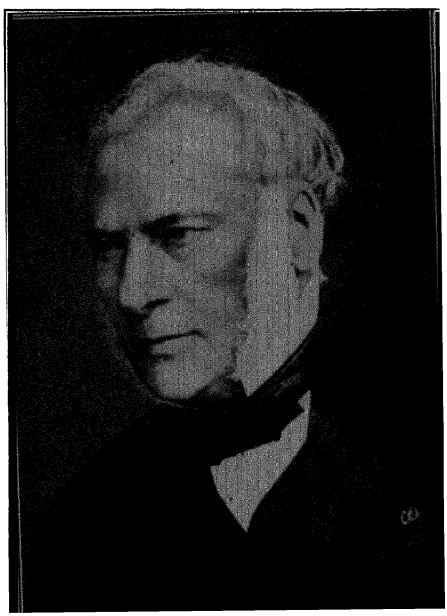


FIG. 6. Jean Baptiste Joseph Dieudonne Boussingault (1802–1887) is credited with the establishment of the first agricultural experiment station in which the chemical laboratory was closely connected with the field experiments.

cal investigations in connection with agriculture. In fact, the establishment of this laboratory marked the start of the *first agricultural experiment station*, although Mary Louise Foster in her "Life of Lavoisier" states that, through the efforts of Lavoisier, the French Government established an experimental farm in Villefrancœur as early as 1778.

Russell states that Boussingault "reintroduced the quantitative methods of de Saussure, weighed and analyzed the manures used and the crops obtained, and, at the end of the rotation, drew up a balance sheet, showing how far the manure had satisfied the needs of the crops and how

far other sources of supply — air, rain and soil — had been drawn upon." He also did very important work upon the assimilation of the free nitrogen of the air by plants. He ascertained many important facts relating to the chemical characteristics of foods, made a comparison as to the quantity of nitrogen in different kinds of feeding stuffs, and compared their values on the basis of the nitrogen content. His study on the production of saltpeter did much to prepare the way for later work on nitri-

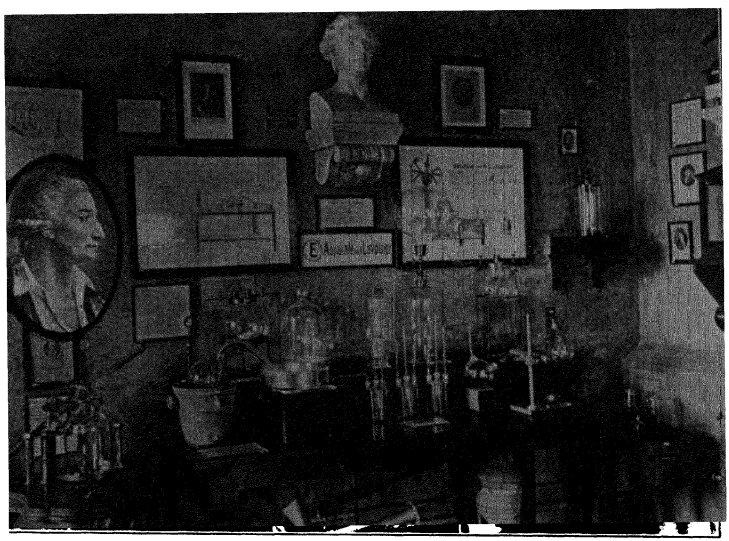


FIG. 7. An exhibit of research apparatus used by the brilliant French chemist, Antoine Laurent Lavoisier (1743–1794). Lavoisier is credited with the establishment of the first experimental farm in 1778. (Courtesy of the Fisher Scientific Company.)

fication. Russell summarized the work of this scientist in the following words: " Boussingault's work covers the whole range of agriculture and deals with the composition of crops at different stages of growth, with the soil, and with the problems in animal nutrition." Much of the earlier work of de Saussure was repeated and verified by Boussingault; during this work many additional facts regarding the chemistry of plant growth were ascertained.

Regarding the source of nitrogen in farm crops he states, " The soil furnishes the crops with mineral alkaline substances, provides them with nitrogen, by ammonia and nitrates, which are in the diluvium which is

the basis of vegetable earth, compounds in which nitrogen exists in stable combination only becoming fertilizing by the effects of time." As to the absorption of gaseous nitrogen of the air by vegetable earth, Boussingault also states, " I am not acquainted with a single irreproachable observation that establishes this. Not only does the earth not absorb gaseous nitrogen, but it gives it off."

No discoveries of importance are recorded during the period 1830 to 1840. In 1840, however, Justus von Liebig's report to the British Association (published later as " Chemistry in Its Application to Agriculture

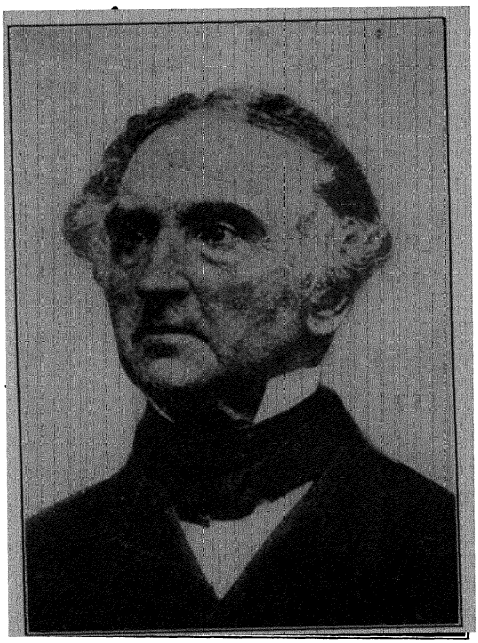


FIG. 8. Justus von Liebig (1803–1873), sometimes called "Father of Agricultural Chemistry." (Courtesy of the McGraw-Hill Book Company.)

and Physiology ") created a great amount of discussion in scientific circles. Liebig was a very forceful writer and seemed to delight in scientific polemical discussions. He berated the plant physiologists for their lack of chemical knowledge and took them to task for ignoring the accumulating experimental evidence that plants receive their carbon supply from the atmosphere rather than from the carbonic acid of the soil. He stated, " All explanations of chemists must remain without fruit and useless because, even to the great leaders in physiology, carbonic acid,

ammonia, acids and bases are sounds without meaning, words without sense, terms of an unknown language, which awake no thought and no association."

Prior to this time many workers adhered to the humus theory of Thaer, which postulated that it was possible for the plant to satisfy its carbon demands entirely from humus or soil organic matter. The publications and lectures of Boussingault and de Saussure had made little impression on the proponents of the humus theory. After Liebig's tirade, however, there were few who dared to oppose his theory of carbon dioxide assimilation. In the first edition of his book Liebig called attention to the fact that farms from which certain products were sold gradually became less productive because of the decrease of nitrogen, but he made one serious mistake. He over-estimated the amount of ammonia present in the atmosphere and under-estimated the value of nitrogen in the soils and manures. A study of the chemical composition of the ash of plants was also made by Liebig. His results led him to propose the mineral theory of plant nutrition. Previous to this time de Saussure had proved that plants contained certain mineral elements, but he had laid no emphasis upon their importance as plant foods. Liebig's publications on the composition of the mineral substances present in plants, and his emphasis of their importance as plant foods, led to the commercial preparation of manures which in later years has developed into the great commercial fertilizer industry.

Liebig's work was unlike that of Boussingault in that it was not conducted in connection with field experiments. It had, nevertheless, a very stimulating influence upon researches in agricultural chemistry, and to him we owe, to a large extent, the collecting and summarizing of the previous work and the pointing out of valuable lines for future investigation. Liebig's great enthusiasm for agricultural investigation may be judged from the following extract: " I shall be happy if I succeed in attracting the attention of men of science to subjects which will merit engaging their talents and energies. Perfect agriculture is the true foundation of trade and industry. It is the foundation of the riches of states, but the rational system of agriculture cannot be formed without the application of scientific principles, for such a system must be based on an exact acquaintance with the needs of the nutrition of vegetables, and with the influence of soils and action of manures upon them. This knowledge must be based on chemistry, which teaches the mode of investigating the composition, and of the study of the character of the different substances from which plants derive their nourishment."

Liebig was a man of wonderful personality. He gathered around him a large following of students, many of whom later became prominent in

scientific work. He is sometimes called the "Father of Agricultural Chemistry." Doubtless it will be of interest to students to know that, though the name of Liebig will go down in history as that of a man of great learning and genius, as a boy in school he was a "sorrow to his parents and a burden to his teachers." It is said that he had no "ear memory," and it was finally necessary to take him from school, at which time his father apprenticed him to an apothecary. In the course of his experimenting he blew the window out of the shop and was dismissed. By hard study in libraries and in the private laboratory in his home he

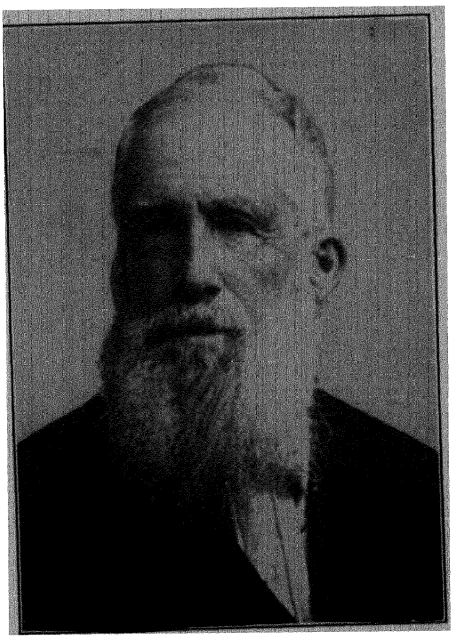


FIG. 9. Sir John Bennet Lawes, Bart., D.C.L., LL.D., F.R.S. Rothamsted Experiment Station in England. (Courtesy of Dr. E. J. Russell.)

obtained such proficiency that he was able to enter college, and at the early age of nineteen obtained the degree of doctor of philosophy. He attracted so much attention that he was given permission to work in the laboratory of the famous Gay-Lussac. These two workers made an important discovery relating to the chemical and physical properties (isomerism) of certain organic compounds. When they obtained the final proof that they had made the discovery they wrapped their arms about each other and waltzed around the laboratory. This personal reference

is made here to show something of the ardor with which Liebig went about his work.

Shortly after Liebig's first work, appeared the investigations at Rothamsted, England, which were begun by John Lawes, a young Englishman who at an early age became interested in chemical investigations. As a boy, experiments made at home seem to have been one of his favorite occupations. He tells us: " At the age of twenty, I gave an order to a London firm to fit up a complete laboratory, and I am afraid it sadly disturbed the peace of my mother to see one of the best rooms of the house fitted up with stoves, retorts, and all the apparatus and reagents necessary for chemical investigation." In 1837, on his home farm at Rothamsted, he commenced experiments, in pots, with agricultural plants and the application of various manures. Lawes was much interested in the results he obtained, and later continued the experiments on a larger scale. He soon found that to carry on his work in a proper manner he needed the assistance of a trained chemist, and he engaged the services of Dr. J. H. Gilbert, one of Liebig's former students, who began his work at Rothamsted in 1843. This marked the establishment of the *second agricultural experiment station;* it is still in operation at the present time, having been endowed with funds by John Lawes. Many of the Rothamsted experiments have been conducted continuously since 1844, and results of the greatest value to agriculture have been obtained as a reward of the earnest, persistent work of Lawes and Gilbert

Lawes was the more practical type of man and directed the agricultural operations on the experimental plots. The execution of the remainder of the experimental work was largely in the hands of Dr. Gilbert, who was a thoroughly trained chemist. Dr. A. D. Hall, one of the recent directors of the Rothamsted station, says of Gilbert: " His special mental characteristics also eminently fitted him for the work subsequently carried out. He was both cautious and painstaking to a remarkable extent, desiring to accumulate a great mass of facts before coming to any certain conclusion upon them. His mode of work was also extremely methodical and a method once adopted after full consideration was continued through many subsequent years, thus giving rise to a long series of results obtained in a perfectly similar manner. The continuation of the same field for more than fifty years and the important results which subsequently followed from an examination of the soil so long under definite cultivation may be cited as an example of Gilbert's methods. Under his care, samples of the grain and straw from each experimental plot in each year were preserved in the laboratory and also the samples of the soil and subsoils of each were repeatedly taken; large portions of each sample were also preserved. At his death the number of samples

stored for future reference in the laboratory and in the adjoining building exceeded 50,000. The bulk of tabulated records prepared by the clerks at the laboratory was correspondingly large. He thus laid the foundation of solid work. The investigations made at Rothamsted on the non-assimilation of atmospheric nitrogen by farm crops, which were published in 1861, were accepted as conclusive evidence upon this much-disputed question. The work of Lawes and Gilbert on manures, nitrifi-

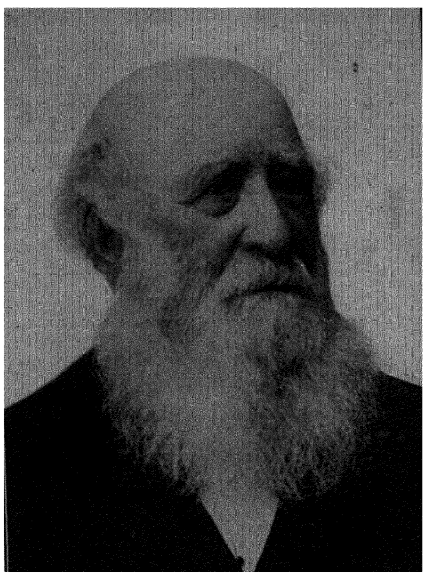


FIG. 10. Sir Joseph Henry Gilbert, M.A., Ph.D., LL.D., F.R.S. (Courtesy of Dr. E. J. Russell.)

cation, the nitrogen supply of crops, and the increase and decrease of nitrogen in the soil when different crops were produced, has had a most important bearing upon the maintaining of the fertility of soils. The general plan of the field experiments at Rothamsted has been to grow some one of the most important crops of rotation separately for many years in succession on the same land without manure, with barnyard manure, and with a great variety of chemical manures. The same kind of manure was applied each year upon the same plot. Experiments with different manures on mixed herbage, on prominent grass lands, on the

effects of fallow, and on the actual course of rotation without manure, and with different manures, have likewise been made."

The association between Lawes and Gilbert in their scientific work is one of the most pleasant recorded of men of science. Dr. Gilbert as a rule spent an hour at Rothamsted every day that Lawes was at home. The plans for the new experiments, the results obtained from day to day, and the drafts of the reports in preparation were thus all discussed by them together. They worked as harmoniously as two brothers, and this lasted for a period of fifty-seven years, or until the death of Dr. Lawes in 1900. Dr. Gilbert died one year later.

Our modern science of bacteriology was, in a large measure, the outgrowth of the early work in agricultural chemistry. As the men studied and worked they found that chemical explanations were not sufficient, and from the earliest times until the present the chemist has made use of all the other sciences, indiscriminately, to aid in the solution of his problems.

The pioneers in agricultural chemistry first confined their attention to the chemical phases of plant growth. Ingen-Housz and de Saussure showed that atmospheric carbon dioxide was the source of carbon in plant compounds. Later Lommel and Pfeffer emphasized the importance of light in the synthesis of organic compounds in the plant, and Sachs, Loew, Baeyer, and others pointed out the important function of the green pigment, chlorophyll, in this synthetic process. It has remained for Willstatter and his students during the past few years to shed further light on the chemical composition of chlorophyll.

The Beginning of Animal Chemistry

From the study of plant protoplasm by Mohl and his school it was but a step to similar studies upon animal tissues by Liebig, Rittenhausen, Chittenden, and others. Among the early investigators who studied the composition of the animal body were Fourcroy, Vauquelin and Berzelius. The chemical composition of bone was studied by Bibra, Mulder and Fremy, and the early chemistry of the albumins was contributed by Graham, Hammarsten, Kossel and Fischer. Although Abderhalden, Fischer and Kossel are pioneers in the study of the decomposition products of proteins, Emil Fischer has been able to build, by synthetic methods, complex protein-like compounds from the simple cleavage products of protein. We will discuss much of this work in detail in later chapters.

In the early publications relating to the consumption of oxygen by animals and its elimination during rest and exertion, we find the names Regnault, Pettenkofer and Voit, as well as those of other investigators equally important.

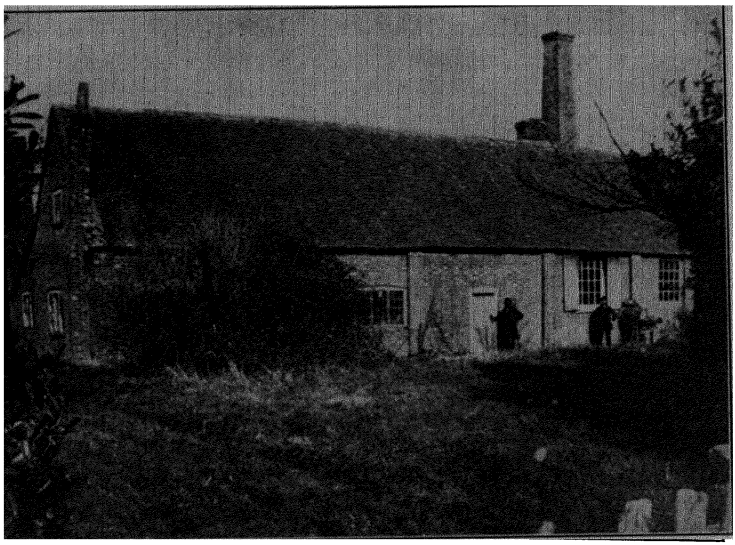


FIG. 11. Old barn at Harpenden, England, where the first experiments were started. This marked the beginning of the Rothamsted Experiment Station. (Courtesy of Dr. E. J. Russell.)

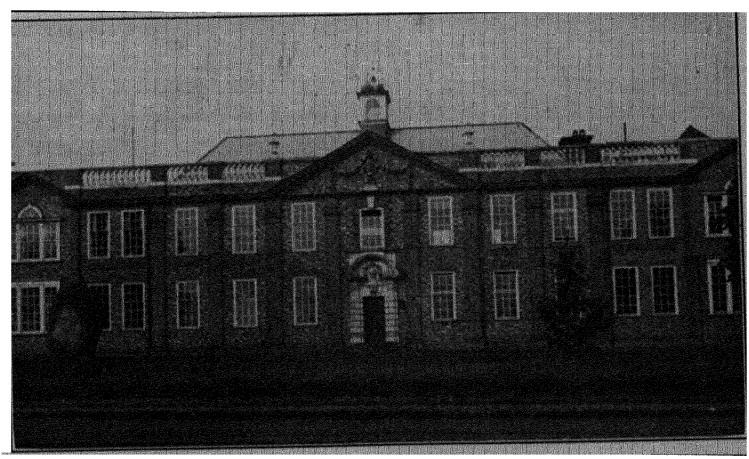


FIG. 12. Present Laboratory of the Rothamsted Experiment Station at Harpenden, England. (Courtesy of Dr. E. J. Russell.)

Liebig was no less prominent in the field of animal nutrition than in the field of plant nutrition. To him is really due the credit for giving the real impetus to the chemical solution of nutrition problems. It was Liebig who first pointed out that albuminous compounds are utilized by the animal body in the formation of tissue, whereas the function of fats and carbohydrates seems to be in the nature of a body fuel for the animal. Kellner, Atwater, Armsby, Benedict and Lusk in more recent

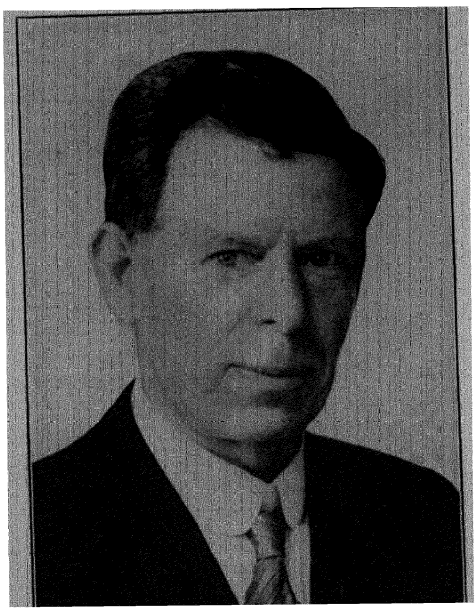


FIG. 13. Dr. E. J. Russell, Director of the Rothamsted Experiment Station and international authority on the chemistry of soils and crop plants.

years have taught us many things regarding energy values of foods and energy requirements of the animal body, utilizing the bomb and respiration calorimeters in their studies.

The classical researches of the eminent French chemist, Louis Pasteur, yielded many contributions of great importance to agriculture. His studies on French wines resulted in the discovery of the true nature of fermentation: that it is invariably due to the presence and growth of micro-organisms. This work inaugurated a new era in the wine-making and brewing industries. This brilliant investigator also ascertained the cause for and found a means of controlling the dreaded silkworm disease

which had practically destroyed the silk industry of France. His experiments with chicken cholera, an epidemic which had destroyed annually 10 per cent of the domestic fowls of his country, developed the use of a preventive method which reduced the death rate to 1 per cent. Next came the successful attempt to deal with the fatal cattle scourge, known as anthrax, which gave to the world an attenuated virus for inoculating animals and reduced the rate of mortality from 10 to 1 per cent. He is equally well known for his discovery of a treatment for rabies, which has resulted in the saving of many human lives. As to the monetary value of these discoveries, Thomas H. Huxley gave as his opinion that in his day it was equal to the war indemnity paid by France to Germany in 1870. It has also been truly pointed out that "the secret power which enabled Pasteur to bring under the domain of scientific laws the phenomena of disease which had so far baffled human endeavor was simply due to the application to these complex problems of the exact methods of chemical and physical research."

The discoveries of Pasteur made possible the work of Schloesing and Muntz which proved the nature of nitrification in soils, a subject that was soon further elucidated by Warington and Winogradski, and also by the findings of Hellriegel and Wilfarth regarding the function of bacteria in the fixation of atmospheric nitrogen by legumes.

We have attempted to show the reader in this brief discussion that the beginning of modern chemistry had its origin in the work of the alchemist, and that the beginning and development of scientific agriculture bears the indelible impress of the agricultural chemist. Up to this point we have described the influence, on agriculture in general, of the investigations of the European chemists. Let us turn our attention, at this point, to the development of agricultural chemistry in America.

Beginning of Agricultural Science in America

Probably the first American publication (1678) which called attention to the chemical phases of agriculture was a paper by John Winthrop, Jr., the first governor of Connecticut. In this paper — "The Description, Culture and Use of Maize" — he called attention to the sugar content and described the syrup obtained therefrom. In 1688 the Rev. John Clayton emphasized certain chemical properties of tobacco. In these early pre-revolutionary days there was a growing interest in chemistry as it related to agriculture, but no chemical work is on record.

One of the earliest chemists who exerted a real influence on American agriculture was Dr. Samuel L. Mitchill, who held the chair of chemistry and agriculture at Columbia College from 1792 to 1801. Professor

Mitchill helped to found the New York Society for the Promotion of Agriculture, Manufactures and the Useful Arts, and through his efforts considerable interest was developed in the use of gypsum as a fertilizer.

In 1806, Dr. Thomas Ewell, of Virginia, published an important treatise in which he stated that "Agriculture is most intimately connected with chemistry. The power of seed to attract and unite to parts of the soil, so as to vegetate or increase in bulk, is purely chemical. Chemical knowledge will teach the gardener and farmer what particular soil is best adapted for particular seed; it will teach the way of forming soils for foreign plants; of making manures to the greatest advantage; of preserving grains, roots, etc.; of destroying the insects; and of correcting the disorders injuring the valuable shrubs."

Sir Humphry Davy's work, mentioned in a previous paragraph, was published in 1813 and went through several American editions. This was the standard book in agricultural chemistry until the appearance of Liebig's well-known work in 1840. Both these men exerted a marked influence on the American agriculture of that period.

In 1810 Dr. Gerard Troost, a Dutch physician, emigrated to this country. He not only made what appear to be the earliest studies of the composition of Pennsylvania soils, but he made valuable contributions to agriculture, mineralogy and geology in Tennessee, where he spent the remainder of his life.

The first chemical bulletin pertaining to agriculture, published by the government, was written by Benjamin Silliman, Sr., Charles Upham Shepard and collaborators. This work, which was devoted to the chemistry of sugar cane, was published in 1833. In 1834 Mr. Shepard was made professor of chemistry at the South Carolina Medical College. While residing at Charleston, Mr. Shepard discovered the calcium phosphate deposits which were destined later to yield enormous revenues in the manufacture of fertilizers.

Edmund Ruffin, in 1821, published an "Essay on Calcareous Manures" which, greatly enlarged, was issued in book form in 1852. This work has been described by Dr. C. A. Browne as a "classic" of its time "which can still be consulted with profit."

In 1842 Dr. Samuel Luther Dana published his "Muck Manual," which exerted a tremendous influence on New England agriculture for the next twenty years. The period 1840 to 1850 was marked by a considerable increase in scientific and industrial activities. Chemists were busy studying the chemistry of soils, crops, foods and fertilizers.

A brilliant young agricultural chemist by the name of John Pitkin Norton was professor of agricultural chemistry at Yale from 1847 to 1852. Although Professor Norton died at the early age of thirty, he

had found time to be an inspiring teacher, a brilliant research worker, and a copious writer. As a result of Norton's influence, Samuel W. Johnson and William H. Brewer, both of whom became celebrated agricultural chemists, became interested in agricultural chemistry. Professor Johnson, who later succeeded Professor Norton at Yale, is probably best

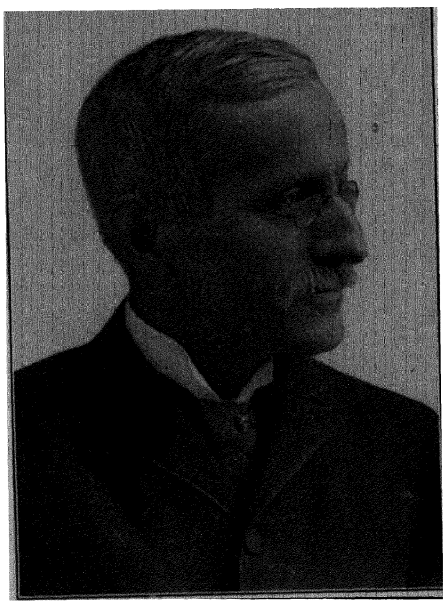


FIG. 14. Dr. Samuel W. Johnson (1830–1909), pioneer American agricultural chemist who is largely responsible for the development of feed and fertilizer control, which is now a part of the governmental organization in the United States. Though Dr. Johnson achieved a reputation as a research worker, he is best known for his ability as organizer, administrator and writer. (Courtesy of Mrs. T. B. Osborne.)

known for his books entitled "How Plants Feed" and "How Crops Grow."

No discussion of American agricultural chemists would be complete without a word or two about Dr. Evan Pugh. Like Professor Norton, Dr. Pugh died early in life. In spite of this, he crowded into his busy young life accomplishments which would have done credit to a man of considerably greater age. Dr. Pugh obtained his graduate chemical training in Europe, and after he had obtained his doctorate he spent two years with Lawes and Gilbert at the Rothamsted Experiment Sta-

tion where, with Lawes and Gilbert, he published his well-known treatise entitled "Sources of the Nitrogen of Vegetation." In 1859 Dr. Pugh returned to the United States in order to accept the presidency of the Pennsylvania State College and headship of agricultural chemistry at State College, Pennsylvania, where he died a few years later at the age of thirty-six years. Although Dr. Pugh's researches were not numer-

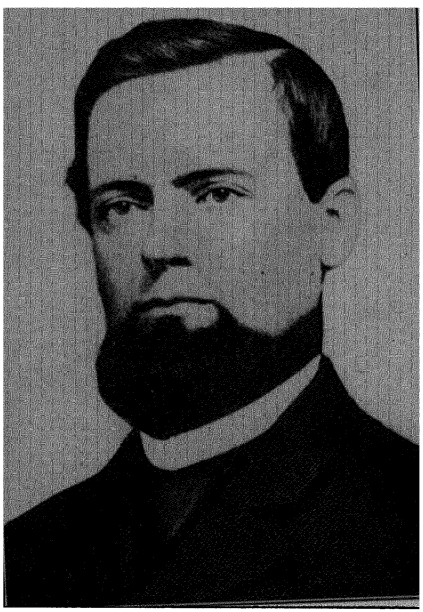


FIG. 15. Dr. Evan Pugh (1828–1864), first President of the Pennsylvania State College. In the field of agricultural chemistry he is best known for his research work on nitrogen utilization by plants, which was conducted at the Rothamsted Agricultural Experiment Station in England. (Courtesy of Sara B. Valentine.)

ous, they were characterized by an unusually high quality of skill and accuracy. His administrative record at the Pennsylvania Agricultural College was no less brilliant, and the organization of the college curriculum, the inauguration of research and the high standards set by Dr. Pugh at that early date will always stand as monuments to his memory.

One of the most important contributions made by the Pennsylvania State College to American agriculture was the establishment, in 1881, of the Soil Fertility Plot Experiments which have been continued to the present day. The fiftieth anniversary of the establishment of these

experiment plots was celebrated at the Pennsylvania State College in July, 1931, and was attended by prominent soil scientists from this and other countries. During the anniversary celebration the plots were dedicated to Dr. Whitman Howard Jordan, who laid them out in 1881. They are now known as the Jordan Soil Fertility Plots. It is to be

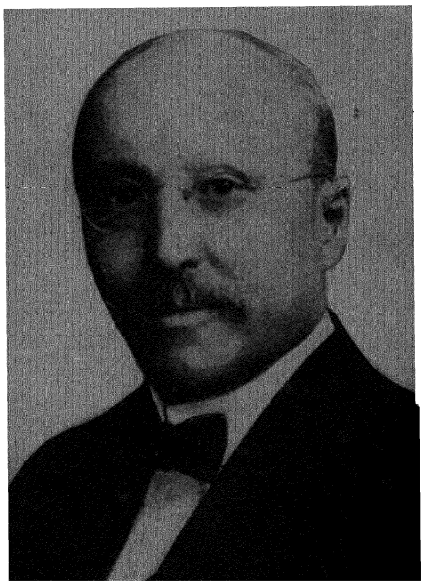


FIG. 16. Dr. Whitman Howard Jordan (1851–1931). Professor of Agricultural Chemistry at the Pennsylvania State College from 1881 to 1885. Dr. Jordan laid out the famous, permanent soil fertility plots which now bear his name. He was a capable research worker, author of many books and scientific papers and a gifted administrator. For many years he was the Director of the New York Agricultural Experiment Station at Geneva, New York. (Courtesy of T. I. Mairs.)

regretted that Dr. Jordan died but a few weeks prior to the time that he was to be the guest of honor at the fiftieth anniversary celebration.

Our brief description of prominent agricultural chemists will not be complete without the mention of Professor John W. Draper, the first president of the American Chemical Society. In 1844 Professor Draper published his "Treatise on the Forces which Produce the Organization of Plants." This work attracted considerable attention, particularly with reference to the photosynthetic processes in plants.

Professor Samuel W. Johnson of Yale undoubtedly exerted a greater influence than any other agricultural chemist of his time. Though Professor Johnson achieved a reputation as a research worker, he is best known for his ability as an organizer, administrator and writer. He is considered one of the most capable critics of agricultural chemical literature of all time. Professor Johnson is known as the founder of chem-

FIG. 17. Partial view of the Jordan Soil Fertility Plots at the Pennsylvania State College.

ical regulatory work in America, owing to his inauguration of fertilizer control work in the State of Connecticut.

If space permitted, the authors of this book would like to discuss here in some detail the invaluable contributions made to American agriculture by outstanding contemporary agricultural chemists. We should like to tell the reader of the work of Professor E. W. Hilgard, Dr. Cyril Hopkins, and others in the field of soil chemistry; of Dr. S. M. Babcock in dairy chemistry and animal nutrition; of Dr. C. A. Goessmann and Dr. Whitman H. Jordan in animal nutrition, fertilizer studies and research administration; of Dr. L. L. Van Slyke in dairy chemistry; of Dr. Henry Prentiss Armsby in animal nutrition; of Dr. William Frear in soils chemistry and pure food work, and many others of equal prominence whose contributions will be described in succeeding chapters.

That the chemist has played an unusually important rôle in American

agricultural development is brought out best by Dr. Harvey W. Wiley, who stated in an address before the American Chemical Society in 1901, " Of the forty-nine directors of the stations at the present time, twenty were professional chemists at the time of their appointment. The selection of so many professional chemists was no mere chance, but evidently had some relation to the dominant position which the science of chemistry holds to the promotion of agricultural research. The list of the

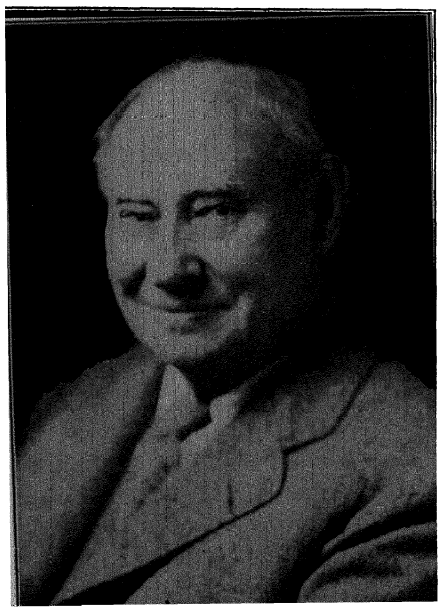


FIG. 18. Dr. Harvey W. Wiley (1844–1930), pioneer American agricultural chemist, who was largely responsible for our present legislation controlling food adulteration and the misbranding of foods.

directors of agricultural experiment stations of Germany shows the same condition of affairs." He goes on to state, " Statistics show that the number of chemists employed in the agricultural experiment stations of the United States is one hundred and fifty-seven, while the botanists number fifty, and the number of entomologists, forty-two."

The report of the Office of Experiment Stations for 1912 shows that the number of scientists employed in that year was as follows: chemists, 250; entomologists, 101; and botanists, 61. In 1915, the number of chemists had increased to 279.

In the United States there has probably been no greater encouragement offered to agricultural science than the grants made at different times by the Federal Government. The Morrill Act in 1862 established our agricultural colleges; the Hatch Act in 1887 established our agricultural experiment stations; and the Adams Act in 1906 provided means for special investigations which have meant much for the promotion of agricultural research. The recent passage of the Purnell Act, which provides additional funds for the prosecution of scientific research in the various state experiment stations, has also served to stimulate many phases of scientific activity as well as the creation of many new research projects.

Another agency which has been of inestimable value to American agriculture is the federal Department of Agriculture at Washington, D.C. With its many bureaus, one of which is the Bureau of Chemistry and Soils, and its excellent scientific personnel, it has not only played a most important rôle in scientific agricultural development, but its researches have helped to better living conditions in every walk of life in every state of the union.

The writers cannot claim to have covered at all adequately the historical development of agricultural chemistry. They have tried to show that chemistry has been the handmaiden of agriculture. In summarizing what we have tried to convey we can do no better than repeat the words of Dr. G. S. Fraps: "The chemist was the first of scientists to turn his attention to agriculture, and his results were so fundamental and of such practical importance, and the science of chemistry was capable of such broad application to agriculture, that for a long time the great body of scientific knowledge regarding agriculture was known as agricultural chemistry. The chemist has not hesitated to avail himself of the sciences of geology, mineralogy, physics, botany or such other sciences as were needed in the solution of the problems at hand. Many of the problems of agriculture are complex and their solution requires the harmonious coöperation of several sciences. Take, for example, the transformation of organic nitrogen to nitrates, a very important process in the soil. This is a chemical change, accomplished by means of micro-organisms, and both chemistry and biology are necessary to give an explanation of this phenomenon, though the explanation has been largely worked out by the chemist."

In the Report of the Director of the Wisconsin Agricultural Experiment Station for 1913, Dr. Russell states, "Chemistry is the basic natural science that has contributed most to the present development of agriculture." Because chemistry has been of such fundamental value in the past in explaining the perplexing questions regarding the composi-

tion of the soil, the relations existing between soil and growing plant, as well as the means of producing better plants and animals, the authors feel that the presentation of such a work is justified. Not only should the student of agriculture be familiar with scientific methods and what these methods have been able to accomplish for the great art of agriculture, but he should also be made to see the immense possibilities for applying the scientific method to future work. In agronomy the chemist will still find great opportunities in the study of the composition of crop plants, soils and fertilizing materials; in animal husbandry and dairy husbandry he will be needed to assist in solution of feeding problems relating to the production of better animals and more and better milk, eggs and meat. He must stand guard over the products used as foods for animals, as well as man, and see that they are always up to standard. In horticulture his methods have been and will be of value in studying fruits and fruit products as well as insecticides and fungicides used in orchard practice. In fact, science and practice have become so interwoven that efficiency demands that the rule-of-thumb method shall no longer be tolerated by the successful farmer.

In succeeding chapters it will be our aim to present the applications of chemical research to agricultural practice, not as permanently established facts in every case, but rather as a means of portraying the continuous growth and transition through which our scientific conceptions are continuously passing.

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CHAPTER II

THE CHEMISTRY OF LIVING MATTER

General Properties. — The art of agriculture has to do, primarily, with the economic production of plants and animals. In the study of agricultural chemistry we must keep this fact in view at all times. Agricultural chemistry may be defined, therefore, as the application of chemistry to agricultural problems. Since biochemistry, which is an important part of agricultural chemistry, is sometimes defined as the "chemistry of living things," it is clear that much of the chemistry with which we are to deal in this book will have to do with life processes.

If we look about us and note the various objects with which we are familiar, we can group all things into two great classes, the living and the non-living or the lifeless. Let us ask ourselves a question at this point — "What properties do all living things possess that are not possessed by those which we have called lifeless?" One property comes to mind immediately, viz., the *power of movement.* To be sure, the plants with which we are familiar cannot move from place to place like animals, but plants, nevertheless, possess the power to move during growth.

We have already mentioned the second property which characterizes living things, viz., *growth.* This property of growth is characterized by synthetic development from within, which is different from the type of growth we see in inanimate crystals which are synthesized by additions from the outside. Another property which characterizes living things is the *power to respire.* Respiration, the power to consume oxygen, give off carbon dioxide and liberate energy, is, therefore, a third characteristic property. All living things respond to changes in their environment, and we say that they possess the property of *irritability.* This is the fourth property not possessed by non-living things. The fifth and possibly the most important property possessed by living matter is the *power of reproduction.*

With the invention of the microscope and the development of the sciences known as cytology and histology we came to the realization that the cell is the biological unit of life. Chemistry and physiology have shown that these tiny units, called cells, may be likened to tiny factories, each receiving its raw materials from which it fabricates not only its own building materials but, in addition, often furnishing raw materials for other cells elsewhere in the organism.

When we examine the cells of plants and animals we find that they differ in certain respects but, in general, we find that they both contain, as their principal constructive substance, a liquid or semi-liquid form of matter known as protoplasm. When we make a careful chemical examination of plant and animal protoplasm we find that they are essentially the same, i.e., they have many chemical and physical characteristics in common.

Immediately a host of questions suggest themselves regarding this protoplasm: what is its function in the cell and what are the chemical activities of the cell itself; how does it receive its raw materials; what changes are produced and how are these chemical changes stimulated and controlled? Many of these questions can be answered by means of chemical research, and it is through the study of problems of this type that we are able to obtain a mental picture of the various phases of growth and development in plants and animals. However, before we can go far in the study of these questions we must ascertain, so far as possible, the chemical composition of the cell and the nature and structure of the constituents as well as their chemical and physical properties. It is logical, therefore, that we turn our attention, for a time, to the chemical compounds that have been found in living cells. In the discussion which follows we shall not attempt to differentiate sharply between plant and animal compounds but rather discuss all compounds found in living tissues regardless of their source and functions. In later chapters these differentiations will become apparent as we discuss the various applications of chemistry to plant and animal growth.

The Chemical State of Living Matter. — When any living tissue is placed in a laboratory oven and allowed to remain at the temperature of boiling water (100° C) for a few hours, we discover that the tissue has lost a proportion of its weight during the drying process. If we analyze the air in the oven we find that it has increased in humidity and the tissue has become dry. In other words, the tissue, during the drying process, has lost *water*. If we place the dry tissue in a platinum dish and heat the dish at a red heat over the gas burner we note that the tissue carbonizes, showing the presence of *carbon compounds*, and, if the heating process is continued long enough, nothing remains but a white or grayish-white *ash*. Chemical examination of this ash yields information regarding some of the mineral elements originally present in the tissue. These simple laboratory manipulations have revealed three important facts, viz., that living tissue contains water, inorganic salts and carbon compounds. We shall now devote a little time to a discussion of some of the more important of these chemical constituents.

Water. — If the reader has been concentrating his mind sufficiently on this and preceding paragraphs, his brain has been receiving certain

stimuli. These stimuli, in turn, have called to mind certain facts learned in preceding courses in chemistry and biology. In other words, memory has been stimulated. The brain, which is the most wonderful tissue we possess, has the property of endowing us with intelligence, memory, love, hate, and all other characteristics which are common to man. And yet when this wonderful tissue is subjected to chemical analysis we find that about 80 per cent of its mass consists of water. This is somewhat disconcerting until we examine other tissues. When this is done we discover that all living tissues are characterized by containing relatively large amounts of water. The following table gives a few typical tissues, chosen at random, and emphasizes the importance of water in living organisms.

TABLE 1

WATER IN SOME TYPICAL TISSUES

Type of tissue	Percentage of water	Type of tissue	Percentage of water
Apples	84	Brain (white matter)	68
Strawberries	90	Brain (gray matter)	84
Alfalfa	72	Muscle	75
Corn (fodder)	79	Liver	76
Asparagus	94	Kidney	78
Lettuce	95	Thymus gland	77
Turnips	89	Adrenal gland	80
Cucumbers	95	Dentine (in teeth)	10

Properties of Water. — One of the most important properties of water from the physiological standpoint is its *solvent power*. In fact, water is the only solvent of importance for inorganic salts, and a number of organic compounds are also readily soluble in it. In fact, we have already inferred that protoplasm is a water solution or suspension of organic and inorganic compounds. Another important property possessed by pure water is its power to dissociate into hydrogen-ions and hydroxyl-ions with the result that water has the property of reacting with many types of compounds. One very important example of this property is the reaction of water with proteins and carbohydrates causing the formation of amino acids and simple sugars. Such reactions are called *hydrolyses*, and the products formed are called *hydrolytic products*.

All students who have studied chemistry and physics are familiar with the fact that the *specific heat* or *heat capacity* of water is high. In other words, a greater amount of heat is required to raise the temperature of a

gram of water one degree Centigrade than is required for almost any other substance. This is a very valuable property from the standpoint of plant and animal metabolism, for the reason that the water in the cells acts as a buffer, preventing extreme changes in temperature within the organism.

Another interesting and valuable property of water is its high *latent heat of vaporization.* Latent heat of vaporization may be roughly defined as the amount of heat required to change a given amount of substance from the liquid to a gaseous state without increasing its temperature. It is clear, therefore, that a substance possessing a high latent heat will require relatively large amounts of heat to produce the change described and, conversely, such a substance will give off relatively large amounts of heat when passing from the gaseous to the liquid state. Water also possesses the property of *conducting heat* with great efficiency. This property is of great importance in regulating the temperature of plants and animals.

In a later discussion we will find that water plays a most important rôle in enabling cellular structures to maintain their form and that it also assists in the formation of protoplasmic membranes. When water plays a part in enabling organisms to maintain their shape or form, two phenomena are considered important, viz., osmosis or osmotic pressure, and the imbibition or absorption of water by tissue colloids. When we study protoplasmic membranes and begin to ask questions regarding cell membranes and membrane permeability, we find that the phenomena of surface tension and surface energy are of extreme scientific interest and importance. Space will not permit further discussion of the properties of water, but sufficient examples have been cited to show that water is of the greatest biological importance, for without it life would not be possible.

Inorganic Salts. — When living tissues are examined for their inorganic constituents it is found that potassium, sodium, calcium, magnesium, iron, sulfates, phosphates, and carbonates are always present and, in fact, they are essential to life. Other elements, such as silicon, manganese, iron, fluorine and iodine, are sometimes present in small amounts, and only recently a prominent French scientist has called our attention to the biological importance of certain elements which, in times past, have been almost ignored because of the fact that they are usually present in but minute traces in living tissues. Such elements are cobalt, manganese and nickel. Recent research work has shown that traces of mineral elements and particularly copper have a definite effect upon the utilization of iron in the regeneration of hemoglobin in red blood cells.

In spite of the fact that the mineral salts are not present in high con-

centrations their importance is often under-estimated. Many of these points will be discussed in later chapters, but it is advisable to point out at this time that the inorganic salts play an important part not only in the construction of tissues but also in controlling chemical and physical changes in plants and animals. Some of these compounds exist as inorganic salts, and some are evidently in chemical and physical combination with organic substances. Salts possess the property of dissociating with the formation of ions which possess the property of conducting the electrical current. It is undoubtedly through the possession of these electrical properties that salts, acids and bases affect the deportment of protoplasm, cells and their membranes. Nerve impulse and muscle contraction in animals, transportation of nutrients and the synthesis of new compounds in plants, are dependent upon the nature and amounts of inorganic substances available in the organism.

Organic Matter. — After water and the other inorganic compounds have been removed from plant and animal tissues the remaining portion is called organic matter; this material is composed of compounds containing carbon, hydrogen, oxygen and nitrogen. These compounds are often referred to as the *compounds of carbon.* When these organic compounds are isolated for the purpose of further study, it is found that they consist of a number of types of compounds of the greatest biological importance. Because of their great importance these compounds will be discussed in greater detail in the following chapters.

CHAPTER III

THE CARBOHYDRATES

Continuing our discussion of the chemistry of plant and animal protoplasm, we shall now turn our attention to those various groups of carbon compounds (carbohydrates, fats and proteins) which are of great biological importance. When the plant puts forth its leaves and starts to " shift for itself " the first types of compounds formed in the leaves are the *carbohydrates*. For this reason, if for no other, we shall begin our discussion of carbon compounds by a brief consideration of this interesting group of substances.

In the early stages of chemical endeavor the chemist observed that the starches and the sugars, when heated slowly in an open dish, charred to form pure carbon, giving off, at the same time, rather large amounts of water. Analyses of the sugars and starches revealed the fact that these compounds contained carbon, hydrogen and oxygen, and that the latter were in the proportion of two to one, as in water. It was natural, therefore, that these early workers should infer that the hydrogen and oxygen were combined as water in these compounds. In other words, it was thought that the starches and the sugars were *hydrates of carbon.* Hence the name *carbohydrate.* Later investigation showed that, although hydrogen and oxygen were combined in the sugars in the proportion of two to one, these compounds were not really hydrates at all. They were found to be aldehyde and ketone derivatives of the polyhydric alcohols.

Although the starches and sugars are not the only substances classified under the heading of carbohydrates, they are by far the most important. For these it is possible to write a type formula, $C_m(H_2O)_n$, which will apply to most of the members of this group. The student, recalling his previous knowledge of organic chemistry, will remember that there are other compounds, also, to which the type formula mentioned above will apply. Examples of such compounds are acetic acid, $C_2H_4O_2$, and lactic acid, $C_3H_6O_3$. These acids, however, differ from the carbohydrates in their chemical structure and behavior.

Carbohydrates, particularly the simpler ones, are easily oxidized. They are, therefore, excellent reducing agents, and it is to these compounds that most of the reducing properties of protoplasm are attributed.

The chemist takes advantage of this fact to reduce solutions of silver and copper salts, in alkaline solution, using it as a test for carbohydrates.

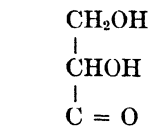
Carbohydrates will oxidize to form acids, indicating the presence of aldehyde $-\overset{\displaystyle H}{\underset{|}{C}}=O$ or carbonyl (ketone) $=C=O$ groups. The former, upon reduction with hydrogen, forms the primary alcohol group $-CH_2OH$; the latter forms a secondary alcohol radical $=CHOH$. The polyhydric alcohols, mentioned in a previous paragraph, are alcohols containing many alcohol groups in the molecule. The simplest carbohydrates are, therefore, polyhydric alcohols containing aldehyde and ketone groups. Those which contain the aldehyde group are known as *aldoses;* those containing the ketone group are called *ketoses.* The ending *-ose* indicates that the substance is a sugar.

Another general characteristic which should be borne in mind is the fact that carbohydrates are *amphoteric,* i.e , they act as weak acids or weak bases. They will combine with stronger acids or bases to form salts. In addition to the aldehyde and ketone groups these compounds contain primary and secondary alcohol groups. Recalling the general chemical characteristics of primary and secondary alcohols and of aldehydes and ketones, the student can see that the simpler carbohydrates will deport themselves just as might be prophesied from the properties of the above-mentioned groups.

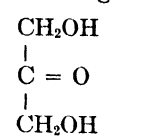
Classification. — For convenience of discussion and study, it is customary to divide the carbohydrates into three large groups. This classification is based upon the number of simple sugars (saccharides) which are obtained when the various carbohydrates undergo hydrolysis. For example, a carbohydrate which yields two molecules of simple sugars on hydrolysis is called a *disaccharide.* Likewise, a carbohydrate which yields three molecules of the simple sugars would be called a *trisaccharide.* Usually all carbohydrates which yield more than three molecules of simple sugars are called *polysaccharides.* It is customary; therefore, to classify the carbohydrates as follows: monosaccharides, disaccharides, trisaccharides, and polysaccharides.

The International Union of Pure and Applied Chemistry, a group of scientists representing most of the civilized nations of the world, has recommended recently that all nations adopt the term *glucid* to designate those compounds which the Americans and the English now call " carbohydrates." Since, at the present time, the recommendations of this committee are purely tentative and since American scientists have raised several valid objections to the new system of nomenclature, we shall continue, throughout this book, to use the accepted terminology.

There are several types of monosaccharides or simple sugars. These are named according to the number of carbon atoms in the molecule. For example, a *diose* sugar contains two carbon atoms. Theoretically, therefore, glycollic aldehyde, $CH_2OH \cdot CHO$, would be a simple diose sugar, whereas *formic aldehyde*, $H - CHO$, would be termed a *monose sugar*. By the same reasoning, *dihydroxy acetone*, $CH_2OH \cdot CO \cdot CH_2OH$, obtained from glycerol, is called a *triose sugar*. *Glyceric aldehyde*, $CH_2OH \cdot CHOH \cdot CHO$, is likewise a member of the *triose* group:



Glyceric aldehyde
(an aldotriose)



Dihydroxy acetone
(a ketotriose)

A sugar containing four carbon groups would be called a tetrose, of which erythrose, $CH_2OH \cdot CHOH \cdot CHOH \cdot CHO$, is an example. The pentoses and hexoses are the most important of the monosaccharides. When it is desirable to indicate the presence of aldehyde or ketone groups in the simple sugars it is customary to prefix *aldo* or *keto* to the name which indicates the number of carbon atoms. For example, an *aldohexose* would be an aldehyde sugar containing six carbon atoms. Likewise, a *ketohexose* would be a ketone sugar containing six carbon atoms.

The following table shows at a glance the general relationships existing among the various carbohydrates.

I. The Monosaccharides

Of this group only two types are of sufficient biological importance to merit detailed discussion, viz, the pentoses and the hexoses. Before we consider these sugars in detail, there are a number of properties that merit emphasis. One very important property possessed by the sugars is that of optical activity.

Optical Activity. — The reader will recall, from his previous knowledge of organic chemistry, that some organic compounds such as lactic and tartaric acids possess the property of bending or rotating rays of polarized light. This property is called *optical rotation*, and such compounds are said to be *optically active*.

An instrument known as a polariscope is used for measuring optical activity; it is so constructed that light from a sodium flame (monochromatic light) is allowed to pass through a quartz prism, known as a Nicol

TABLE 2

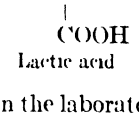
CLASSIFICATION OF THE CARBOHYDRATES

Group name	Subgroup	Name	Occurrence
I. Monosaccharides	aldodiose	glycollic aldehyde	
	aldotriose ketotriose	glyceric aldehyde dihydroxy acetone	
	aldotetrose ketotetrose	erythrose *d*-erythrulose	
	aldopentose	arabinose	hydrolysis of cherry gum
	aldopentose	xylose	hydrolysis of wood or straw
	aldopentose	ribose	hydrolysis of nucleo proteins
	ketopentose	arabinulose	
	aldohexose	glucose	honey, ripe fruit juices
	aldohexose	galactose	hydrolysis of milk sugar
	aldohexose	mannose	hydrolysis of rare polysaccharides
	ketohexose	fructose	honey, ripe fruit juices
	ketohexose	sorbose	from sorbitol

Group name	Name	Yields on hydrolysis	Occurrence
II Disaccharides $C_{12}H_{22}O_{11}$	maltose	glucose+glucose	malted grains
	sucrose	glucose+fructose	sugar cane and sugar beet
	lactose	glucose+galactose	milk
	trehalose	glucose+glucose	
	melibiose	glucose+galactose	
III. Trisaccharides $C_{18}H_{32}O_{16}$	raffinose	sucrose+galactose	cotton seed and sugar beet
IV Polysaccharides	dextrins	glucose	
	starches	glucose	seeds and tubers
	cellulose	glucose	woody portion of plants
	glycogen	glucose	liver and muscles
	mucilages	pentoses, hexoses	flax seed
	gums	pentoses, hexoses, acids	plant exudations
	pectins	pentoses, hexoses, acids	fruits
	inulin	fructose	dahlias and artichokes

prism or polarizing prism. The light transmitted by this prism vibrates in one plane and is known as *polarized light.* At a distance from the polarizing prism is placed another, known as the analyzing prism. These prisms are arranged in such a manner that one of them can be rotated while the other remains in a fixed position. When one of them is rotated, a point is found where the polarized light is absorbed and cannot be seen by the observer. If at this point a solution of optically active substance is placed between the prisms, the plane of light is twisted to one side and some light is visible to the observer. It is necessary, therefore, for the observer to turn or rotate the analyzing prism to the right or left until the light again disappears. If it is necessary to turn the analyzer to the right to accomplish this, the optically active solution under examination is said to be *dextro-rotatory.* If, on the other hand, the analyzer is turned to the left, the solution is said to be *levo-rotatory.*

The optical activity (i.e., the property of rotating polarized light) of carbon compounds is due to the presence of *asymmetric carbon atoms.* These are carbon atoms which have four different or unlike atoms or atomic groups attached to them. For example, lactic acid, represented by the following formula, contains an asymmetric carbon atom to which are attached four different or unlike radicals, the asymmetric carbon atom being indicated by black-face type:



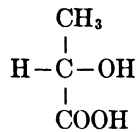
Lactic acid

When lactic acid is studied in the laboratory two optically active forms are found: *d-lactic acid,* which is dextro-rotatory, and *l-lactic acid,* which is levo-rotatory. Since these lactic acids are isomeric with each other and contain the same number of atoms and atomic groups of the same kind, they are said to be *stereo-isomers.* This term refers to the geometrical arrangement of the atoms and atomic groups in space.

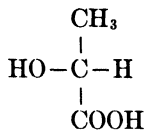
Again using the lactic acids to demonstrate this form of isomerism, we can imagine that the various groups or radicals are so attached that we cannot superimpose one molecule upon another so that they coincide. This will be clear to the reader if we ask that he attempt to superimpose his left hand upon his friend's left hand with the palms of both hands facing in the same direction. It is found that they are not "isomeric" but identical, since they coincide, i.e., thumb is over thumb, index finger over index finger, etc. If, however, he attempts to superimpose his own left hand upon his own right hand, he will find that it is impossible to

make them coincide unless he bring his hands palm upon palm. This shows, however, that the hands are not identical but are really *mirror images* one of the other. In other words, the left hand is an isomer of the right hand.

It is by similar reasoning that the chemist has explained the existence of two lactic acids, each one being a mirror image or stereo-isomer of the other, as indicated in the following formulae:



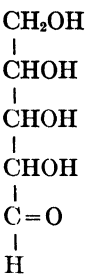
d-Lactic acid



l-Lactic acid

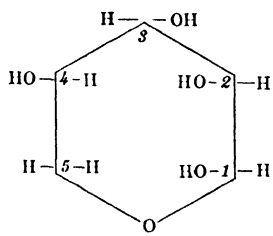
Our brief discussion of carbohydrates and other organic compounds is made largely with the view of refreshing the reader's mind relative to some of the more important chemical facts that he has studied previously in organic chemistry. We have pointed out briefly that carbohydrates may be optically active and that many isomers should occur in nature or should be capable of being synthesized. If we refer to the previous table on carbohydrate classification and examine the portion devoted to monosaccharides, it will be noted that there are really many groups and classes. In reality, not many are of real importance, the pentoses and hexoses being of the greatest biological interest.

The Pentoses ($C_5H_{10}O_5$). — As the name indicates, these sugars contain five carbon atoms or five carbon-containing radicals. These sugars are of some agricultural significance, although they cannot compare in importance with the hexoses. The pentoses do not occur in plants in the free state but are in combination with other compounds. These complex pentose-forming compounds are known as *pentosans*, and they may be considered as anhydrides of the pentoses, since the hydrolytic action of water will cause the molecule to split, yielding pentose sugars, such as *arabinose* and *xylose*. The complex anhydrides that yield arabinose by hydrolysis are known as *arabans*, and those that yield xylose are known as *xylans*. The latter may be obtained from straw, oat hulls, corn cobs, and many other woody or fibrous materials. Arabinose is obtained from cherry gum and gum arabic. These sugars are not fermentable with yeast and are not utilized by the animal to a very appreciable extent. They are reducing sugars and, for that reason, they are usually given the following structure:

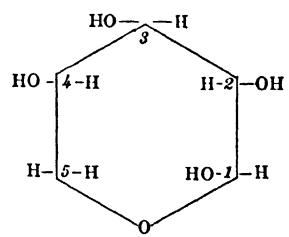


A pentose sugar (aldehydic)

Recent work in organic chemistry indicates that these (and certain other sugars) are really ring structures where oxygen acts as a bridge to close the ring. This conception calls for the following type of formula for arabinose and xylose and shows why they differ in properties in spite of the fact that they contain identical groups or radicals. The radicals are merely arranged differently, in space, which makes these sugars isomeric:



Xylose ($C_5H_{10}O_5$)



Arabinose ($C_5H_{10}O_5$)

The Hexoses ($C_6H_{12}O_6$). — These sugars are of great importance, being widely distributed in nature. They occur naturally in many types of plant materials, and one of them (glucose) is a normal constituent of blood. At least three hexose sugars merit description, i.e., glucose, fructose and galactose.

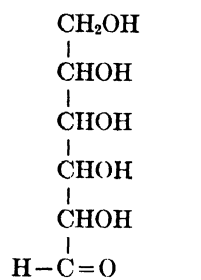
Glucose. — This sugar (also known as *d*-glucose, dextrose, grape sugar, starch sugar or corn sugar) is undoubtedly the most important of the hexoses. It is found in ripe fruits, sweet corn, onions and honey. It is usually found associated with fructose, owing to the fact that these two sugars are formed when the disaccharide, sucrose, is broken down by hydrolysis. Glucose is also formed when certain polysaccharides (glucosans) are hydrolyzed.

Glucose is sweet to the taste, but less so than cane sugar. It is soluble in water and fermentable with yeast. On account of its structure,

it is easily oxidized in the animal organism. There is little question but that it is undoubtedly the most important transport form of sugar that we have. When glucose is subjected to the action of yeast it is fermented, forming ethyl alcohol and carbon dioxide:

$$\underset{\text{glucose}}{C_6H_{12}O_6} + \text{yeast} \rightarrow 2\ \underset{\substack{\text{ethyl}\\\text{alcohol}}}{C_2H_5OH} + 2\ \underset{\substack{\text{carbon}\\\text{dioxide}}}{CO_2}$$

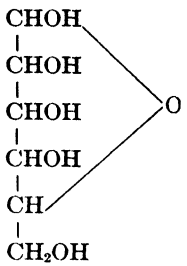
When glucose is allowed to react with acids, acid anhydrides or acid chlorides, esters are formed in which five acid radicals combine forming a penta-ester, indicating that there are five hydroxyl groups in glucose. The aldehyde group may be *oxidized*, in which case gluconic acid is formed, and, in case the aldehyde group is *reduced*, a corresponding alcohol, sorbitol, is formed. These and other chemical reactions lead us to believe that glucose may exist as a straight chain compound and that its formula may be written as follows, without attempting to indicate the special relationships of the hydrogen and hydroxyl groups:



Straight chain or aldehydic formula for glucose

On the other hand, it is quite evident that certain chemical reactions that should occur (if this is the correct formula) do not do so. Furthermore, when glucose is dissolved in water and placed in a polariscope, it is found that the optical activity gradually changes on standing. This leads us to the belief that glucose must be undergoing, in solution, some sort of intermolecular rearrangement.

It is thought that glucose exists not only as a straight chain compound but also as a cyclic compound in which oxygen acts as a bridge connecting the carbon atoms. This structure is called the oxide form, and it is probable that glucose exists in the aldehyde and oxide form, in the same solution:

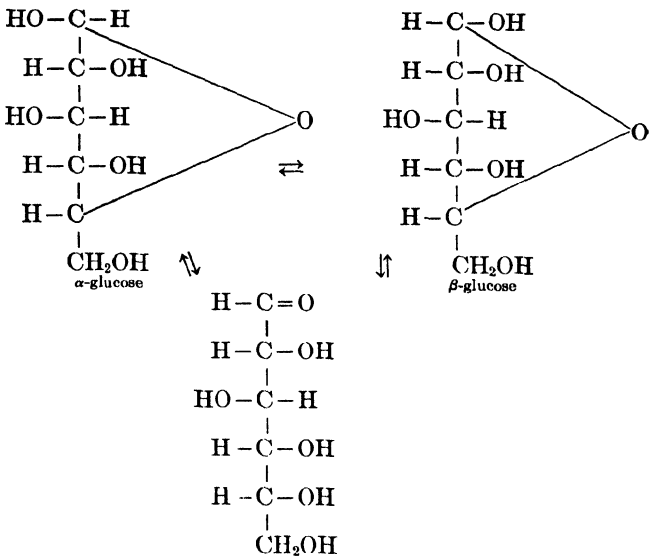


Amylene (oxidic) form of glucose

The classic work of Irvine indicates that we are not safe in ascribing any particular structural formula to glucose for the reason that this sugar may exist in many forms. For example, we have shown that glucose may exist as an aldehydic straight-chain structure, which has been the orthodox way to represent this sugar. We have also shown that glucose can exist as an amylene (oxidic) structure in which the first and fifth carbons are connected by the oxygen bridge. Irvine has shown that a butylene (oxidic) structure may exist in which carbons (1) and (4) are connected by the oxygen linkage. He also states that it is possible that other oxygen bridges may be formed between other carbon atoms, forming other structural forms. As a matter of convenience, most authors use the straight-chain aldehydic form or the amylene oxidic structure when referring to this important sugar.

When glucose changes in optical activity, the process is called *mutarotation*, and it is possible that glucose exists in two different oxide structures known as α- and β-glucose. These, of course, are isomers of one another.

The following formulae probably indicate the three forms in which glucose may exist in solution:



Fructose. — This ketohexose sugar is known also as levulose and fruit sugar. It is isomeric with glucose but differs in its chemical and physical properties. Fructose is strongly levo-rotatory, soluble in water and is much sweeter than glucose. It occurs naturally as a constituent of honey and is also found associated with glucose in sweet fruits. When cane sugar is hydrolyzed by acids or by enzyme action, glucose and fructose are found in equal amounts. This process of sucrose hydrolysis is known as " inversion," and the resulting mixture is sometimes called " invert sugar."

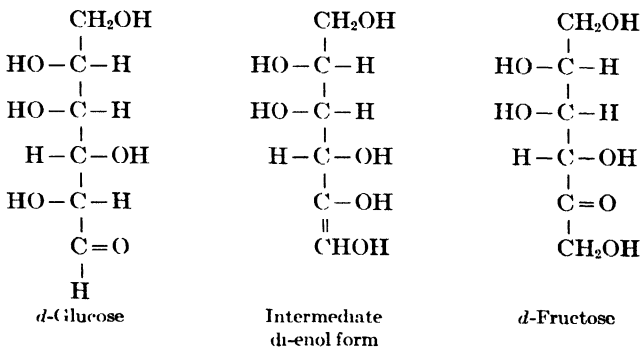
$$\underset{\text{sucrose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underbrace{\underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{fructose}}{C_6H_{12}O_6}}_{\text{invert sugar}}$$

Fructose may be prepared, uncontaminated with glucose, by hydrolyzing inulin, a polysaccharide obtained from dahlia bulbs and artichokes.

Originally, fructose was known as *levulose* and glucose as *dextrose* to emphasize the optical properties. Research work, however, brought out the fact that it was theoretically possible to postulate the existence of sixteen isomeric aldohexoses, and that these should be arranged in pairs — each sugar (in each pair) should be, structurally, the mirror image of the other. Subsequent work proved that this theory was correct. As a result we now have *d*- and *l*-glucose, *d*- and *l*-galactose, etc. The terms *d*- and *l*- do not necessarily imply dextro- and levo-rotation but are now

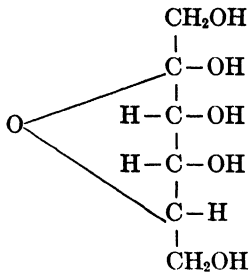
used in a generic structural sense rather than in the purely optical sense. As a result it is possible, as in the case of the hydrolysis of cane sugar, to have a levo-rotatory *d*-fructose. It became clear, therefore, that the words dextrose and levulose had lost much of their original meaning. For the reasons just enumerated, scientists have adopted the terms *d*- and *l*-glucose, *d*- and *l*-fructose, and the terms dextrose and levulose are seldom found in modern scientific literature.

Whereas glucose is an aldohexose, fructose is characterized by the presence of a carbonyl or ketone group. It is quite apparent that glucose and fructose are mutually convertible one into the other. This is explained on the theory that an intermediate di-enol compound is formed which is readily convertible into glucose or fructose. The following formulae show this relationship and are written to emphasize the aldehydic and ketonic properties of these sugars:



The di-enol form of glucose, just described, is called the 1, 2 di-enol form because the double bond exists between the first and second carbon atoms. Two other di-enol forms are possible, viz ,.2, 3 di-enol glucose and 3, 4 di-enol glucose. Since the existence of these double bonds affects the asymmetric properties of the carbon atoms involved, the reader can understand why glucose solutions change in optical properties during the formation of these isomeric forms during mutarotation.

Structurally, the situation with fructose is much the same as with glucose, viz., that this sugar undoubtedly exists in oxidic forms, of which the butylene type is most representative:



Oxidic form of fructose

Galactose. — This hexose sugar does not occur free in nature but may be found in combination with other substances. Its most important source is the disaccharide lactose or milk sugar. When lactose is hydrolyzed it yields *d*-glucose and *d*-galactose. Galactose is an aldehyde sugar, less soluble than glucose, and much less sweet to the taste.

Mannose. — This sugar does not occur free in nature, at least in any quantity, and is best obtained by hydrolyzing the mannans, sometimes called the mannosans. Mannose is a dextro-rotatory aldohexose. It is not of outstanding importance.

Other Hexose Sugars. — Although glucose, fructose and galactose have been emphasized in our discussion of the hexoses, the reader must realize that this has been done because of their importance. Eight other hexose sugars exist or can be made, each one being capable (theoretically) of existing in two forms, i.e., *d* and *l*. These isomeric hexoses are mannose, idose, gulose, talose, allose, altrose, sorbose, and tagatose. All the isomers have been found or prepared with the exception of *l*-allose, *l*-altrose, and *l*-tagatose.

II. The Disaccharides ($C_{12}H_{22}O_{11}$)

The disaccharides may be considered anhydrides of the monosaccharides since the latter are formed by the interaction of the former with water. The hydrolytic cleavage of the disaccharides may be brought about by acids or by enzymes which act specifically on each sugar. Only three disaccharide sugars that occur in nature are of sufficient importance to merit discussion, viz., sucrose, maltose and lactose.

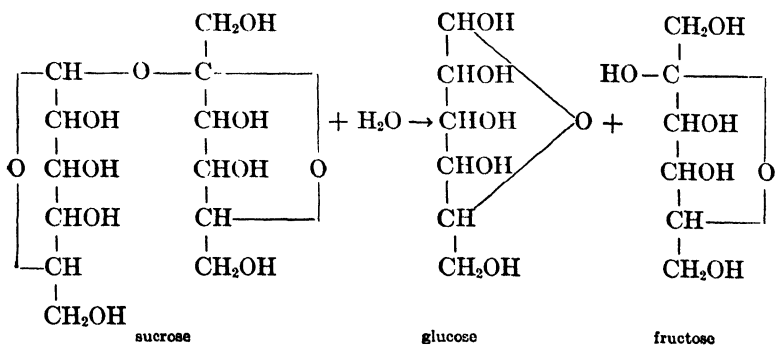
Sucrose. — This sugar is known under various names such as cane sugar, beet sugar and saccharose. It is the most important of the disaccharides and occurs widely distributed throughout the vegetable kingdom, usually associated with greater or less amounts of glucose and fructose. From the commercial standpoint the sugar cane, the sorghum cane, the sugar beet, the sugar maple and the sugar palm are the best sources. In this country the largest amount of sugar is obtained from

the sugar cane and the sugar beet, the former containing as much as 20 per cent and the latter from 10 to 25 per cent of sucrose. Sucrose also occurs in varying quantities in the fruits and juices of many plants. For example, the pineapple contains about 11 per cent of sucrose, and fruits like the strawberry, the apricot, ripe bananas and apples usually contain about 5 to 6 per cent of this carbohydrate.

The carbohydrate content of the dilute nectar of flowers is almost entirely composed of sucrose. During the evaporation and concentration of this liquid by the honey bee, the sucrose is acted upon by enzymes which convert the sucrose to honey, which is a mixture of glucose and fructose.

Sucrose crystallizes in large anhydrous crystals which are easily soluble in water. It is readily hydrolyzed by acids and by enzymes called *invertases*. In water solution sucrose is quite strongly dextro-rotatory, a property which becomes very useful in measuring the amount of this sugar in cane and beet juices at the factory. When sucrose, which possesses a specific rotation of $+66.5°$ at 20° C., is hydrolyzed, the resulting mixture of sugars is found to be levo-rotatory. This is due to the fact that glucose possesses a specific rotation of $+52.6°$ whereas fructose is strongly levo-rotatory, i.e., $-93°$. Consequently, the monosaccharides resulting from sucrose hydrolysis have changed the optical rotation of the solution. As pointed out in a previous paragraph, this process is sometimes called *inversion*, and the resulting mixture of glucose and fructose is called *invert* sugar.

Sucrose is not fermented by yeast until it has been inverted to glucose and fructose, nor does it give characteristic chemical reactions for aldehyde and ketone groups. As a result of the researches of Irvine, the following formula for sucrose is accepted as the best to express the structure of this complex disaccharide, since it can be shown that this structure can be split into *d*-glucose and *d*-fructose:

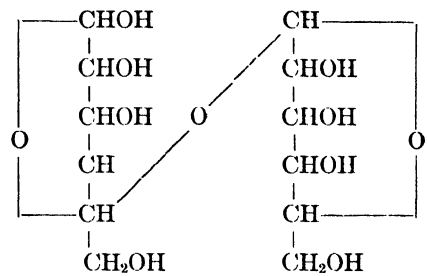


Maltose. — Although this sugar possesses the same empirical formula as sucrose ($C_{12}H_{22}O_{11}$), it differs from sucrose in many of its properties. Maltose does not occur as abundantly in nature as sucrose. It is found in small concentrations in the cell sap of metabolizing plant tissues, and in larger concentration in germinating cereals and malted grains when the enzyme diastase exerts its hydrolytic action on starch. For this reason maltose is also known as *malt sugar*. Some maltose is formed in the salivary digestion of starch owing to the action of the salivary enzymes.

The crystalline form of maltose contains one molecule of water of crystallization; it is soluble in water and shows marked ability to reduce Fehling's solution. It is strongly dextro-rotatory, having a specific rotation of +138°. Maltose will not ferment with yeast until it has been hydrolyzed to its constituent monosaccharides. When hydrolysis occurs, maltose yields two molecules of glucose:

$$\underset{\text{maltose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow 2\ \underset{\text{glucose}}{C_6H_{12}O_6}$$

Maltose, like glucose, exhibits mutarotation in solution, which means that the oxygen bridge and molecular structure changes, forming alpha and beta forms. The following formula represents the maltose molecule, according to Irvine:



Maltose, a gluco-glucoside

Lactose, or milk sugar, occurs in the milk of all mammals, averaging about 6 per cent in human milk and about 4.5 per cent in cows' milk. Lactose appears to be synthesized in the mammary gland from the glucose carried in the blood to the gland. This sugar is not so soluble as sucrose and is much less sweet to the taste. On hydrolysis, lactose yields glucose and galactose:

$$\underset{\text{lactose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{galactose}}{C_6H_{12}O_6}$$

Other disaccharides are *trehalose* and *melibiose*. The latter will be mentioned under raffinose when we consider the chemistry of trisaccharides.

III. The Trisaccharides ($C_{18}H_{32}O_{16}$)

From an economic standpoint, this group of carbohydrates is relatively unimportant. They are of scientific interest, however, since they yield three monosaccharide molecules on hydrolysis.

The only member of this group that will be mentioned is *raffinose*. This sugar is found in small amounts in sugar beets, cottonseed, barley and other plants. It is dextro-rotatory and may be hydrolyzed to form glucose, fructose and galactose, the three important hexoses. This happens in stages, however, as shown by the following equations:

$$\underset{\text{raffinose}}{C_{12}H_{32}O_{16}} + H_2O \rightarrow \underset{\text{fructose}}{C_6H_{12}O_6} + \underset{\text{melibiose}}{C_{12}H_{22}O_{11}}$$

$$\underset{\text{melibiose}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{galactose}}{C_6H_{12}O_6}$$

Since raffinose is composed of the monosaccharides glucose, fructose and galactose, it is obvious that partial hydrolysis may yield sucrose plus galactose or melibiose plus fructose, depending upon the point in the raffinose molecule where the first hydrolytic cleavage happens to take place.

IV. The Polysaccharides

From the standpoint of plant economy the polysaccharides are the most important of the carbohydrates. This is particularly true from the standpoint of storage or reserve carbohydrates. Cellulose, starch, and inulin are important storage forms found in plants; the animal stores relatively large amounts of carbohydrate in the liver and muscles in the form of glycogen or animal starch. Chemically, these compounds are anhydrides of the simple sugars, since the latter may be formed from the former by the hydrolytic action of water. As a class, the polysaccharides are colorless, odorless, and almost tasteless amorphous compounds. They are characterized by rather large molecules, although recent research has indicated that they may not be as complex as has been thought.

Instead of forming true solutions, which is characteristic of the sugars, most of these compounds form sticky or gelatinous solutions. It is for this reason that they are purified and studied with great difficulty; owing to their colloidal nature it is most difficult to make accurate determinations of their molecular weights. For this reason, writers have usually written the empirical formulae for the pentosans $(C_5H_8O_4)_x$ and for the hexosans $(C_6H_{10}O_5)_x$ to indicate that the exact number of monosaccharide units in the polysaccharide molecule was not known. As has already been inferred, the polysaccharides yield, on hydrolysis, many molecules of the simple sugars. Some polysaccharides yield pentoses and are known as *pentosans*. Others are peculiar in that they yield only hexose

sugars, and consequently they are termed *hexosans* to emphasize this fact. Some of the hexosans yield only glucose and are called *glucosans*; a *fructosan* is that type of polysaccharide which yields only fructose on hydrolysis. For convenience of discussion we shall consider the polysaccharides in relation to their products of hydrolysis.

The Pentosans. — These complex compounds are discussed first because they are characterized by yielding pentose sugars during hydrolysis. The pentosans are substances of doubtful composition existing in plant tissues; they may be isolated most satisfactorily from the woody or fibrous portion of the plant, such as straw, oat hulls and corn cobs. It is not possible to write graphical or structural formulae for them, although it is possible to give them an empirical formula $(C_5H_8O_4)_x$ which is quite representative of the group. Those pentosans which yield the simple sugar xylose are known as *xylans*. Those which are found in cherry gum and gum arabic are called *arabans*, since they yield the pentose sugar arabinose on hydrolysis

The Hexosans. — This group of compounds is undoubtedly the most important, from an agricultural standpoint, of all the polysaccharides. The reason for this statement lies in the fact that the hexosans include the starches, glycogen, inulin and cellulose, which are the most important storage forms of carbohydrate All these substances may be hydrolyzed to yield monosaccharides, all of which are hexose sugars. Some, which yield glucose, are called *glucosans*, whereas those which yield fructose are known as *fructosans*.

The Glucosans. The Starches. $(C_6H_{10}O_5)_x$. — This important type of carbohydrate occurs abundantly in nature in seeds, roots, bulbs and tubers, and in the stems and leaves of most plants Although starches from different sources possess about the same chemical properties, the physical properties are variable. In some plants, like the rice plant, the starch grains are small, whereas in others, like the potato, the grains are large and present a different appearance under the microscope. It is by microscopic methods that the food and feed chemists are able to detect adulterations of one starch-containing food with another.

The following table shows the percentage of starch in some of the common seeds and tubers. The percentage is based on air-dry material.

TABLE 3

STARCH IN SOME COMMON FOODS

Wheat flour	66 55	Barley	62 00
Corn meal	71 00	Potato tuber	75 50
Oat meal	56 23	Beans	42 70
Rice	79 40	Peas	40 50

Starch grains do not dissolve in water but form colloidal suspensions. When such starch suspensions are heated to boiling, the granules rupture, forming a colloidal solution which is usually called starch paste. The fact that starch nitrate and similar esters are capable of formation indicates quite clearly that starch must contain hydroxy or alcohol groups. When starch is treated with starch-splitting enzymes (amylases) or with boiling mineral acids, it is hydrolyzed to sugars. The hydrolytic action of enzymes is much more rapid on starch paste than on the unchanged starch granules. Although this is undoubtedly one of the important functions of cooking in the preparation of human foods, research work has shown that most animals, including human beings, are capable of digesting large amounts of raw starch.

Owing to the colloidal nature of starch and other hexosans, methods for determining their molecular weights are most difficult to devise. No matter how carefully starch preparations are made, it is impossible to remove a small trace of phosphorus which seems to be a part of the starch molecule. When fermentation occurs, however, this element is split off as a phosphate radical. Most authorities are convinced that the starch molecule is large, some authors stating that the molecular weight of starch is about 34,000. Recent investigations, however, especially those of Ling and Nanji, and Irvine and Pringsheim, seem to indicate that the starch molecule is not quite so complex as has been thought. In fact, these workers are convinced that starch is either a polymerized anhydro- tri- or hexa-saccharide. It will appear, therefore, that it is still safe to write the formula for starch $(C_6H_{10}O_5)_x$ until the problem is solved.

Some authors believe it to be a complex polymerized anhydro-trisaccharide, in which case it might be written $(C_{18}H_{30}O_{15})_x$. The following formulae have been suggested by Irvine. Fig. 19 represents his conception of the structure of the true starch (amylose) unit; Fig. 20 portrays the structure of the cellulose outer layer (amylopectin) which is said to cover the starch granule.

When starch is hydrolyzed by enzymes, acids or heat a large number of products of hydrolysis are formed. These products (dextrins), prior to the formation of glucose, still retain many of the characteristics of starch. They are still complex in structure, although they are less complex than starch. They form colloidal solutions but are more soluble than starch.

The Dextrins. — These by-products of starch may also be represented by the formula $(C_6H_{10}O_5)_x$. A number of dextrins have been studied, the more important being amylodextrin, erythrodextrin, achroödextrin, and maltodextrin. When these compounds are hydrolyzed they form

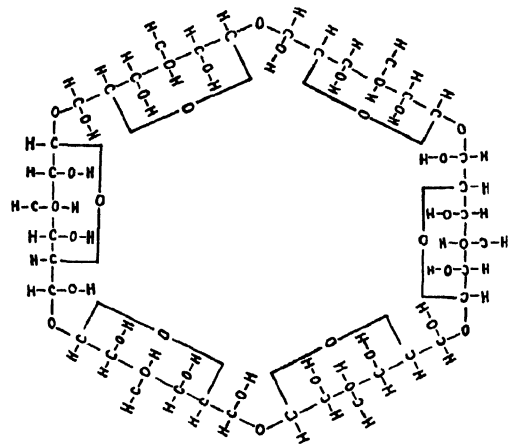


FIG. 19. Structure of starch molecule (amylose). After the specifications of Sir John Irvine.

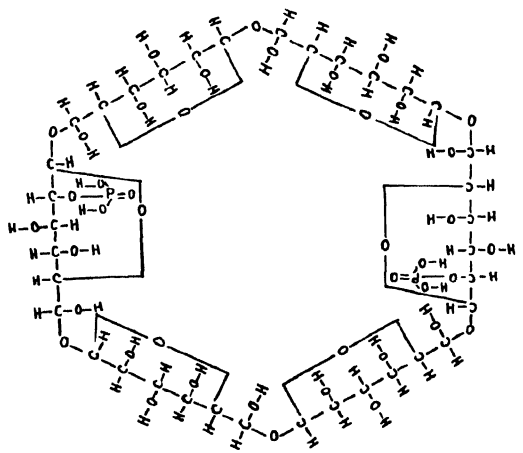


FIG. 20. Structure of starch (amylopectin). After the specifications of Sir John Irvine.

maltose and finally glucose. The reader will note, therefore, that the final product of starch and dextrin hydrolysis is the monosaccharide, glucose. In physical properties and in appearance, the dextrins are quite similar to starch, but they form sticky solutions with water which give them "gummy" properties. One commercial form, known as British gum, is made by heating starch at 230 to 260° C. This form of dextrin is used extensively as an adhesive on paper labels, envelope flaps, etc. A similar hydrolytic change occurs when starched clothes are ironed, the heated iron forming dextrin, which imparts stiffness to the fabric

Inulin — Another hexosan of some scientific interest and importance is inulin Like starch and dextrin, it is also a white amorphous powder, which forms a colloidal suspension in hot water Whereas the starches and dextrins tend to rotate the plane of polarized light to the right, inulin is levo-rotatory Starch forms a blue compound with iodine; inulin fails to do so Inulin is found in commercial quantities in the artichoke tuber and in the bulbs of the dahlia plant This polysaccharide is peculiar in that it forms fructose on hydrolysis and is, therefore, called a *fructosan* No authoritative work has been done to determine the structure of inulin, and, like starch and dextrin, it is usually represented by the empirical formula $(C_6H_{10}O_5)_x$

Glycogen is another hexosan that should be mentioned briefly at this point This polysaccharide is the principal storage carbohydrate in the animal body, and on account of this fact it is sometimes called "animal starch" Glycogen is also a constituent of yeast and certain fungi. It is quite similar to starch in many ways, since it is a white, amorphous, tasteless powder which is capable of producing glucose when hydrolyzed. Glycogen, however, does not form a blue compound with iodine. It does form colloidal solutions with water and it can be precipitated from water with alcohol

Glycogen is found in the lower forms of animals, such as the oyster, as well as in the higher animals In the latter it is stored in relatively large amounts in the liver, although an appreciable amount may be found in the muscles and other tissues It is difficult to give figures on the glycogen content of tissues because it is so variable The reason for this lies in the fact that, if the animal has been active, glycogen is utilized so promptly after storage that much of it may be utilized, in which case the glycogen content would be low; on the other hand, animals closely confined in small cages, where they cannot be active, may store large amounts of this polysaccharide. The constitution of glycogen has not been determined, and therefore we will represent it by the formula $(C_6H_{10}O_5)_x$.

Mannans or Mannosans. — These compounds are anhydrides of the monosaccharide, mannose. On account of their relative unimportance, they are merely mentioned here.

The Celluloses. — This important group of polysaccharides is known as *hexosans* or *glucosans* owing to the fact that glucose is formed on complete hydrolysis. Cellulose is the chief constituent of wood, the fibrous portion of plants, and the walls of most plant cells. The types of cellulose differ in toughness and strength, depending upon the age and type of the plant. Wood fiber, cotton and linen are good sources of cellulose. Cellulose is quite resistant to the action of most enzymes, dilute acids and alkalies, and is digested with considerable difficulty by most animals, although the ruminants and the horse are able to utilize appreciable amounts. Books on feeding stuffs often refer to this material as " crude fiber " or the " indigestible carbohydrate " of the feed.

It is clear that, although cellulose does not constitute an important source of energy for man, it may serve as an important source of energy for certain types of live stock. Aside from its value as a source of energy it plays an important rôle in the nutrition of all animals by serving as " bulk " and by facilitating the elimination of food residues. Cellulose exists in the purest form in cotton, where it appears as white, amorphous fibers. It is insoluble in all the ordinary solvents, but it can be made to form a thick solution when treated with Schweitzer's reagent, which is composed of copper ammonium hydroxide The cotton can be reprecipitated from this solution by the addition of a strong mineral acid Zinc chloride in solution is also a solvent for cellulose.

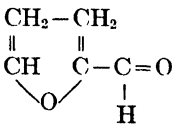
In woody tissues, such as we find in the wood of trees, cellulose is in combination with substances of unknown composition called *lignins* Such combinations are known as *ligno-celluloses* In the manufacture of paper from wood, the wood is cooked in large digesters in the presence of water, sulfur dioxide and lime. This removes the lignin, leaving the long cellulose fibers in the form of wood pulp, which is impure cellulose.

When cellulose is treated with alkali it gelatinizes and the fibers become translucent, imparting to the finished product a silky luster. This product is known as *mercerized* cotton, since its discoverer was an Englishman named Mercer. Parchment paper is made by treating certain types of paper with sulfuric acid. The artificial silk of today is made from *viscose*, which, in turn, is made from cellulose by the action of sodium hydroxide and carbon disulfide. This viscous material is forced through tiny openings into an acid solution of sodium bisulfate which changes the sodium-cellulose compound back to cellulose again. Artificial silks are also made from nitrated cellulose, but the viscose type is rapidly taking its place.

When cellulose is treated with nitric acid in the presence of sulfuric acid, cellulose esters or nitrates of cellulose are formed. Celluloid, gun cotton and pyroxylin lacquers for automobiles are made from the nitrates of cellulose, and esters of cellulose and acetic acid also find a wide use in the manufacture of leather substitutes, moving picture films, aeroplane varnishes, etc. The lower cellulose nitrates are dissolved in alcohol and ether and sold on the market as collodion or " new skin." The fact that cellulose forms esters with nitric and acetic acid shows that the molecule contains alcohol or hydroxyl groups. Since cellulose also forms glucose on hydrolysis, it is thought that its structure must be somewhat similar to that of starch. Like the sugars, cellulose has acidic properties, for it forms combinations with sodium, copper and similar bases.

During the past few years considerable research work has been done, by the United States Government and the Iowa State College, in cooperation with industrial agencies, with the view of finding commercial outlets for cellulose in corn stalks or stover and in cereal straws. To date the work has been most encouraging, the results showing that wall board, artificial lumber, paper, rayon, cellulose nitrates and similar products can be made from these agricultural materials In fact, straw board, card board, wall board, etc , are already commercial products, and experimental issues of books and agricultural papers have appeared, the paper-stock of which was made from corn stalks. It is thought that, as the cost of wood pulp increases, it will not be many years before the paper-making industries will be using corn-stalk-cellulose in commercial quantities

In addition, a number of chemical by-products, which are made from oat hulls and similar cellulose waste products, are becoming of commercial importance. Of these, furfural seems to hold the greatest promise, for it is already being used as a substitute for formaldehyde in certain industries. Only those products which contain pentosans will yield furfural:



Furfural or furfuraldehyde

Compounds Allied to the Carbohydrates

There are a number of other carbohydrates or carbohydrate-like materials the chemistry of which is not fully understood. A few of these will now be considered.

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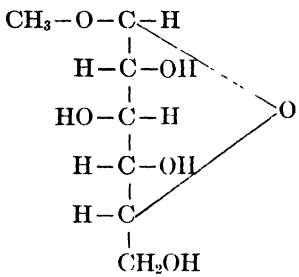
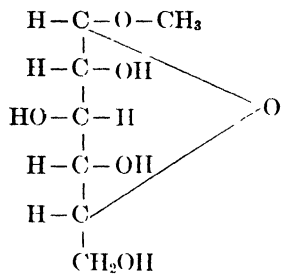
Glucosides. — The term *glucoside* is used to represent a group of plant compounds containing glucose and one or more basic or acidic organic compounds in chemical combination. Examples are known, however, where fructose, rather than glucose, is present in the molecule.

The glucosides are usually intensely bitter, colorless, crystalline compounds yielding solutions which are levo-rotatory in respect to polarized light. These compounds are readily hydrolyzed by acids and by certain enzymes. It is thought that in the normal growing plant the enzyme and glucoside substrate are separated, but that under pathological conditions they are brought in contact, causing the tissues to undergo decomposition.

Glucosides may be classed according to whether the alpha or beta form of glucose combines with the non-glucose residue:



Methyl glucoside (alpha)



Methyl glucoside (beta)

It is possible to list under the heading of glucosides a great number of heterogeneous compounds such as disaccharides, tannins, plant pigments and other compounds. For example, maltose may be regarded as an alpha-glucoside of *d*-glucose.

Many true glucosides have been isolated and identified. Among these may be mentioned *arbutin*. This compound comprises about 1.3 per cent of the dry matter of pear leaves and when hydrolyzed yields the following products:

$$\underset{\text{arbutin}}{C_{12}H_{16}O_7} + H_2O = \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{hydroquinone}}{C_6H_6O_2}$$

Another well-known glucoside is *amygdalin*, which is found in the kernels of the peach and cherry, and in similar seeds. In the kernels of the cherry laurel, amygdalin comprises 4.0 per cent of the dry matter. On hydrolysis amygdalin yields the following products:

$$\underset{\text{amygdalin}}{C_{20}H_{27}NO_{11}} + 2\,H_2O = \underset{\substack{\text{benz-}\\\text{aldehyde}}}{C_7H_6O} + 2\,\underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\substack{\text{prussic}\\\text{acid}}}{HCN}$$

Owing to the fact that individual glucosides vary so markedly from a chemical standpoint, it cannot be assumed that they bear the same relationship to plant growth. They are similar in one respect: all contain sugars and thus may constitute a reserve supply of food material. It appears, however, that the main physiological importance of these substances is to form temporary inactive " paired compounds " with a great variety of metabolic substances which are elaborated by living plants and which may otherwise interfere with certain physiological activities.

Hemicelluloses. — The hemicelluloses are a group of substances entering into the composition of the plant cell membrane, which are soluble in dilute alkalies and are easily hydrolyzed by dilute mineral acids, yielding pentoses, in addition to hexoses The hemicelluloses are partly structural and partly reserve materials. As reserve compounds they occur in the kernels of seeds, especially those which are poor in fat and starch. Their hydrolytic products are mainly hexose sugars Structural hemicelluloses, which are found in wood, straw and related substances, consist largely of pentose residues.

Gums. — Gums are generally looked upon as exudation products of plants; they are salts of a complex acid, the molecule of which, according to Sands and Klaas, seems to be built around a nucleus of uronic acid. On hydrolysis, gums yield a mixture of pentose and hexose sugars These sugars are attached to the uronic acid nucleus in such a way that it is possible to hydrolyze off one kind of sugar more or less completely under suitable conditions without removing the others.

The general chemical composition of a gum is very seldom constant and appears to vary in different seasons. Though the different sugars present in the molecule function as bases, certain mineral elements, which are more strongly basic, are usually present also For this reason, calcium, magnesium or potassium may combine with the acid complex. The inorganic content of gums, however, is never constant.

Pectins. — Pectins are colloidal polysaccharides of high molecular weight and rather complex composition. On hydrolysis, galacturonic acid, galactose, arabinose, methyl alcohol, and acetic acid have been obtained. At the present time, according to Willaman, three pectic substances are known, namely, *protopectin*, *pectin*, and *pectic acid*.

Protopectin is the mother substance of this group of compounds; it occurs in the cell walls of plants and was formerly known as *pectose*. Protopectin can be hydrolyzed by the enzyme *protopectinase* into a soluble product known as *pectin*. Such a transformation occurs during the ripening of fruits. The resultant pectin is the best known of all the pectic substances and is the pectin of commerce. It contains more than 10 per cent of methyl alcohol combined as methoxy groups or as methyl

ester groups. On removal of these groups, which may be accomplished by boiling with dilute acids, a compound is formed which is known as *pectic acid.*

Mucilages. — Mucilages are colloidal substances which appear to be sulfuric acid esters, where the ester group is a complex polysaccharide. Carrageen or " Irish moss " and agar-agar are among the best-known members of this group of compounds; the latter may be prepared from seaweeds. On acid hydrolysis mucilages appear to yield galactose or an isomer of this sugar, mannose, and fucose, which is a methyl pentose. Further work is necessary before the chemical composition of these compounds will be known.

In the following chapter we shall consider the chemistry of the fats and related compounds.

REFERENCES FOR GENERAL READING

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CHAPTER IV

THE FATS AND RELATED COMPOUNDS

When food materials are treated with ether, alcohol, petroleum ether or chloroform, various substances are dissolved. When the volatile solvent is allowed to evaporate and we examine the residue, we find that we have a number of different types of substances such as fats, oils, waxes, alcohols, and a number of other compounds.

For convenience of classification and discussion, we shall consider all these peculiar so-called ether-soluble compounds under the heading of "lipids." The Committee on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry has recommended the term *lipid* to refer to this group of fatty materials but it is not yet officially recognized

Properties. — Although the various lipids are often unrelated chemically, they have a number of properties in common. First, as we have pointed out in a preceding paragraph, they are all soluble in the so-called fat solvents; second, they are all greasy or fat-like in their general properties; and third, they are all relatively insoluble in water. The following classification based on that proposed by Gies will summarize in a brief way the outstanding facts that are known about these substances:

TABLE 4

CLASSIFICATION OF LIPIDS

LIPIDS. — Constituents of protoplasm having a greasy feel; soluble in alcohol-ether.

1. (*a*) Fats Neutral esters of glycerol and fatty acids which are solid at 20° C.
   (*b*) Fatty acids
2. *Fatty oils* Neutral esters of glycerol and fatty acids liquid at 20° C.
   (*a*) Drying oils Harden on exposure to light and air. *Example* linseed oil.
   (*b*) Semi-drying oils. Thicken slowly on exposure to light and air. *Example:* cottonseed oil.
   (*c*) Non-drying oils. Remain liquid on exposure to light and air. *Example:* olive oil.

3. *Waxes.* Esters of sterols and fatty acids *Examples* beeswax, carnauba wax, sperm oil, spermaceti.

4. *Sterols.* Alcohols, generally of the terpene group, solid at ordinary temperatures. *Examples:* cholesterol, phytosterol, cetyl alcohol, myricyl alcohol, etc. (Oxidation products are terpenic acids.)

5. *Phospho-lipids.* Phosphatides. Fatty substances yielding on hydrolysis phosphoric acid and fatty acids.

(*a*) Mono-amino-monophospholipids. *Examples* lecithin, cephalin.
(*b*) Di-amino-monophospholipids.
(*c*) Mono-amino-diphospholipids.

6. *Glyco-lipids.* Fatty substances yielding on hydrolysis fatty acids and a carbohydrate, generally glucose or galactose. Contain no phosphorus. *Examples* cerebron, kerasin, phrenosin.

7. *Sulfo-lipids.* Fatty substances yielding on hydrolysis fatty acids and sulfuric acid. Sulfatide of brain. *Example* protagon. Nature still undetermined.

8. *Amino-lipids.* Fatty substances containing amino nitrogen and fatty acids. Contain no phosphorus. Not well characterized. *Example* bregenin.

The Fats and Fatty (or Fixed) Oils

These groups of lipids, from the standpoint of occurrence in nature and from the standpoint of commercial value, are probably the most important of all the groups enumerated above. Although the fats and oils differ in physical and chemical properties, they possess so many points of similarity that it is convenient to discuss them together. The fats and oils are characterized by their greasy properties, and both types produce permanent translucent grease spots on paper. The main outstanding difference in properties lies in the fact that the fats are generally solid at room temperature, whereas the oils are liquid under similar conditions. The reader has learned in previous courses in organic chemistry that these compounds are neutral esters of the tri-hydroxy-alcohol, glycerol, and certain of the higher fatty acids such as palmitic, stearic, and oleic acids. Another generalization that can be made, at this point, is that in the fats the saturated fatty acids predominate, whereas unsaturated fatty acids are peculiar to the oils.

From animal sources we find such fats as butter, lard, and tallow occurring in a fairly pure state. Fewer solid fats are found in the plant kingdom, although a number are obtained from plants, for example, cocoa fat and certain fractions of palm oil and cottonseed oil.

The vegetable oils are most commonly obtained from seeds such as the cottonseed and the flax or linseed; fish oils and oleo oil, obtained from beef tissues, are the most common animal oils of commercial importance. These oils differ from the mineral (hydrocarbon) oils in that they cannot be distilled without undergoing decomposition. The fats and oils may be hydrolyzed with enzymes, acids, or by steam to form glycerol and free fatty acids. They are also capable of saponification with alkalies, in which case salts of the fatty acids are formed and glycerol is liberated:

Saponification of Stearin

$$\begin{array}{l} CH_2-OOC\cdot(CH_2)_{16}\cdot CH_3 \\ | \\ CH-OOC(CH_2)_{16}\cdot CH_3 + 3\,NaOH \rightarrow \\ | \\ CH_2-OOC(CH_2)_{16}\cdot CH_3 \\ \text{stearin} \end{array} \quad \begin{array}{l} CH_2OH \\ | \\ CHOH + 3\,NaOOC\cdot(CH_2)_{16}\cdot CH_3 \\ | \\ CH_2OH \\ \text{glycerol} \end{array} \quad \begin{array}{l} \\ \\ \text{sodium stearate} \\ \text{(a soap)} \end{array}$$

If glycerol combines with but one kind of fatty acid it is said to be a *pure* fat or oil. If, however, glycerol combines with more than one kind of fatty acid, it is said to be a *mixed* fat or oil or a mixed glyceride. The mixed glycerides are more common than was believed formerly. Myristo-palmito-olein has been isolated from cocoa butter, stearo-palmito-olein from tallow, and palmito-stearo-olein from butter Some of our best authorities contend that the mixed glycerides make up the major portion of our natural fats.

Two factors influence the properties of a fat or oil, i.e., the size of the fatty acid molecules and the amount of unsaturated or saturated fatty acids present In general, it may be said that, the greater the proportion of saturated fatty acids to unsaturated fatty acids, the greater the tendency toward hardness or solidness at room temperature. Conversely, if unsaturation is great, the glycerides tend to become liquid at ordinary temperatures. Another generalization may be made, viz., the greater the molecular weight of the fatty acid the harder the fat. When glycerin is combined with nothing but stearic acid the fat is called *stearin* Similarly, the fat *butyrin* contains butyric acid, and *palmitin* contains palmitic acid. When stearin or any other fat is treated with steam, free glycerol and free fatty acids are formed according to the following equation:

$$\begin{array}{l} CH_2-OOC(CH_2)_{16}\cdot CH_3 \\ | \\ CH-OOC(CH_2)_{16}\cdot CH_3 + 3\,HOH - \\ | \\ CH_2-OOC(CH_2)_{16}\cdot CH_3 \\ \text{stearin} \end{array} \quad \begin{array}{l} \text{water} \end{array} \quad \begin{array}{l} CH_2OH \\ | \\ CHOH + 3\,CH_3(CH_2)_{16}\cdot COOH \\ | \\ CH_2OH \\ \text{glycerol} \end{array} \quad \begin{array}{l} \\ \\ \text{stearic acid} \end{array}$$

Glycerol. — Since glycerol is an alcohol containing primary and secondary alcohol groups, we may expect it to deport itself chemically in a manner similar to that of other primary and secondary alcohols. For example, by oxidation in easy stages we might expect, and in reality we do obtain, the following products:

$CH_2OH \cdot CHOH \cdot CHO$	glyceric aldehyde
$CH_2OH \cdot CHOH \cdot COOH$	glyceric acid
$COOH \cdot CHOH \cdot COOH$	tartronic acid

If the secondary alcohol group is oxidized to the carbonyl radical, a ketone is formed known as dihydroxy acetone, $CH_2OH \cdot CO \cdot CH_2OH$. When glycerol is heated at high temperatures, as in the case of fats spilled on the top of a hot stove, water is removed and acrylic aldehyde (acrolein) and acrylic acid are formed. These substances are very irritating and cause the eyes to smart and burn:

$$\underset{\text{glycerol}}{CH_2OH \cdot CHOH \cdot CH_2OH} - 2\,HOH \rightarrow$$

$$\underset{\text{acrylic aldehyde}}{CH_2 = CH \cdot CHO} + O \rightarrow \underset{\text{acrylic acid}}{CH_2 = CH \cdot COOH}$$

The applications of these facts will be discussed in later chapters.

The Fatty Acids. — The fatty acids may be classified according to their chemical properties:

TABLE 5

SATURATED FATTY ACIDS

1. *The Acetic Acid Series* $C_nH_{2n}O_2$

Formic	$H\ COOH$
Acetic	$CH_3\ COOH$
Propionic	$CH_3\ CH_2\ COOH$
Butyric	$CH_3\ (CH_2)_2 \cdot COOH$
Valeric	$CH_3\ (CH_2)_3\ COOH$
Caproic	$CH_3\ (CH_2)_4\ COOH$
Caprylic	$CH_3\ (CH_2)_6\ COOH$
Capric	$CH_3\ (CH_2)_8\ COOH$
Lauric	$CH_3\ (CH_2)_{10}\ COOH$
Myristic	$CH_3\ (CH_2)_{12}\ COOH$
Palmitic	$CH_3\ (CH_2)_{14}\ COOH$
Stearic	$CH_3\ (CH_2)_{16} \cdot COOH$
Arachidic	$CH_3 \cdot (CH_2)_{18} \cdot COOH$
Behenic	$CH_3 \cdot (CH_2)_{20}\ COOH$
Lignoceric	$CH_3 \cdot (CH_2)_{22} \cdot COOH$

UNSATURATED FATTY ACIDS

2 *The Oleic Acid Series* $C_nH_{2n-2}O_2$

(containing one set of double bonds)

Tiglic	$C_5H_8O_2$
Physetoleic	$C_{16}H_{30}O_2$
Oleic	$C_{18}H_{34}O_2$
Rapic	$C_{18}H_{34}O_2$
Erucic	$C_{22}H_{42}O_2$

3. *The Linolic Series* $C_nH_{2n-4}O_2$

(containing two sets of double bonds)

Linolic	$C_{18}H_{32}O_2$
Tariric	$C_{18}H_{32}O_2$
Eleomargaric	$C_{18}H_{32}O_2$

4. *Linolenic Series* $C_nH_{2n-6}O_2$

(containing three sets of double bonds)

Linolenic	$C_{18}H_{30}O_2$
Iso-linolenic	$C_{18}H_{30}O_2$

5. *Series* $C_nH_{2n-8}O_2$

Clupanodonic	$C_{14}H_{28}O_2$

6. *Hydroxy Acids* (mono-)

Ricinoleic	$C_{18}H_{34}O_3$
Cerebronic	$C_{25}H_{50}O_3$

7. *Hydroxy Acids* (di-)

Dihydroxystearic	$C_{18}H_{36}O_4$

Unsaturation. — When the constitution of the unsaturated fatty acids is studied, it is found that the chain contains one or more unsaturated groups which confer higher chemical activity at that point in the chain. Three of the most common unsaturated fatty acids may be represented by the following formulae:

oleic acid $CH_3(CH_2)_7CH = CH(CH_2)_7COOH$

linolic acid $CH_3(CH_2)_6CH = CH - CH = CH(CH_2)_6COOH$

linolenic acid

$$CH_3(CH_2)_5CH = CH - CH = CH - CH = CH - (CH_2)_5COOH$$

This unsaturated linkage is easily broken. As a result, oxygen may enter it and form an ethylene linkage which gives resinous properties to such fatty acids. When oils are rich in unsaturated acids of this type, they dry rapidly, owing to this oxygen absorption. Such oils are called drying oils; examples are linseed oil and China wood oil.

Fat Constants. — These constants are used to identify a fatty acid or to identify fats containing certain fatty acids. When a food chemist is confronted with the possibility that an expensive oil, such as olive oil, has been diluted with a cheaper oil, such as cottonseed oil, these constants become very useful. The fat constants are physical and chemical measurements which seem to remain fairly constant for oils and fats prepared properly from the same sources. Among the constants which are determined most commonly are (1) specific gravity, (2) melting point, (3) iodine absorption number, (4) Reichert-Meissl number, and (5) saponification number.

The *melting point*, though not an extremely accurate determination, is a helpful guide in detecting adulteration of oils or fats and gives a rough idea of the possible amount of unsaturated fatty acids present.

Often, the chemist, instead of determining the melting point of a fat, will determine the melting point of the fatty acids. This is done by saponifying the fat with alkali and treating the soap with mineral acid. This liberates the fatty acids, which can be isolated and used for the melting-point determinations, a few of which are given in the following table:

TABLE 6

THE MELTING POINTS OF THE FATTY ACIDS IN SOME COMMON FATS AND OILS

	degrees Centigrade		*degrees Centigrade*
Olive oil	21–30	Cacao butter	48–53
Cottonseed	34–40	Cocoanut oil	24–25
Wheat oil	39–40	Beef fat	38–42
Linseed oil	17–24	Tallow	41–49
Castor oil	13	Cod-liver oil	21–25

The iodine absorption number is obtained by allowing one gram of oil or fat to react with an iodine-chloride or bromide solution of known strength. Each unsaturated group unites with two atoms of iodine, as indicated below:

$$\underset{\text{oleic acid}}{CH_3(CH_2)_7 - CH = CH - (CH_2)_7 - COOH} + \underset{\text{iodine}}{2\ I} \rightarrow$$

$$CH_3(CH_2)_7CHI - \underset{\text{di-iodo oleic acid}}{CHI - (CH_2)_7COOH}$$

By titrating the excess iodine and calculating the amount absorbed, the chemist may obtain valuable information regarding the degree of unsaturation of a fat or oil. The iodine number is expressed in parts of iodine per 100 parts of oil. For example, the iodine number for the non-drying oil, castor oil, is about 85; for the semi-drying oil, corn oil, 113 to 125; and for linseed oil, which is one of the best drying oils, from 175 to 205.

The following table gives the iodine values of a number of the more important fats and oils:

TABLE 7

IODINE NUMBERS FOR SOME COMMON FATS AND OILS

Olive oil	77– 95	Cocoanut oil	8 2– 9 5
Cottonseed oil	104–116	Beef fat	36 – 42
Wheat oil	115	Butter fat	28 – 42
Linseed oil	175–201	Tallow	33 – 48
Castor oil	84– 85	Cod-liver oil	154 –170

The iodine number is very useful in industrial work since it is an index of the drying capacity of an oil, a property which is of value in the preparation of paints. In our discussion of unsaturated fatty acids we

touched on this point, viz., that unsaturated fatty acids possessed the property of absorbing oxygen. An oil which is rich in unsaturated fatty acids will absorb oxygen readily and form a resinous skin characteristic of good oil paints. This absorption of oxygen may become so rapid that sufficient heat is formed to bring the oil to its kindling point. This often happens when oil-soaked rags are thrown aside and forgotten. This process is known as *spontaneous combustion* and is the cause of many disastrous fires. Oils may be classified into three groups, depending on their ability to absorb oxygen, viz., *drying oils*, of which linseed oil is an example; *semi-drying oils*, of which cottonseed oil is an example; and *non-drying oils*, of which olive oil is representative.

The Reichert-Meissl number is an index of the amount of volatile fatty acids of *low molecular weight*. A known weight of fat is saponified and the soap is decomposed with dilute sulfuric acid, liberating the free fatty acids, which are distilled with live steam. Those that have from one to ten carbon atoms are quite volatile with steam and may be distilled and thereby separated and titrated with $N/10$ KOH. The number of cubic centimeters of $N/10$ alkali required to neutralize the volatile fatty acids in five grams of oil or fat is called the *Reichert-Meissl number*. Most fats have no volatile fatty acids, but butter fat is an exception, having a Reichert-Meissl number of 21 to 33 Cocoanut oil and palm oil, on the other hand, have numbers ranging from 5 to 8. If butter fat is adulterated with other fats or oils the lowering of the Reichert-Meissl number is of great help in detecting the adulteration.

The saponification number is another very useful analytical constant which gives the food chemist valuable information. This test is based on the fact that all fatty acids, regardless of molecular weight, are monobasic, each acid molecule uniting with but one potassium atom in the formation of soap A known weight of fat or oil is saponified with an excess of an alcoholic solution of potassium hydroxide of known strength. When the saponification reaction is complete, the excess alkali is determined by titration, and the amount of potassium hydroxide which united with the fatty acids is calculated. The *saponification number* is the number of milligrams of potassium hydroxide required to saponify one gram of oil or fat.

If the oil or fat is composed very largely of stearic, palmitic, and oleic acids of high molecular weight, there will be fewer molecules of fatty acids per gram of fat. Consequently, the number of potassium ions in combination will be relatively few. The saponification number of a fat or oil, therefore, is an index of the *relative size of the fatty acid molecules* in the fat. Rape-seed oil contains appreciable quantities of erucic acid, $C_{22}H_{42}O_2$, and, as a result, possesses a saponification value of about 175.

Oleomargarine averages about 195, since it contains relatively large amounts of the higher fatty acids. Butter fat, however, with its butyric, caproic and caprylic acids, has a saponification value that averages close to 227.

The following table gives some idea of the saponification values of some of the common fats and oils:

TABLE 8

SAPONIFICATION NUMBERS OF SOME COMMON FATS AND OILS

Olive oil	185–196	Cocoanut oil	246–262
Cottonseed oil	191–196	Beef fat	196
Wheat oil	183–190	Tallow	193–198
Linseed oil	190–201	Cod-liver oil	179–190
Castor oil	175–183	Cacao butter	192–195

A number of other fat constants are used by chemists in the study of fats and oils, but those mentioned will suffice to give the reader some idea of the chemical properties of this interesting and valuable group of lipids.

The Waxes

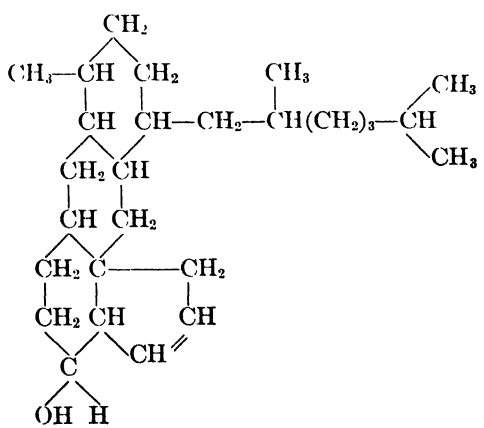
These compounds, like the fixed oils and fats, are esters containing fatty acids. They differ from the glycerides, however, in one important respect, viz., that they do not contain glycerol. Instead of the trihydroxy alcohol, glycerol, they contain monobasic alcohols known as sterols. The waxes are of both animal and vegetable origin and their solubilities are similar to those of the fats and oils. They may be saponified like the fats but with much greater difficulty. The waxes occur quite extensively in nature, but, as a rule, they never occur abundantly. Beeswax, which is one of the best known of this group, contains myricyl alcohol, $C_{30}H_{62}OH$, combined with palmitic, $C_{16}H_{32}O_2$, cerotic, $C_{26}H_{52}O_2$, and melissic, $C_{30}H_{60}O_2$, acids. Wool wax or lanolin contains at least three higher alcohols (sterols), viz., carnaubyl alcohol, $C_{24}H_{49}OH$, cholesterol, $C_{27}H_{45}OH$, and iso-cholesterol, $C_{27}H_{45}OH$. These sterols are combined in lanolin with myristic, $C_{14}H_{28}O_2$, lano-palmitic, $C_{16}H_{32}O_3$, and lano-ceric, $C_{30}H_{60}O_4$, acids. Waxes are found in thin layers covering the surfaces of the stems or stalks of many plants. It is thought that these waxes function as a protective water-proof coating. The "bloom" on many fruits has been identified as a wax or waxlike substance.

The Sterols

The sterols have been mentioned in our discussion of the waxes. These alcohols are solid at ordinary temperatures and are easily crystallized. They are soluble in ether and chloroform, and for that reason the ether

extract of plant and animal materials usually contains sterols and waxes in addition to the fats and fixed oils. Hot alcohol is also a solvent for many of the sterols.

Cholesterol, $C_{27}H_{45}OH$, is probably the best-known member of the group. It may be obtained in large amounts by extracting pulverized human gallstones with hot alcohol containing some potassium alcoholate. Cholesterol may be crystallized from alcoholic solution in the form of rhombic crystals, which, in chloroform solution, are levo-rotatory. When heated in vacuo at about 300° C. cholesterol may be distilled or sublimed without decomposition. It is easily dissolved in fats and in bile. On account of the fact that it is an alcohol and not an ester, it cannot be saponified, consequently it can be separated from fats, after saponification. Cholesterol is widely distributed in nature; it can be found in many animal tissues, particularly in egg yolk and the brain and other nerve tissues. On account of its alcoholic properties it forms esters readily. Although its structure has not been established with certainty it would appear that cholesterol is an unsaturated secondary alcohol. It is related to the terpenes and probably possesses at least two six-carbon rings. Considerable interest has centered about cholesterol since the discovery that foods can be made anti-rachitic (rickets curing) when irradiated with ultraviolet light. For a time it was thought that cholesterol was the compound that was activated. It has been shown recently, however, that cholesterol, in the pure state, cannot be activated. Associated with cholesterol is another sterol, called ergosterol, which is now known to be the activated compound and is thought to be the parent substance of vitamin D. The formula (for cholesterol) given below is the best that has been proposed:



Ergosterol. — This sterol, the parent substance of vitamin D, was first isolated from ergot; it has been found to exist in a number of food materials in amounts so small that its presence has remained undetected until recently. It occurs associated with cholesterols and phytosterols in plant and animal tissues, and its separation is a matter of considerable difficulty. In chemical composition, ergosterol does not seem to be strikingly different from cholesterol, although it is clear that it contains three pairs of double bonds and, as a consequence, is much more unsaturated than cholesterol, which may explain its greater activation by ultraviolet light.

The Phytosterols are solid alcohols existing in plants. It is these alcohols that play an important part in the formation of the plant waxes. Little is known regarding their structure, and undoubtedly, a number of phytosterols exist, they have not been wholly differentiated, although the group can be represented by the empirical formula, $C_{27}H_{46}O$. *Sitosterol* is another sterol found in the plant kingdom. It is found in wheat embryo and has been given the empirical formula, $C_{26}H_{44}O$. *Stigmasterol*, $C_{30}H_{48}O$, *ergosterol*, and *cucurbitol*, $C_{21}H_{40}O_4$, are plant sterols which have been isolated from the calabar bean, yeast, and watermelon seeds, respectively.

The Phospholipids. — From a biological standpoint the phospholipids are, without doubt, the most important members of the lipid group. They are found in all plant and animal tissues, particularly those tissues which have to do with reproduction. The properties of protoplasm, upon which all life processes depend, are intimately correlated with the properties of the phospholipids. We will learn in subsequent chapters that the physical properties of the sterols, the phospholipids, and the proteins play the major rôle in the formation and maintenance of that mysterious semi-fluid state of matter known as protoplasm

The phospholipids are, as the name indicates, fatty or fat-like substances containing phosphoric acid in combination. They always contain fatty acids, and most of them contain glycerol. The most typical ones contain nitrogenous bases, the most common of which is choline. This combination of phosphoric acid, choline, and fatty acids gives us a clue to the peculiar properties of these compounds, for phosphoric acid and choline possess a decided affinity for water, whereas fatty acids are relatively insoluble in water. As a result, these interesting compounds have the property (with cholesterol and protein) of absorbing relatively large amounts of water which, in this process of absorption, loses its usual solvent properties. Consequently, these semi-fluid emulsions become what Mathews has termed " the physical substratum " of protoplasm. Inasmuch as the chemistry of the phospholipids is still in the develop-

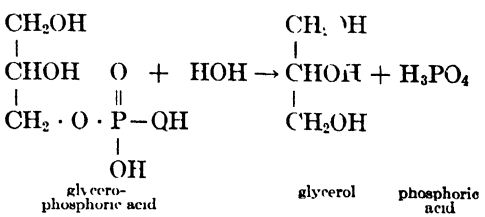
mental stage, we shall discuss only those which seem to be typical of the group and whose chemistry is fairly clearly established.

Lecithin is undoubtedly the best-known member of this group. It is found in all actively growing tissues, in nervous tissues, and in the yolk of eggs. Although little is known, of a definite nature, regarding its functions in the organism, it is of undoubted importance, for nature has seen fit to place it in all growing tissues and particularly in those tissues which are the most vital to life and reproduction. The term lecithin does not apply to a single individual but rather to a group of chemically related compounds of which lecithin, as we will discuss it, is but one. When lecithin is prepared in fairly pure form it makes waxy plates which are soluble in ether and other fat solvents. On hydrolysis it yields saturated and unsaturated fatty acids, glycerol, phosphoric acid, and a nitrogenous base. As a result, we can obtain a mental picture of its probable composition by consulting the following diagram:

glycerol { 1. fatty acid (saturated)
2. fatty acid (unsaturated)
3. phosphoric acid (nitrogenous base) (choline)

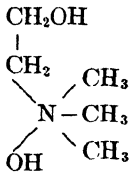
Levene, of the Rockefeller Institute for Medical Research, has isolated four types of lecithin from liver. In egg yolk he was able to isolate three of these compounds. In liver he found glycerol, phosphoric acid, and a nitrogenous base with (*a*) palmitic and oleic acids, (*b*) stearic and oleic acids, (*c*) palmitic and a twenty-carbon acid called arachidonic, and (*d*) stearic and arachidonic acid. In egg yolk he found palmitic and stearic acids associated with oleic, linoleic, and arachidonic acids.

Further study of the structure of the molecule showed that glycerophosphoric acid was present, which, upon hydrolysis, yielded glycerol and phosphoric acid:



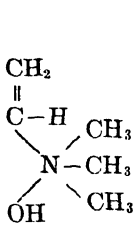
A study of the nature of the nitrogenous fraction proved the existence of choline. If the reader will recall his organic chemistry, he will remember that choline is a substituted ammonium hydroxide whose chemical name is tri-methyl-hydroxy ethyl ammonium hydroxide, in which the

four hydrogens of NH_4OH have been replaced by three methyl, CH_3, radicals and a hydroxy ethyl, C_2H_4OH, group:

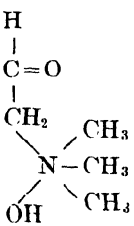


Choline

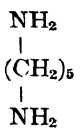
At this point we may again refer to the chemistry of the amines and the substituted ammonium hydroxides by showing the relationship of choline to neurine, muscarine, and cadaverine:



Neurine

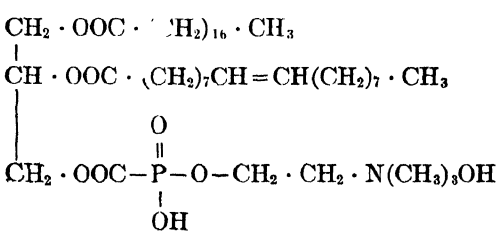


Muscarine



Cadaverine

All these compounds possess toxic properties when ingested by animals. Muscarine is found in poisonous mushrooms, and cadaverine is one of several similar substances, known as ptomaines, which are formed during food spoilage by the action of anaerobic bacteria on protein-containing foods. As a result of the findings to which we have just referred, it is possible to represent a typical lecithin by the following formula:



A representative lecithin

Other members of the phospholipid group are *kephalin* (found in the brain and nervous tissue), *cuorin* (found in heart tissue), and *sphingomyelin* (found in brain and other body tissues).

The Glycolipids. — As their name implies, these lipids, on hydrolysis, yield carbohydrates, usually galactose. Although their chemistry is not clear, it is certain that they do not contain phosphorus. In solubility they differ somewhat from the other lipids. They are found in brain tissue, for which reason they are sometimes called the " cerebrosides." *Phrenosin* and *kerasin* are members of this group. Owing to the facts that these brain lipids contain galactose and that milk (which also contains galactose) is so necessary in the development of the young, some scientists have postulated that this monosaccharide must be indispensable for the growing child, although most physiological research seems to indicate that galactose is not essential, per se, but can be manufactured from glucose by the body cells.

The *sulfo-lipids* or *sulfatides* are ill-defined compounds which occur in the brain. One of these, known as *protogon*, yields fatty acids and sulfuric acid, on hydrolysis.

The *amino-lipids* are also fat-like materials which are characterized by the presence of amino groups.

The classification of essential oils has always been a problem for writers of chemistry textbooks because these aromatic materials are related to no particular group or family of organic compounds and yet they are all related in one way or another to many groups of organic substances. For example, some volatile oils contain hydrocarbons which give them their characteristic properties, others contain alcohols or aldehydes or esters, etc.

As a consequence, some authors have classified them with the lipids for the reason that they are similar to certain lipids in some of their physical properties We have not classified them with lipids, but we feel that this is a logical point at which to discuss these interesting compounds.

The Essential or Volatile Oils

The characterized odor and taste of nearly all plant products are due, in a large measure, to certain oils which differ in chemical composition and properties from the vegetable and animal oils just described. These aromatic oils are known as essential or volatile oils.

Essential oils probably have no physiological function in the plant, but they do seem to be of service to many plants in attracting desirable insects and thereby effecting fertilization, and it is [illegible] that some pungent essential oils seem to repel certain [illegible] insects.

Essential or volatile oils are, as a rule, insoluble in water; when fresh, they are colorless or yellow in color, and most of them darken on exposure to air and light. Like the vegetable and animal oils, they possess

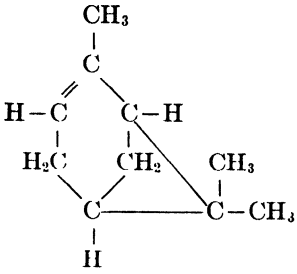
greasy properties and produce a translucent spot on paper. Unlike the glycerides of the fatty acids, however, the translucent spot is not permanent, owing to the volatile properties of these compounds. As a rule, the essential oils are soluble in alcohol, ether and chloroform, which is one reason for including them in the lipid classification. Most of them can be distilled without change; this is not characteristic of the fixed oils, which decompose prior to volatilization. Volatile oils are soluble in the fixed oils, and advantage is taken of this property in the manufacture of certain rare volatile oils, like the oil of rose.

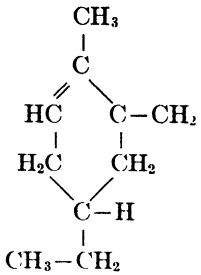
Some volatile oils may be obtained from plant tissues by expression; some are separated by distillation with or without previous fermentation of the plant tissue; others are dissolved in a fixed oil and the mixture subsequently extracted and distilled; and some are extracted by volatile solvents which are later separated by fractional distillation.

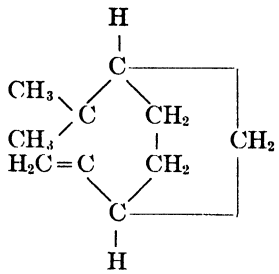
Chemically, the essential oils are so varied and heterogeneous that no satisfactory system of classification can be evolved. The best we can do is to discuss the chemical nature and properties of a few representative members of this group of plant substances, which have been of interest and value to man for thousands of years as perfumes and flavoring agents. Some of these oils are characterized by the presence of cyclic hydrocarbons, giving them volatile and aromatic properties; others may contain alcohols, aldehydes, ketones, esters of alcohols and acids or other organic compounds.

In a general discussion of essential oils, the hydrocarbon groups of compounds deserve special mention. These compounds, many of which have the general formula, $C_{10}H_{16}$, are known as terpenes. The terpenes do not necessarily contribute to the aromatic properties of the essential oils, at least not to the characteristic aromatic properties. In fact, the same terpenes are found in many essential oils, for example, oil of nutmeg, oil of spearmint and oil of rosemary all contain a terpene which is also the chief constituent of turpentine.

Pinene is an important terpene found in the oil of turpentine. As the formula below indicates, this compound is a cyclic hydrocarbon having the empirical formula $C_{10}H_{16}$ and is unsaturated. Other isomeric terpenes are limonene and camphene:


Pinene


Limonene

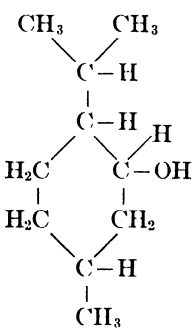

Camphene

Another group known as *sesquiterpenes*, having the empirical formula, C_5H_8, and the molecular formula, $C_{15}H_{24}$, are also found in essential oils.

Some Common Essential Oils. *Oil of Bitter Almonds.* — This oil, obtained from almond kernels, exists in the almond as a glucoside which is hydrolyzed by the enzyme emulsin, yielding benzaldehyde, hydrocyanic acid and glucose. When the essential oil has been carefully separated, it is found to be almost pure benzaldehyde, $C_6H_5 \cdot CHO$.

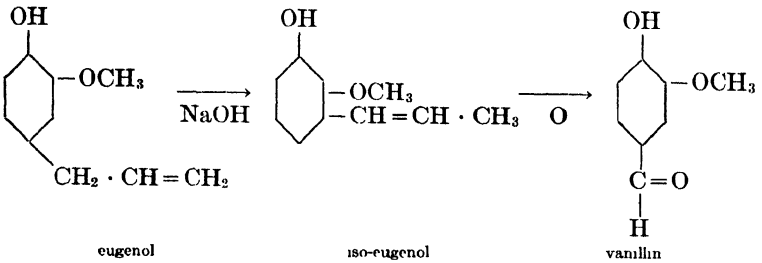
Oil of wintergreen is obtained from the teaberry or wintergreen plant and consists almost entirely of methyl salicylate, $C_6H_4OH \cdot COO \cdot CH_3$, an ester of methyl alcohol and salicylic acid

Oil of peppermint is a mixture of alcohols, terpenes, aldehydes and esters One constituent, an alcohol known as menthol, is of considerable pharmaceutical value:


Menthol

Oil of cloves is prepared from the buds of the clove tree; it is characterized by its content of eugenol, an aromatic alcohol, associated with a small amount of sesquiterpenes. It is used in the preparation of pharmaceuticals and in the manufacture of perfumes and flavoring extracts. For example, when eugenol is treated with alkali, iso-eugenol is formed.

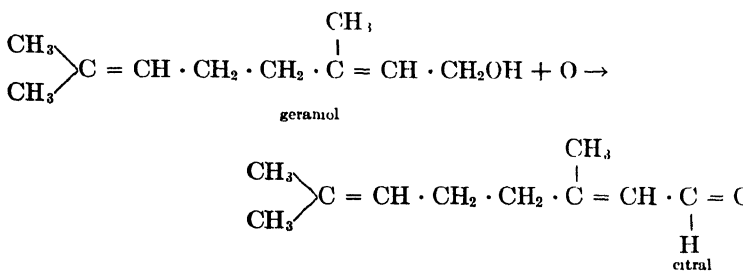
The latter compound may be oxidized to form *vanillin*, the flavoring principle in vanilla extracts:



eugenol iso-eugenol vanillin

Oil of lemon is another essential oil used in large quantities in the preparation of flavoring extracts and fruit syrups. It contains relatively large amounts of limonene, which has been mentioned above, and an unsaturated aldehyde known as citral. This compound is an oxidation product of an unsaturated branched-chain primary alcohol called geraniol.

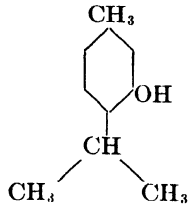
Geraniol is a volatile compound with an odor of flowers used in the manufacture of perfumes. It is also a constituent of the oil of geranium, oil of lemon grass and citronella oil. This compound, like many others in this group, possesses a relatively high boiling point, but the aromatic properties are due to appreciable vapor pressures at ordinary temperatures. Citral may be formed from geraniol by oxidation:



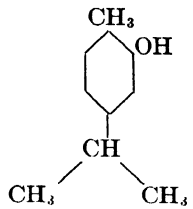
geraniol citral

Oil of roses, sometimes known as attar of roses, is obtained from southern Europe. It is extremely expensive, and for this reason is often adulterated with cheaper oils, such as oil of geranium. Chemically, oil of roses is composed largely of two aliphatic alcohols, geraniol and citronella.

Oil of thyme is of interest, primarily, because of its content of important pharmaceutical compounds known as thymol and carvacrol, isomeric phenol derivatives:



p-Isopropyl-*m*-cresol
Thymol



p-Isopropyl-*o*-cresol
Carvacrol

Oil of turpentine is a complex mixture of substances of which pinene, $C_{10}H_{16}$, is the most important. It is obtained by distilling the exudate of the long-leaf pine. The distillate is known as turpentine or spirits of turpentine, and the non-volatile residue is called rosin.

Oil of mustard, which is used in medicine, is obtained by allowing black mustard seeds to ferment. A glucoside called sinigrin (potassium myronate) is hydrolyzed during fermentation, yielding potassium acid sulfate, glucose and allyl-iso-thiocyanate, $CH_2=CH \cdot CH_2 \cdot N=C=S$, which is separated by distillation and is known as oil of mustard.

This brief description of a few common essential oils will give the reader some idea of the complex nature of these substances. With the advance of our knowledge of organic chemistry, the manufacturer of perfumes and flavoring extracts is becoming less dependent upon the natural sources for many of these products, for they can be made synthetically by the manufacturing organic chemist.

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CHAPTER V

THE PROTEINS

We shall learn, in later chapters, that proteins are essential constituents of all living cells, in fact without them there would be no life. Whereas the carbohydrates and the fats serve as the chief sources of energy in most living organisms, we will find that the proteins play a most important structural rôle, particularly in the animal kingdom. Proteins received their name about 1838 from a Dutch chemist by the name of Mülder. The name is taken from a Greek verb which means "to take first place," for Mulder thought that proteins were of prime importance in the constitution of cell protoplasm.

Plants are able to synthesize these important chemical substances from simple nitrogenous compounds absorbed from the soil, and from the water and carbon dioxide assimilated by roots and leaves. The animal, on the other hand, does not seem to be able to build these complex molecules from such simple materials but must obtain the proteins from plant sources and synthesize the protein tissues from the digestion products.

Since proteins are of such great importance to animals, we find that the farmer grows many plants because of the commercial value of the protein which they contain. Feed manufacturers also produce animal foods, from plant and animal sources, which are sold, primarily, because they contain these important nitrogenous compounds in relatively large amounts. In later chapters we will learn that not all proteins are of the same value for the growing animal. This difference in the biological value of proteins lies in the fact that some proteins are rich in certain "building stones" called amino acids whereas other proteins are devoid of some of these acids or contain them in very small amounts Since this is the case, it seems logical and necessary to devote a little time to the consideration of the nature, properties and structure of the proteins and their hydrolytic products, the amino acids.

General Properties and Composition of Proteins

When the chemist desires to study the chemistry of proteins, he must first separate and isolate them in the pure state, if possible. This is a most difficult task for the reason that nearly all proteins form sticky, col-

loidal solutions with water. Methods have been evolved, however, based upon the differences in solubility in various reagents; for example, some proteins are soluble in dilute salt solutions, others are dissolved by alkalies or other solvents. In this way food materials may be extracted and the protein fractions separated. These fractions may then be precipitated with various reagents, dissolved and reprecipitated until the protein is fairly pure.

Analysis of these fractions shows that they all contain nitrogen, carbon, hydrogen and oxygen; some contain phosphorus and sulfur, and in a few cases other elements, such as iron, are found in combination. Although the proteins vary in composition, most of the typical ones consist of carbon, about 50 per cent; nitrogen, 16 per cent; hydrogen, 7 per cent; oxygen, 22 per cent; sulfur, from 0 to 3 per cent; and phosphorus, from 0 to 4 per cent

TABLE 9

COMPOSITION OF SOME TYPICAL PROTEINS

	C	H	N	S	O	P	Fe
Gliadin from wheat	52 72	6 86	17 66	1 03	21 73	..	...
Edestin from hempseed	51 36	7 01	18 65	88	22 10	..	...
Gelatin from bones	49 38	6 80	17 97	70	25 13	. .	..
Fibrin from blood	52 68	6 83	16 91	1 10	22 48	.	.
Hemoglobin from blood	52 66	7 25	17 70	45	19 54		40
Casein from cow's milk	53 00	7 00	15 70	80	22 65	85	

The physical properties of proteins will be discussed in the next chapter.

If we were to define the proteins we would keep in mind the following: (1) They are complex compounds of high molecular weight containing carbon, nitrogen, hydrogen, and oxygen; usually sulfur; occasionally phosphorus; and sometimes iron. (2) As we shall learn later, they are colloidal in nature and will not pass through membranes (3) They may act chemically as bases, combining with acids, and like the acids, may combine with bases, i e., they are amphoteric. (4) Upon hydrolysis they pass through a number of stages, yielding simpler compounds, and finally, when hydrolysis is complete, it is found that the hydrolysate is made up almost entirely of amino acids. (5) The proteins are complex combinations of amino acids, linked one to another.

Classification of Proteins

There are, at present, two systems of protein classification, the American and the English systems. We shall use the American classification and terminology but shall give the English equivalents where it is possible to do so. Both systems of classification are based on the chemical

properties and solubilities of the various proteins. The reader should keep in mind that both classifications are more or less arbitrary and, although they are not entirely satisfactory, they help us to visualize the proteins as a group.

Both classifications divide the proteins into three large classes:

1. Simple Proteins.
2. Conjugated Proteins.
3. Derived (American) or Hydrolyzed (English) Proteins.

The first two classes represent proteins as they exist in nature; the third class contains those proteins which have been changed or modified by enzymes or chemical reagents.

TABLE 10

THE AMERICAN CLASSIFICATION OF PROTEINS

A. THE SIMPLE PROTEINS. — Protein substances which yield only α-amino acids or their derivatives on hydrolysis.

1. *Albumins.* Simple proteins, soluble in water and coagulated by heat. *Examples* egg albumin, lactalbumin (milk), serum albumin (blood), leucosin (wheat), legumelin (peas).

2. *Globulins.* Simple proteins, insoluble in pure water but soluble in neutral salt solutions. *Examples* edestin (hemp seed), phaseolin (beans), legumin (beans and peas), arachin and conarachin (peanuts), muscle globulin, serum globulin.

3. *Glutelins.* Simple proteins, insoluble in all neutral solvents but soluble in dilute alkalies and acids. Glutenin of wheat is the best-known member of this group.

4. *Alcohol-soluble proteins,* sometimes called *Prolamines* or *Gliadins* (English). Simple proteins, insoluble in water but soluble in 70 to 80 per cent alcohol. Insoluble in absolute alcohol. *Examples* gliadin (wheat), zein (corn), hordein (barley).

5. *Albuminoids,* called *scleroproteins* in Great Britain. Simple proteins occurring in the skeletal structures of animals and in skin, hair, horn, etc. These proteins (keratin, elastin, collagen) are hard and tough and are insoluble in practically all solvents. They are not used as food proteins. Collagen, however, yields gelatin on boiling.

6. *Histones.* Soluble in water; insoluble in dilute ammonia; in the absence of ammonium salts they are insoluble in an excess of ammonia; coagulated by heating, and coagulum dissolves in dilute acids. *Examples* thymus histone and globin from hemoglobin. Basic amino acids predominate in the hydrolysate.

7. *Protamines.* Less complex than the preceding groups. Soluble in water; not coagulated by heat; basic in reaction and the hydrolysate is rich in diamino (basic) acids. They have no importance as food proteins. Found in ripe fish sperm.

B. THE CONJUGATED PROTEINS. — These protein materials are united to some other molecule or molecules otherwise than as a salt.

1. *Nucleoproteins.* Protein compounds in which nucleic acid is combined with protein. *Examples.* nuclein and nucleo-histone.

2. *Glycoproteins* or *Glucoproteins* (English). As the name indicates, the protein molecule is united to a substance containing carbohydrate groups. *Example:* mucins in mucous secretions.

3. *Phosphoproteins.* Compounds containing phosphorus in combination with proteins, but not as a nucleo-protein or as a lecithin combination. *Examples.* ovo-vitellin from egg yolk and casein from milk.

4 *Hemoglobins* or *Chromoproteins* (English). Combinations of hematin (or other colored groups) and protein. *Example·* hemoglobin from blood.

5 *Lecithoproteins.* Compounds of lecithins and related substances, with proteins. Found in cytoplasm and limiting membranes. *Example.* tissue fibrinogen.

C. Derived Proteins — This group contains all decomposition products of the naturally occurring proteins These products may be formed by heat, water, enzymes, or chemical reagents, and they may include products which scarcely differ, in properties or size of molecule, from the original natural protein molecule. They may include such simple substances as the amino acids Those which have been changed the least are called "primary derived proteins"; those which have undergone considerable cleavage are known as "secondary derived proteins"

1. *Primary protein derivatives*
   (*a*) Proteans This term refers to those protein compounds which have been but very slightly modified by water, dilute acids or alkalies, or by enzymes. They are insoluble in water *Examples* fibrin (coagulated blood), casein (curdled milk), edestan
   (*b*) Metaproteins Products of the further action of acids and alkalies, which are soluble in acids and alkalies but not in neutral solvents *Examples* acid-metaprotein, alkali-metaprotein
   (*c*) Coagulated proteins Insoluble protein products resulting from the action of heat, alcohol, etc *Examples* cooked egg albumin.
2 *Secondary protein derivatives* These are simpler products formed by further hydrolysis of the protein molecule
   (*a*) Proteoses or Albumoses (English). Soluble in water, not coagulated by heat Precipitated by saturating their solutions with ammonium sulfate. Commercial peptone used in bacteriology contains considerable protein in the form of proteose.
   (*b*) Peptones Simpler products of protein hydrolysis Soluble in water, not coagulated by heat and not precipitated by saturated solutions of ammonium sulfate
   (*c*) Peptids Much simpler than the peptones Really combinations of two or more amino acids. If several amino acid molecules are combined, the peptid is called a "polypeptid" Likewise, combinations of four, three or two amino acids are termed "tetra-," "tri-" and "di-" peptids, respectively.

Products of Protein Hydrolysis

When proteins are boiled for many hours with 10 per cent sulfuric acid or 30 per cent hydrochloric acid, the sticky colloidal properties disappear and the proteins go into solution. Similar changes take place when protein-digesting enzymes are allowed to react with protein suspensions at 25 to 40° C. Further study of the resulting solution shows that substances have been formed which are capable of crystallization. Analysis of the solution shows that the water-free solids weigh more than the

original protein, and that the oxygen and hydrogen contents of the hydrolysate are higher and the carbon and nitrogen contents are lower than was the case in the original protein material. In other words, hydrogen and oxygen have been added in the form of *water* during the cleavage process. For this reason, the process of protein cleavage is called hydrolysis. Reasoning conversely, it would appear that the protein molecule must be a sort of anhydride. Examination of the crystalline products in the hydrolysate shows that the mixture is composed of amino acids. It would appear, therefore, that proteins are anhydrides of the amino acids, most of which are α-amino acids:

TABLE 11

AMINO ACIDS FROM SOME TYPICAL PROTEINS

(Expressed in Percentages)

	Gliadin (wheat)	Zein (maize)	Egg albumin	Gelatin	Casein	Hemoglobin
Glycocoll	0 02			25 5	0 45	
Alanine	2 00	9 79	2 22	8 7	1 85	4 2
Serine	0 13	1 02	?	0 4	0 5	0 6
Cystine	0 45	?	?		0 07	0 3
Valine	0 21	1 88	2 50		7 95	
Leucine	5 61	19 55	10 71	7 1	9 7	29 0
Phenyl alanine	2 35	6 55	5 07	1 4	3 88	4 2
Tyrosine	1 20	3 55	1 77		4 5	4 63
Aspartic acid	0 58	1 71	2 20	3 4	1 77	4 4
Glutaminic acid	42 98	26 17	9 10	5 8	21 77	1 7
Arginine	3 16	1 55	4 91	8 2	3 81	5 4
Lysine			3 76	5 9	7 62	4 3
Histidine	0 61	0 43	1 71	0 9	2 59	11 0
Proline	7 06	9 04	3 56	9 5	7 63	2 3
Tryptophane	Present				1 5	2 61

About twenty-three amino acids have been isolated from proteins, and since, in later chapters, we shall refer to the amino acids and since their type and structure determine the biological value of protein, a short discussion of the more important amino acids should be helpful at this point.

The Amino Acids

From the standpoint of organic structure these compounds are, at the same time, amines and acids. As a result, we should expect them to possess many of the properties of both types of compounds. One of the

principal characteristics of amines is their base-forming property or their ability to form salts with acids. Without going into a detailed discussion of the physical chemistry of the amino acids and theories regarding the internal salt structure of these compounds, let it suffice to say that we may consider them to be substances which possess both basic and acidic properties, in solution, owing to the presence of amino and carboxyl groups. Such substances are said to be "amphoteric."

Using the simplest amino acid, amino acetic acid, as an example in the following equations, it can be seen why these substances are amphoteric:

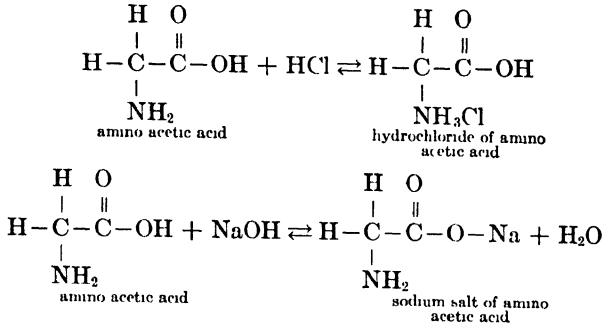


TABLE 12

CLASSIFICATION OF THE AMINO ACIDS

The amino acids are classified according to their structure and according to the number of amino and carboxyl groups they possess.

Monoamino-monocarboxylic acids

(1) Glycocoll, amino acetic acid, CH_2NH_2COOH
(2) Alanine, α-amino propionic acid, CH_3CHNH_2COOH
(3) Serine, β-hydroxy-α-amino propionic acid, $CH_2OH \cdot CHNH_2COOH$
(4) Cysteine, β-thio-α-amino propionic acid, $CH_2SH \cdot CHNH_2COOH$
(5) Cystine, di (-β-thio-α-amino propionic acid),

$$COOH \cdot CHNH_2CH_2S \cdot SCH_2CHNH_2COOH^*$$

(6) α-amino butyric acid, $CH_3CH_2CHNH_2COOH$
(7) Valine, α-amino isovaleric acid, $(CH_3)_2CH \cdot CHNH_2COOH$
(8) α-amino valeric acid, $CH_3CH_2CH_2CHNH_2COOH$
(9) Leucine, α-amino isocaproic acid, $(CH_3)_2CH \cdot CH_2CHNH_2 \cdot COOH$
(10) Isoleucine, α-amino-β-methyl valeric acid,

$$CH_3CH_2CH(CH_3)CHNH_2COOH$$

(11) Caprine, α-amino normal caproic acid, $CH_3(CH_2)_3CHNH_2COOH$
(12) Phenyl alanine, β-phenyl-α-amino propionic acid, $C_6H_5CH_2CHNH_2COOH$
(13) Tyrosine, β-para hydroxy phenyl-α-amino propionic acid,

$$HO \cdot C_6H_4CH_2CHNH_2COOH$$

* This is really a diamino-dicarboxylic acid.

Monoamino dicarboxylic acids:

(14) Aspartic acid, amino succinic acid, $COOH \cdot CH_2CHNH_2COOH$

(15) Glutaminic acid, α-amino glutaric acid, $COOH \cdot CH_2CH_2CHNH_2COOH$

Diamino monocarboxylic acids:

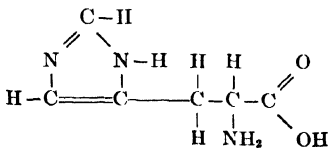
(16) Arginine, α-amino-δ-guanidine valeric acid,

$$NH_2C(.NH) \cdot NH \cdot CH_2CH_2CH_2CHNH_2COOH$$

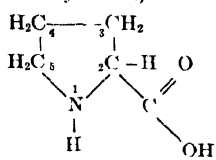
(17) Lysine, α ϵ-diamino caproic acid, $NH_2CH_2CH_2CH_2CH_2CHNH_2COOH$

Acids containing heterocyclic ring structures *

(18) Histidine, β-imidazole-α-amino propionic acid,

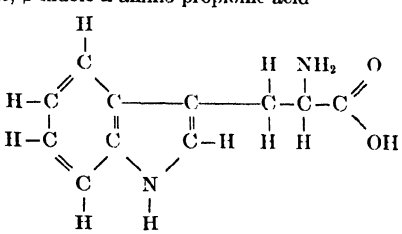


(19) Proline, 2-pyrrolidine carboxylic acid,



(20) Hydroxproline, same as above with one hydroxyl group substituted for one hydrogen atom in the fourth position

(21) Tryptophane, β-indole-α-amino propionic acid

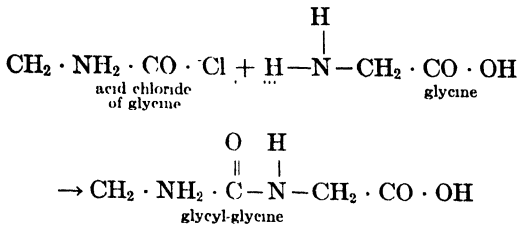


(22) A recently discovered sulfur containing amino acid, $C_5H_{11}NSO_2$, of unknown structure.

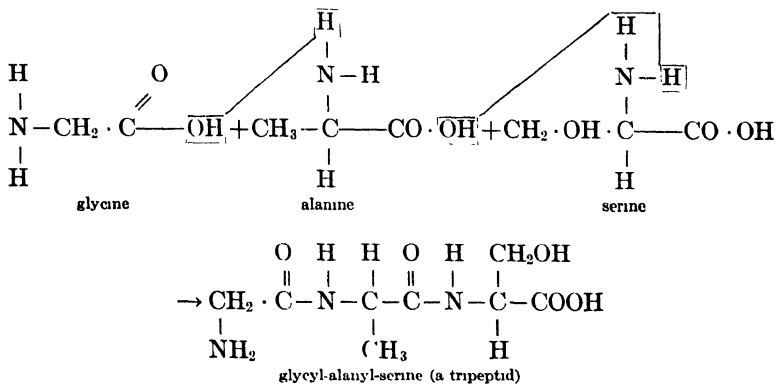
Formation of Peptids from Amino Acids. — When the amino groups of amino acids are protected by benzoyl or other radicals, it is possible to form acid chlorides. By the formation of esters of amino acids and their subsequent reaction with acid chlorides of the amino acids, it has been possible to form derivatives of the polypeptids, which, after treatment with ammonia, followed by hydrolysis, yield polypeptids.

* Ring structures containing more than one kind of atom in the ring.

Although the following equations do not actually represent the complex steps in laboratory procedure, they will show the reader, in a systematic way, how the amino acids are thought to combine during peptid synthesis:



Attention is called to the fact that the $-\overset{\overset{O}{\|}}{C}-\overset{\overset{H}{|}}{N}-$ linkage is characteristic of these compounds and it is undoubtedly this amide type of linkage that makes the protein molecule possible. When three amino acids combine, the compound is known as a tripeptid, an example of which is glycyl-alanyl-serine:

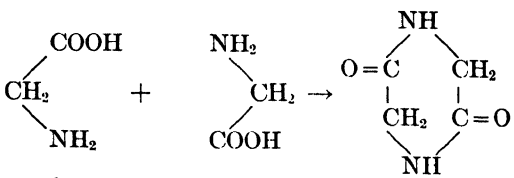


By suitable chemical procedure it is possible to cause this tripeptid to unite with another tripeptid to form a hexapeptid. Although the $-CO-NH-$ linkage is undoubtedly the most important one, there is ample proof that amino acids may react and combine in other ways. Artificial polypeptids containing as many as fifteen glycine and three leucine molecules have been prepared in the laboratory. These synthetic polypeptids exhibit many of the reactions which are characteristic of natural polypeptids.

Protein Structure

After the CO · NH linkage had been established by F. Hofmeister and E. Fischer, it was natural that theories should arise relative to the part this linkage might play in protein structure. As a result, the first theory advanced was the straight-chain theory which we have just described. This hypothesis has been very useful, and through its application many valuable researches have been prosecuted. E. Fischer was able, as we indicated in a previous paragraph, to cause eighteen amino acids to unite to form a complex polypeptid, and Abderhalden has succeeded in causing nineteen amino acids to combine to form these protein-like compounds.

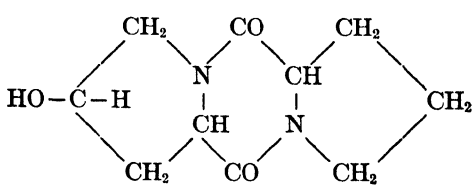
It was not long, however, before evidence began to accumulate to show that cyclic groups could be isolated from proteins, and it is now quite well established that the amino acids may form anhydrides through the formation (and loss) of water by the interaction of carboxyl and amino groups. For example, two molecules of glycine (amino acetic acid) may react to form a di-keto-piperazine:



glycine glycine 2, 5-dioxopiperazine

Likewise, other amino acids may form such anhydrides, and it is possible for such structures to be formed in which the amino acids may be different, i.e., an amino acid may form an anhydride with itself and with each of the other amino acids. The reader can begin to appreciate the mathematical possibilities involved, and it is not difficult to understand how nature can make so many proteins from so few amino acids.

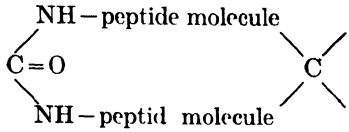
Not only can protein linkages of the CO · NH type occur, but it is quite well established that anhydride formation can occur where three or more amino acids unite. Dakin has isolated hydroxyprolyl-proline from gelatin which is of interest because it contains three rings in the molecule:



Hydroxyprolyl-proline

Dakin and some European workers are also of the opinion that the pyrrol ring may be a fundamental unit in protein structure.

Brigl and Held argued that the peptid and the di-keto-piperazine theories were at fault, since they would yield a proportion of oxygen to nitrogen that did not actually exist in natural protein. To overcome this objection, these authors introduced the " ureide " theory of protein structure, which postulates that polypeptid chains of varying length may be linked together by carbonyl groups:

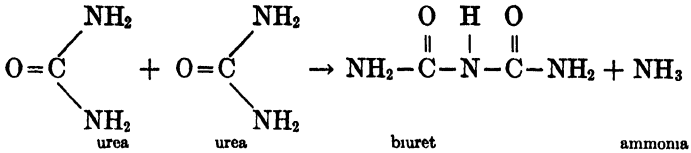


In a book of this type it is impossible to go into further detail regarding protein structure, but we have mentioned a few of the prevailing theories to show the reader that no single theory is completely satisfactory and that further research is necessary before the problems of this interesting and baffling phase of organic chemistry can be solved. When this is accomplished much will be learned regarding the biochemical and biological importance of proteins and their disintegration products.

Color Reactions of the Proteins. — Now that we have discussed the chemistry of the amino acids, we are in a position to discuss the so-called " protein tests." The proteins and their hydrolytic products undergo color reactions, with certain chemical reagents, which have become useful in identifying protein in food products In reality, however, the color tests are not " protein tests " but are characteristic of certain chemical groups or linkages which are often found in proteins. A few of the more common tests are given below to show the chemical principles upon which these tests are based

The Biuret Reaction. — When protein solutions are made alkaline with sodium or potassium hydroxide and a drop or two of a solution of copper sulfate added, a violet coloration ensues which is known as the " biuret test." This has become a more or less specific test for protein and its cleavage products, since it gives this color only in the presence of CO—NH linkages. Other compounds than proteins give this test, and *biuret*, from which the test gets its name, is a good example.

When two molecules of urea are condensed, and ammonia is lost, a substance called " biuret " (biurea) is formed. This compound, as well as a number of other non-protein compounds, exhibits the violet color with copper sulfate and alkali:



The Millon Test. — When Millon's reagent (consisting of a mixture of mercuric nitrate and nitrite in nitric acid) is allowed to react with protein, a red compound is formed. The reagent forms these colored compounds only in the presence of monohydroxyphenyl groups ($-C_6H_4OH$) such as we find in the amino acid tyrosine. Any protein, therefore, like gelatin, which does not contain tyrosine, will not respond to this test, but non-proteins such as phenol, C_6H_5OH, or salicylic acid, $C_6H_4OH \cdot COOH$, will cause the reaction described. It will be seen that this test is not specific for proteins.

The Xanthoproteic Test. — This is another common test which is characterized by a yellow color when protein solutions or suspensions are heated with dilute nitric acid and neutralized with ammonium hydroxide. The color thus formed is due to the nitration of the benzene nuclei in the protein molecule. There are three amino acids which contain these benzene nuclei, i.e., tyrosine, phenyl alanine and tryptophane. The colored compounds formed are undoubtedly similar to picric acid, $C_6H_2(NO_3)_3OH$, and its salts.

There are a number of other types of organic compounds such as the purines, nucleic acids and certain aromatic compounds that could be discussed with profit at this point. We have discussed the most important type-compounds, however, and when occasion requires we will discuss the others in later chapters.

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CHAPTER VI

THE PHYSICAL STATE OF MATTER

In the preceding chapters we have considered the chemistry of living matter, emphasizing the *chemical properties* of the various types of compounds and stressing the *chemical composition* of plant and animal materials.

In this chapter we shall consider the more important *physical* and *physico-chemical properties* of protoplasm and its various constituents. In the elementary science courses we have studied the various physical laws, and possibly many of us were not backward in expressing our opinions relative to the time and energy required in the study of laws which we thought had nothing to do with the solution of agricultural problems. We find, however, that we can make excellent use of all these laws when we begin to study the chemistry and physics of respiration and all the problems that this interesting subject suggests. The solubility of gases in body fluids, the effect of salts on the solubility of gases, the effect of temperature and pressure on respiration and gas absorption — are vital questions that can be answered only in terms of physics, physical chemistry and mathematics.

When we begin to study the various body fluids and tissues we are impressed with the relatively large number of variables that regulate our physiological processes and that control that mysterious phenomenon we call life For example, it is impossible to go far in the study of the cell and protoplasmic activity without coming to the realization that the physical sciences are of great importance if we wish really to get at the facts, and we are forced to consider, therefore, atomic structure, molecular structure, dissociation, electrical charges, conductivity, surface tension, adsorption, hydrogen-ion concentration, permeability of membranes, the colloidal state and a host of interesting phenomena that we can scarcely more than touch upon in a textbook of this type. The writers feel that their labors will not have been in vain if they are able to convince the student that vital, interesting and understandable relationships exist between agriculture and biology, on the one hand, and the so-called pure sciences, on the other. It would appear to be axiomatic that, the better we understand the fundamental laws of chemistry and physics, the better we shall appreciate, understand and control the biological changes going on within us and around us.

The modern conception of the atom has modified many of our ideas with reference to the deportment of chemical substances. We now conceive the atom to consist of a central nucleus which is positively charged and which possesses an infinitely greater mass than the negatively charged electrons which surround it. According to this conception, the electrons revolve in much the same way as planets revolve about the sun. Some of these electrons revolve in a fixed sphere or shell not far from the nucleus, but they are not sufficient in number to neutralize the positively charged nucleus. These are called *nuclear electrons*. Other electrons, known as *planetary electrons*, revolve about the nucleus but at a greater distance than those just described. It is the outer layer of these planetary electrons, known as *valence electrons*, which possess the " balance of power " so far as neutralizing the positive nucleus is concerned, and it is these, chiefly, that may be gained or lost during chemical reactions.

In the case of hydrogen, it is thought that the hydrogen atom consists of a nucleus or *proton* to which is attached one planetary electron. Helium is thought to consist, essentially, of four hydrogen atoms which act as the helium nucleus or proton, and rotating about this nucleus are two planetary electrons. Nitrogen is thought to yield three helium atoms and two hydrogen atoms. In other words, the conviction is growing that all the elements have evoluted by the condensation of hydrogen atoms. It is thought that some of the higher elements can be " bombarded " or undergo radioactive disintegration in such a manner that they may lose hydrogen atoms, resulting in the formation of the adjoining element in the periodic table.

The foregoing is known as the *physicist's atom*, and the electrons are conceived to be rotating continuously at an enormous speed. The chemist has difficulty in applying this conception to the union of atoms to form molecules. As a result of the study of crystals by the use of the X-ray, the chemist has modified the physicist's atom, arguing that the rotating electrons may be vibrating in such a manner that their average positions cause them to assume positions at the corners of imaginary cubes. The valence electrons may vary from one (in hydrogen) to eight (in neon). When an element contains eight valence electrons the corners of the cube are saturated. Since the electrons are generally arranged in octets; new cubes are started as soon as the corners of the old cubes are filled. It is postulated that the proton or nucleus occupies the center of the cube. When sodium (which contains one valence electron) unites with chlorine (which contains seven valence electrons) stabilization occurs by the acceptance of an electron by chlorine (from the sodium atom), thereby forming a stable composite structure consisting of cubes, the corners of which are saturated.

Since salts in solution are said to ionize and conduct an electrical current, the proponents of the atomic structure theory, just described, explain the ionization of a salt, such as sodium chloride, by stating that water disrupts the cubical structure, liberating the positively charged sodium and the negatively charged chlorine in active ionic form.

Some Properties of Solutions

Dissociation. — Aqueous solutions may be divided into two classes, viz., those which conduct the electrical current and those which do not. If two wires connected with an electric lighting circuit and with an electric lamp are placed in pure water the lamp does not light. If a small amount of sodium chloride is dissolved, at this point, the lamp begins to glow, showing that the current is being conducted by the ions formed. Solutions of cane sugar, alcohol or glycerol will not cause the lamp to light. We say that sodium chloride is an *electrolyte* and that alcohol is a *non-electrolyte* for the reasons just stated. In general, salts, acids and alkalies are electrolytes. The electrolytes are said to dissociate into positively and negatively charged particles or ions which cause the electrical current to flow. From a biological standpoint this ionic conception is of the utmost importance, since many biological phenomena are explained in the light of this theory. Many substances, although soluble in water, are weakly ionized but other substances are very highly ionized. In general, it may be said that the reaction of a substance in solution is related to the amount of the substance in ionic form. The weak acids and alkalies are slightly ionized; the strong acids, alkalies and most salts possess a high degree of ionization. Water will ionize, of itself, into hydrogen and hydroxyl ions, but the number of ions in proportion to molecular water is very small. Water, therefore, is a very poor conductor of electricity and for this reason becomes an excellent insulator, unless it contains dissolved electrolytes

Surface Tension. — Another property, of biological importance, possessed by liquids is that of surface tension. In a general way we might define surface tension as the resistance of a surface film of liquid to rupture. Expressing it another way, we may say that an attraction exists between the molecules of a liquid by which an internal pressure is produced. This is shown when a liquid is suspended in another liquid of the same density and the former assumes the form of spherical globules, a free surface being formed and the globule tending to form the smallest possible surface. We may imagine, therefore, that the surface layer of a liquid acts as an elastic film or skin which, like rubber, tends to contract and become smaller. This force which acts to decrease the surface may

exist at the interface between gases and liquids, between gases and solids, between liquids and solids, or between two immiscible liquids. When liquid-vapor interfaces are considered, the term *surface tension* is used; the term *interfacial tension* refers to those types represented by liquid-liquid or solid-liquid interfaces.

Capillary forces are due to surface tension but the reader must remember that water or other liquids will not ascend capillary tubes unless the tubes are wetted by the liquid under consideration. For example, a glass is wetted by water but it is not wetted by mercury. As a result water ascends a capillary tube while mercury does not. The forces exerted by capillarity are of great practical importance in nature — the most common example being the movement of soil water, by wetting of soil particles, resulting in the manifestation of interfacial tension by capillarity. This is due, of course, to the tendency for surface decrease which is a spontaneous reaction. When ordinary soaps are dissolved in water, the interfacial tension of the latter is lowered. If an oil is present the soap tends to decrease the interfacial tension of the water, thereby forming droplets of oil in the water.

Free acids and bases, particularly organic acids and bases, usually decrease the surface tension of water. Electrolytes, as a rule, have little effect. Non-electrolytes such as aldehydes, monohydroxy alcohols, ketones and esters of the organic acids tend to lower the surface tension of water. Bile salts, lecithin, starch and glycogen also possess a lowering effect. As a result, we might expect that physiological solutions, such as milk, urine, blood serum and bile, would have a lower surface tension than that possessed by pure water, and such has been found to be the case.

Those substances which tend to lower surface tension become concentrated in the surface layers (positive adsorption); those which tend to raise surface tension are found in greater concentration in the body of the solution. When egg albumin lowers the surface tension of water, it also increases its viscosity. Concentration of the albumin in the surface layer leads to the development of a film. If air is passed through the mixture the formation of a froth or foam is brought about through the formation of viscous films. Foaming or frothing may be said to be encouraged by increased viscosity, and any substance which will lower viscosity will discourage the formation of foam. In other words, any substance which lowers viscosity and increases surface tension will discourage the formation of foam. Since proteins, cholesterol and lecithin are capable of lowering surface tension and since these substances are found in all protoplasm, it is natural to expect that we should find proteins and these lipids concentrated at the surface. Modern biology

teaches us that this is undoubtedly the mechanism of cell membrane formation.

Osmosis. — Another property of solutions which plays a most important part in all living tissues is that of osmosis and the creation of osmotic pressure. In our study of general chemistry we learned that, when two gases are brought in contact, they diffuse, one into the other, by reason of molecular motion, tending eventually to come to equilibrium in a uniform mixture. The difference in partial pressures of the two gases produces a force which is measurable.

In solutions we observe a similar phenomenon when two aqueous mixtures of different concentration are brought together. Owing to greater friction between the molecules of the solute and the solvent the diffusion will progress with less rapidity, but eventually the solutions tend to come to an equilibrium point where the mixture is uniform throughout.

If a semi-permeable membrane, such as parchment paper, is introduced between the two liquids, a pressure (osmotic pressure) is set up. This pressure is explained by the fact that the membrane is more permeable to certain molecules and less permeable to others. For example, if pure water is on one side of the membrane and a solution of sucrose is on the other, the water molecules will pass through faster than the sugar molecules. This does not continue indefinitely, however. After a time the pressure reaches a maximum and starts to decrease, because of the reduction of the differences in concentration on the two sides of the membrane, due to the diffusion of the solute molecules.

When we consider the osmotic pressure produced by pure salts in solution, we find some very interesting relationships. In our early science courses we were taught that Avogadro's law states that the molecular weight of any pure gas will always occupy about 22.4 liters of space at standard conditions of temperature and pressure. We learned, also, that Boyle's law states that the volume of a gas varies inversely with the pressure exerted upon it. The law of Charles states that, at constant pressures, gas volumes vary in proportion to the variations in (absolute) temperature. In other words, we know that there are definite mathematical laws which show definite relationships between molecular weight, volume, temperature and pressure of gases.

When we study dilute solutions we find that a similar situation exists. In fact, we find that, within certain limits, the dilute solutions obey the gas laws. We found in our study of organic chemistry that it was possible to determine the molecular weights of unknown gases by the application of the gas laws and by using the Victor Meyer vapor density method. We were told that solids which could not be volatilized could be dissolved in a known quantity of any solvent (usually water), and that

the molecular weight of any soluble solid could be determined by measuring the rise in boiling point or the depression in freezing point. We learned also that the boiling-point rise and the freezing-point depression were due to the number of particles of the solute present per unit volume of solvent. In cases of non-electrolytes the direct determination of molecular weight is possible by these methods. In the case of electrolytes, the results are always higher than they should be, owing to the fact that ions exert just as much effect as molecules. Consequently, we must know the degree of ionization of an electrolyte if we desire an accurate calculation of molecular weight.

When we study the behavior of electrolytes and non-electrolytes in solution on diffusion pressure or osmotic pressure, we find that they obey the same general laws. We can find definite relationships between osmotic pressure, temperature, volume, concentration and molecular weight. Ionization also plays a rôle in osmosis, the number of ions influencing the pressure obtained. Although these laws do not apply to concentrated solutions, they do apply with considerable accuracy to dilute solutions which are tenth-molar, or less, in concentration. Since all biological fluids are dilute solutions, we find that all these laws become of immediate use to the biochemist and biologist.

It is due, in part, to osmotic pressure that the protoplasmic contents of plant cells swell and press against the cellulose walls, causing a certain tenseness of the plant tissues, known as " turgor." Turgor is responsible for keeping many plants stiff and upright; when turgor is lost, wilting occurs.

Theories of Membrane Action

Many theories have been advanced relative to the mechanics of membrane permeability. It has been known that certain simple compounds pass through most membranes quite readily, while the more complex compounds, with high molecular weights, do so with difficulty and some of them are incapable of diffusion.

1. The sieve theory of membrane action pictures the membranes as porous partitions, separating liquids. If the size of the molecule is sufficiently small to pass the pores of the membrane, it is said to be permeable to the substance. If the molecule is unusually large or if aggregates of molecules clump together to form large masses (colloids), the pores are not large enough and the substances cannot diffuse and the membrane is said to be impermeable.

2. The membrane solution theory is another attempt to explain the semi-permeable nature of parchment paper and similar membranes. This theory assumes that only those substances can pass through a mem-

brane that are soluble in it. Those which cannot dissolve in the membrane cannot pass through. This would allow membranes to show *preferential* or *selective permeability* toward chemical substances. For example, when rubber is used as a membrane separating pyridine and water, no water passes to the pyridine but the pyridine readily diffuses to the water. The theory explains this phenomenon by pointing to the fact that pyridine dissolves in the rubber, whereas the water does not.

3. The capillary theory might be the name given to a third explanation of membrane action. Supporters of this theory prefer to picture semipermeable membranes as if they consisted of thousands of fine capillary tubes. These tubes are supposed to be so small that large capillary forces are possible. In some respects, this theory does not differ appreciably from the *porosity* or *sieve* theory. It is entirely possible that all these theories are correct within certain limits, and that differences in surface tension between solvent and membrane also play a most important rôle in the process of diffusion.

Properties of Fluids

We have learned that many chemical substances dissociate to form ions and that acids are characterized by excess hydrogen ions and bases by excess hydroxyl ions. The so-called "strength" of acids and bases is proportional to the amount or concentration of hydrogen or hydroxy ions in solution. For example, 25 milliliters (cc.) of $N/1$ NaOH will exactly neutralize 25 milliliters of $N/1$ acetic or hydrochloric acid and yet this volume of the latter acid contains almost 100 times as many hydrogen ions as the former. Acetic acid is known as a "weak acid" whereas hydrochloric acid is said to be "strong," owing to the high concentration of hydrogen ions.

When hydrogen ions predominate over hydroxyl ions the solution is *acid*; when hydroxyl ions predominate, the solution is *alkaline*; and when the hydrogen ions exactly equal the hydroxyl ions the solution is *neutral*. Even pure water, as we have pointed out in a previous paragraph, dissociates into ions. Its power of dissociation is extremely low, however, and the number of hydroxyl and hydrogen ions are equal, which explains why pure water is neutral in reaction.

Molecules, which ionize, are continuously forming ions, and ions are, in turn, forming molecules again. In other words, the reaction is a reversible one in which nature strives, eventually, to arrive at a point of equilibrium. The *law of mass action* states that, in reversible reactions, the ratio of the product of the concentrations of the reacting substances (raised to the proper powers), on one side of the equation, to the product of the substances on the other side, is a constant.

In the case of water this law can be expressed by $\frac{[H^+]\,[OH^-]}{[H_2O]} = K$, where $[H^+]$ represents the concentration of hydrogen ions, $[OH^-]$ the concentration of hydroxyl ions, and $[H_2O]$ the concentration of undissociated water molecules. Since the latter is a constant, the expression becomes $[H^+] \times [OH^-] = K_w$, which means that the product of the concentrations of the H and OH ions is a constant. The value of K_w has been found to be 1×10^{-14}. The concentration of either ion, therefore, would be $\sqrt{1 \times 10^{-14}} = 1 \times 10^{-7}$ N. Expressed fractionally, this means that water at 24° C. is 1/10,000,000 normal with respect to H^+ and OH^- ions, and that 1000 milliliters of water contain 1/10,000,000 equivalents of hydrogen or hydroxyl ions. This means, also, that a neutral solution possesses 1×10^{-7} mole or gram equivalent weight of hydrogen and hydroxyl ions. Hydrogen-ion concentration is often expressed "C_H," which is an abbreviation for the expression "hydrogen-ion concentration." The expression $[H^+] \times [OH^-] = 1 \times 10^{-14}$ holds for dilute aqueous solutions. Hence it follows that if the OH^- ion concentration is 10^{-6} normal (alkaline), the H^+ ion concentrations will be 10^{-8} normal. This is the fundamental reason for expressing alkalinity in terms of hydrogen-ion concentration

TABLE 13

ACIDITY AND ALKALINITY OF SOLUTIONS

C_H or $[H^+]$	pH		pOH	C_H or $[OH^-]$
$N/10$	1	acidity	13	
$N/1000$	3		11	
$N/1{,}000{,}000$	6		8	
$N/10{,}000{,}000$	7	neutrality	7	$N/10{,}000{,}000$
	8	alkalinity	6	$N/1{,}000{,}000$
	11		3	$N/1000$
	13		1	$N/10$

The reader will come in contact with another expression which is used in most scientific periodicals which expresses the same idea in a different and more convenient way. This expression is "pH" and is the nega-

tive logarithm of the C_H. In other words, water, which has a C_H of 1×10^{-7}, has a *p*H of 7, since -7 is the logarithm of the above number. This means that all neutral solutions must possess a *p*H of 7. As the *p*H increases, solutions are increasingly alkaline in reaction; *p*H readings less than 7 indicate increasing acidity. Similar calculations can be made for *p*OH but, since *p*H really expresses the same information, it has become the custom to express either hydrogen- or hydroxyl-ion concentration in terms of *p*H, since $p\text{H} + p\text{OH} = 14$.

The preceding table will give some idea of the relationship of C_H to *p*H.

Buffer Substances. — In living organisms, acids and alkalies are continuously being liberated. It is found that very slight changes in hydrogen-ion concentration in the animal body are harmful. One writer has put this idea into simple, understandable terms by stating that death will ensue if the blood should change in hydrogen-ion concentration as much as tap water differs from distilled water. In other words, the animal organism must keep a very delicate balance between hydrogen- and hydroxyl-ion concentration When the matter is studied by delicate chemical methods it is found that blood and the body fluids always show a *p*H of about 7, which is the neutral point so far as acidity and alkalinity are concerned.

How can the organism neutralize the excess of acids and alkalies in the body and keep the body fluids so near the neutral point? The answer is that the body contains *buffer substances* which can absorb relatively large amounts of acids and alkalies without undergoing much change in acidity or alkalinity. Such buffers are the phosphates, the proteins, the carbonates and the bicarbonates To make this point more clear, let us take some concrete examples Ordinary di-sodium phosphate, Na_2HPO_4, which is nearly neutral in reaction, may react with hydrochloric acid, according to the following equation, forming mono-sodium phosphate and sodium chloride, both of which are also practically neutral:

$$Na_2HPO_4 + HCl \rightleftarrows NaH_2PO_4 + NaCl$$

In other words, fluids containing the one phosphate may receive acids, and in a similar manner fluids containing the other phosphate may receive alkalies, without appreciable changes in acidity or alkalinity.

Proteins are thought to act in a similar manner, combining with alkalies and acids without appreciable changes in reaction. The presence of carbonates and bicarbonates in fluids tends to neutralize strong acids and decrease the ionization of carbonic acid as it is formed in the body, with the result that acidity is not allowed to increase as a result of CO_2 production.

The Colloidal State

We have learned in previous chapters that chemical compounds of biological and agricultural importance can be arranged in classes or groups according to their physical and chemical properties. Before it is possible to discuss these compounds from the standpoint of their physiological behavior in the cells or tissues, we must know more concerning the environment in which they exist and the state or condition in which the compounds are apt to occur. It is, therefore, both advisable and necessary to dwell, at this point, upon the *colloidal condition* or *state* in which matter is found, in order that we may better understand the discussions which are to follow

Thomas Graham is given credit for giving impetus to this particular field of physico-chemical research. Between the years 1861 and 1864, Thomas Graham published a number of papers giving the results of his studies on the diffusion of certain substances through membranes. Some of these substances passed through membranes readily; others either diffused at a very slow rate or failed to do so entirely. Generally speaking, those that diffused readily were capable of crystallization, whereas those that did not were substances like glue, gelatin and the gums, always without form or amorphous in the solid state. To the first class he gave the name "crystalloids," and to the other the name "colloids" (from *kolla*, meaning glue). The reader can more readily understand this when the following experiment is described. Common table salt and starch paste are placed in water in a parchment bag and the bag is suspended in distilled water. Chemical tests show that the salt passes through into the distilled water readily, whereas the colloidal starch remains in the paper bag. We say that the starch cannot pass through the membrane owing to its colloidal condition.

Graham continued his studies and found that colloids could be divided into two classes: (1) *sols* or *hydrosols*, which are capable of forming apparently homogeneous solutions; and (2) *gels* or *hydrogels*, which are semi-solids. He found that, by varying the conditions, sols could be converted to gels and gels could be changed to sols. Subsequent studies led to very interesting and important results in this field, in which new terms were coined and new viewpoints created. The word "colloid" is now used as a noun in the same manner that the terms "gas," "liquid," and "solid" are used. The word colloid refers to the state or condition in which a chemical substance may exist. We learned in our courses in elementary inorganic chemistry that chemical substances were capable of existing in *true* solution in molecular and ionized form. Those particles which were not visible under microscopes of highest power passed readily through filter paper and parchment paper, manifested osmotic

pressure, did not deflect light rays when passed through their solutions and were said to be in molecular motion.

Colloidal substances, however, may occur in (colloidal) solution or as suspensions, and, when in this state, chemical substances do not exhibit properties of true solutions. For example, the molecules may form aggregates of such size that light does not pass directly through their solutions or suspensions but is deflected or reflected by the particles in the liquid. Colloidal solutions pass through filter paper but do not pass through parchment paper, and the particles of suspended solids are of such size that they will not pass through filter paper. The view is held, therefore, that most colloidal solutions are homogeneous solutions which contain substances in finely divided condition, each particle being considered an aggregate of many molecules. We should not leave the impression, however, that all colloidal particles are made up of molecular aggregates. In the case of albumin, the evidence seems to indicate that this protein may exist in colloidal solution in molecular form. In other words, the molecule may be sufficiently large to bring it into the colloidal realm without the necessity of postulating an aggregation of molecules. Recent work at the New York Experiment Station indicates that colloidal casein may also exist in molecular form. When the molecular aggregates increase in size they eventually become so large that they form *mechanical suspensions*, which settle out readily. *Colloidal particles* do not do this but may remain suspended for long periods. It may be seen from this brief description that no sharp line can be drawn between the different states of chemical substances.

To give the reader some idea of the relative size of colloidal particles, it may be said that we may classify dispersed matter into three arbitrary divisions, i.e., (1) molecular and ionic, (2) colloidal, and (3) what might be termed gross dispersion. This arbitrary division is based on the relative size of the particles. Those particles which are less than 1 millimicron in diameter (1/1,000,000 millimeter) are said to be molecular in size. Such particles are not visible in the ultra microscope. Particles which are greater than 1 millimicron in size but less than 1/10 micron are said to be colloidal. These particles are too small to be seen in the high-power microscope and yet are sufficiently large to be visible in the ultra microscope. All particles which exceed 1/10 micron (1/10,000 millimeter) are visible under high-power microscopes and are of sufficient size to be considered as possessing size or "mass." The term "mass" is used here in the layman's sense. The scientist considers that everything has mass, even the tiny electron.

Some Properties of Colloids. — Nearly every one has seen dust particles floating in the air when sunlight is allowed to penetrate a darkened

room through a crack or aperture in the wall. The light strikes these dust particles in such a manner that some of the light is reflected from the dust particles to the eye, making the dust particles appear as bright specks in the air.

A similar phenomenon is noted when a ray of light is passed laterally through suspensions of colloidal materials in water. If water alone is used, the light-beam passes through unhampered; but if the water contains a colloidal suspension of clay or similar material the light is usually reflected by the colloidal particles, producing a cone-shaped brightened area in the solution known as a " Tyndall cone." The phenomenon is called the " Tyndall effect." The reader will understand, of course, that the Tyndall effect will not be visible unless the refractive indexes of particle and liquid are different. If they do not differ, no Tyndall effect can be noted.

There are at least two phases to a colloidal system. The colloidal material, which is suspended or dispersed, is known as the *disperse phase.* These particles are discontinuous and are distributed throughout another medium known as the *continuous phase* or *dispersion medium.* In the case of a colloidal clay suspended in water, the clay particles are called the *disperse phase* and the water is known as the *continuous phase.*

It should be emphasized at this point that it is not necessary to visualize solids in liquids as the only type of colloidal system, for it is possible to have colloidal systems where solids are suspended in solids (colors in precious stones); solids in gases (smoke hazes); liquids in solids (opal); liquids in gases (fogs); and liquids in liquids (such as finely dispersed oil emulsions). A suspended solid is often referred to as a *suspensoid*; a hydrated dispersed particle, such as is found in emulsions, is called an *emulsoid.* Such colloidal systems may be studied by means of the *ultra* microscope, an instrument which is so constructed that a beam of light is allowed to pass through the colloidal solution at right angles to the axis of the microscope. No light can reach the eye of the observer except that which is reflected or refracted by the colloidal particles.

When true solutions of the so-called crystalloids like salts or sugars are separated from water by a semi-permeable membrane, a pressure is observed, known as *osmotic pressure.* When colloidal solutions are studied under the same conditions it is found that they do not diffuse, and, as a consequence, they exert little or no osmotic pressure, owing to the relatively large size of the colloidal particles as compared with molecules and ions.

When particles are large, they can be made smaller and more thoroughly dispersed in a number of ways, i.e., by mechanical grinding, by

chemical methods, or by electrical methods. This process, by which particles are dispersed in a very fine state of division, is known as *peptization.* For example, gold wire may be *peptized* (vaporized) by forming an electrical arc between gold wires under the surface of water. A portion of the gold from the anode vaporizes and, failing to reach the cathode, disperses in the water, forming beautiful colored suspensions of colloidal gold, which remain suspended for long periods. In reality, it is the gaseous phase that coagulates to form colloidal gold particles. When coagulated egg albumin is treated with pepsin (enzyme) in dilute hydrochloric acid, a portion of the egg white forms a fine milky colloidal suspension. We say that the large aggregates have *peptized* or have been made truly colloidal. The origin of this term is due to Sir Thomas Graham, who called the above change " peptonization " (formation of soluble peptones), which has been shortened to " peptization " and adopted to describe this phenomenon.

Conversely, a peptized colloid may be treated so that the tiny particles form larger and larger aggregates and the colloid passes from the peptized *sol* form to that of a semi-solid, known as a *gel.* This process is known as *condensation* and is the reverse of peptization. *Coagulation* of colloids is also condensation and may be brought about by heat, certain enzymes and certain chemical reagents. A gel may be formed also by hydration of the substance. For example, when gelatin passes from a semi-liquid state to the semi-rigid form that we call " gel," it is thought that water molecules are added in large numbers (hydration or imbibition), forming a large water shell about the gelatin particles.

All colloids possess an electrical charge which may be either positive or negative. When colloidal suspensions are subjected to the action of direct electric current, the colloidal particles migrate slowly to cathode or anode, depending upon the type of charge they bear. Some colloids bear greater electrical charges than others and, therefore, migrate more rapidly. This speed of migration can be measured by the use of a specially constructed cell and a microscope. This phenomenon is known as *electrophoresis* or *cataphoresis.* Recent work on the electrophoresis of bacteria by Falk, Lisse, Tittsler and others indicates that there are many interesting biological relationships between quantity of electrical charge and the biological deportment of the organism.

When a colloidal membrane is arranged so that a liquid is forced through it under the influence of an electrical current, the process is called *electro-endosmosis.* Like all colloids, such membranes are charged with electricity, and usually this charge is negative in nature. The water always migrates in the direction of the pole which carries the same charge as the membrane. Hydrogen-ion concentration may determine the

magnitude of the charge, and there must be a difference in potential between the membrane and its medium, if electro-osmosis takes place. Other ions in the system also affect the magnitude of the charge. If the hydrogen-ion concentration is changed, there will be found, with most colloids, a certain hydrogen-ion concentration where the charge becomes zero. At this point, which is called the *iso-electric point*, no electro-osmosis can occur and, in the case of colloidal solutions, the particles no longer repel one another, consequently, they collect in aggregates and precipitate. As a result of the latter phenomenon, it is possible to coagulate or precipitate negatively charged colloids by neutralizing the negative charges by the addition of positively charged ions and vice versa. An excess of such ion-carrying reagent may reverse the charge and peptize the precipitate, forming a dispersed colloid again, which possesses an opposite charge. The iso-electric point of dispersed colloidal particles can be determined cataphoretically.

Certain colloids, such as gelatin, possess the property of becoming *hydrated*, i.e., they may imbibe large amounts of water to form gels. This process is known as *imbibition*. Sols may consist of substances which have imbibed relatively large amounts of water. These may be " salted out " with certain salts which dehydrate the colloids and may change the sign or magnitude of the charge. A water-loving colloid is known as a *hydrophilic colloid*; one that does not imbibe water is known as a *hydrophobic colloid*.

Some colloids, like gelatin, agar or gum arabic, imbibe considerable water. This is invariably followed by *swelling*, some substances swelling to 300 to 350 times their original volumes. This is a property of great biological value in plant and animal life and is of considerable importance in many industrial processes. Emulsoids may also imbibe an appreciable amount of the dispersion medium. Colloids increase not only in volume, when imbibition occurs, but also in weight. Gortner and his co-workers have shown that viscosity increases as imbibition occurs, and they have used viscosity measurements as an index of water absorption by colloids. These authors have used this method to detect the baking quality of wheat flours — a property which is dependent upon the nature and quality of the wheat proteins, with reference to their imbibitional capacity.

In a previous chapter we learned that those substances which decrease surface tension tend to concentrate in the surface layers; therefore, when this occurs, substances tend to concentrate in the surrounding film or adhere to the colloids, in the manner described; when this situation exists the substances are said to be *surface tension active* and are *adsorbed*. The process is known as *adsorption*.

Adsorption plays a most important rôle in biology and industry, for there is scarcely a phase of life that is not affected by the concentration of substances on colloid surfaces by the adsorption process. The decolorization of sugar solutions in industry by charcoal is a case in point, the enormous surface offered by the finely divided charcoal being capable of adsorbing large amounts of colored impurities. Adsorption is, therefore, a surface phenomenon which is probably physical and chemical in nature. To give the reader some idea of the enormous surfaces that finely divided colloidal particles may possess, let us take for example the red blood corpuscles, which are much larger than colloids and which possess much less surface area.

Assuming that there are five million red blood corpuscles in one cubic millimeter of blood and assuming the average diameter of a single corpuscle to be 0 007 millimeter in diameter, it is calculated that the red corpuscles in 5000 cubic centimeters of blood (about this amount for a man weighing 150 pounds) would offer a surface area for adsorption purposes of 18,750 square feet or nearly one-half of an acre. It is not difficult to understand, therefore, why certain colloidal materials are capable of adsorbing relatively large quantities of chemical substances. Colloids not only adsorb foreign materials but they also mutually adsorb one another, and for this reason it is almost impossible to purify chemical substances which have a tendency to exist in the colloidal state, on account of the adsorption of extraneous materials. For this reason, as we have pointed out elsewhere, it is very difficult to obtain proteins in sufficiently pure form to study them with the same accuracy that is possible in the case of easily crystallizable substances.

It has been found that colloids have the power, on account of their surface area, to hasten or accelerate chemical reactions. This catalytic property is common to many of the colloids but more especially to a class of substances known as *enzymes* which will be considered in the following chapter. The catalytic effect of finely divided substances is undoubtedly due to the enormous surface area exposed, which, by the process of adsorption, brings reactive materials into such close contact that reactions are stimulated.

In closing our very brief discussion of colloids we should mention a few of the outstanding properties of emulsions, which, as we have already inferred, are dispersions of minute droplets of liquids in liquids. Usually it is very difficult to make a stable emulsion with two immiscible liquids unless a third substance known as a *stabilizer* or *protective colloid* is added. For example, in the preparation of kerosene-water emulsions, used in spraying, it is impossible to keep the water and kerosene from separating unless a little soap is added. When this is done the kerosene droplets

remain dispersed in a stable emulsion for long periods of time. Likewise, gelatin, albumin and gum arabic are used in pharmaceutical and industrial practice to stabilize colloidal solutions of many types. Scientists are of the belief that good emulsifying agents are those colloids which tend to concentrate at the liquid-liquid interface by strong adsorption.

Most emulsions with which we are familiar consist of oil dispersed in water. Since the alkali soaps are soluble in water, they tend to form oil-in-water emulsions. On the other hand, the alkaline earth or heavy metal soaps are less soluble in water and are peptized by oil, which results in the formation of a rather unstable emulsion consisting of water dispersed in oil. Cream is an example of an oil-in-water emulsion; butter is an example of a water-in-oil emulsion, in which water is the disperse phase and fat the continuous phase.

In the following chapter we will consider some of the properties of enzymes, substances which are produced by living organisms and which have the property of stimulating or hastening certain types of chemical reactions.

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CHAPTER VII

ENZYMES

Two types of chemical change are taking place continuously in all living tissue. The first type is termed *anabolic* change and has to do with the formation of new complex tissue from simpler substances; the second is known as *catabolic* change and is characterized by the destruction of the complex tissue and the formation of simpler chemical units. The terms applied to the two processes which we have just mentioned are *anabolism* and *catabolism*, respectively; they include all chemical reactions of a synthetic or destructive nature. The term *metabolism* is applied to all chemical changes taking place in plant or animal tissue irrespective of their nature. Thus, metabolism includes both anabolic and catabolic changes.

Many of the chemical reactions to which we have just referred are very complex and little understood; this holds true, more especially, for the synthetic type It is possible to study the nature of certain destructive changes in the laboratory. For example, the chemist is able, by means of high temperatures and strong acids and alkalies, to cause complex biochemical substances to break down to their simpler units, and it is these units which have been studied to the greatest advantage. Some of the units derived from the decomposition of proteins are amino acids, water, ammonia and carbon dioxide; the fats have yielded glycerol, fatty acids, carbon dioxide and water. In the laboratory, the chemist is required to use very drastic measures to bring about these decompositions, whereas the plant and animal are able to bring about the most complex chemical reactions with comparative ease. What is it, we may ask, that stimulates these changes? If it is a substance, can it be isolated? What is its nature? These are questions which science has been asking for many years, and to some of her questions she has received but evasive answers. A study of biochemical changes, however, has brought out the fact that certain peculiar organic substances possess the property of accelerating chemical reactions in a manner similar to that of the inorganic catalysts. The name *enzyme* or *ferment* has been given to this class of substances, the name enzyme being derived from a Greek word meaning " in yeast," for the yeast enzyme, which brings about alcoholic fermentation, was among the first enzymes studied.

For many centuries, fermentive changes have been a matter of common observation. Man has observed that, during fermentation, gases are liberated and generally make themselves manifest by the formation of bubbles. Thus, the word fermentation was derived from the Latin word, *fervere* (to boil). The substances causing these changes became known as *ferments* and later as enzymes.

The older classification speaks of *organized* and *unorganized ferments*. Organized ferments were thought to be capable of reproducing themselves whereas the unorganized ferments were considered to be nonliving. This idea was more or less prevalent until the year 1897, when the epoch-marking publications of Buchner established some new and interesting facts. Büchner ground the cells of the yeast plant with sand, treated this mixture with water, and filtered the mass. He found that the clear filtrate or liquid obtained was capable of producing fermentive changes in sugar solutions just as efficiently as the original yeast cells. This was contradictory to the ideas prevailing at that time, for the presence of the cell itself was thought to be necessary for ferment action. Other investigators found that enzymatic changes may occur that are not accompanied by the production of gas. For example, fats may be split into two simpler compounds (glycerol and fatty acids), and the higher carbohydrates may be hydrolyzed to the simple sugars, such as glucose and fructose.

An enzyme has been defined as a catalyst, elaborated by plant and animal cells, which, under definite conditions, facilitates chemical changes without undergoing chemical change or entering into the composition of the final products. The substance upon which the enzyme acts is termed the *substrate*. For many years it was thought that enzymes probably do not initiate chemical changes, but merely facilitate changes already in operation. Some workers believe, however, that enzymes differ from inorganic catalysts in that they may, under proper conditions, actually initiate chemical reactions. The latter view is growing in scientific favor.

Up to the present time, little authentic scientific information is available concerning the chemical nature of enzymes. The principal reason for our lack of knowledge in regard to the chemistry of the enzyme molecule is the fact that enzymes exist in the colloidal state and, consequently, are difficult of purification. We cannot name or classify enzymes, therefore, on the basis of chemical composition, with the result that we are forced to do the next best thing, i.e., to name and classify them according to the types of chemical change with which they are associated or the chemical substance (substrate) upon which they act.

The nomenclature generally followed at the present time is that sug-

PART II. THE PLANT

CHAPTER VIII

SEED GERMINATION

The seed is an organized structure which contains a miniature living plant, the *embryo*. The tissues surrounding the embryo contain, in a more or less unavailable form, all the materials necessary for its existence. Having no power to synthesize its own food and possessing but limited absorptive capacity, the embryo must depend for its nourishment upon the food materials stored within the seed coat. When the seed is placed in the proper environment, the embryo becomes, eventually, a more highly developed structure, which begins to assume some of the characteristics of the parent plant. This new organism is known as a *seedling*, and the process involved in the formation of the seedling is designated as the *germination process*. This process is of fundamental importance both from the standpoint of the student and of the agriculturist.

The Chemical Composition of Seeds. — A knowledge of the chemical composition of seeds is essential to a thorough understanding of the process of germination. Fortunately, a considerable amount of such data is now available. It has been shown that there are always present variable quantities of those elements and compounds which make up the real living matter of both plant and animal organisms — namely, protoplasm — the composition of which has already been discussed.

Organic compounds, such as fats, proteins and carbohydrates, predominate in seeds. Upon removal of these and other related compounds by ignition, there remains behind a not inconsiderable quantity of inorganic material or ash. This ash is found to contain a number of basic elements which are present as phosphates, sulfates, carbonates, and in some cases as chlorides. It must not be assumed, however, that these inorganic compounds pre-existed as such in the living seeds. Normally, these ash-forming materials are present in both organic and inorganic forms.

The percentage composition of seeds produced by one species of plant under ideal environmental conditions is uniformly constant, although slight differences may be noted between varieties. Marked differences

do occur, however, between the seeds of different plant species, especially in respect to organic constituents. (See Table 58, p. 328.)

All living seeds contain water, which, as we have already learned, is of paramount importance to all living organisms. The quantity of water present, previous to germination, however, should never be very high. Although the importance of water in the process of germination can hardly be over-estimated, it is likewise true that, if too large a quantity

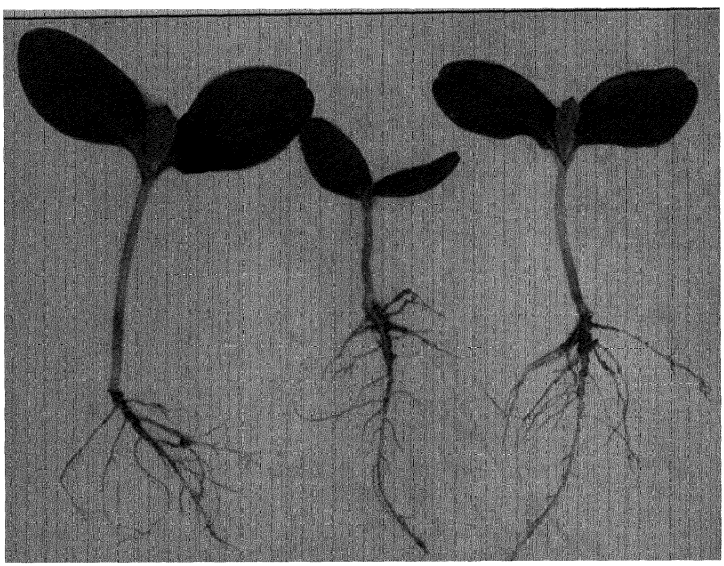

FIG. 21. Showing relative development of seedlings in sand impregnated with fertilizers, left; in sand alone, center; and in compost, right. (Courtesy of the Pennsylvania State College.)

is present during the storage period, a number of chemical changes follow which are apt to lower the viability. The respiration of stored seeds, under otherwise suitable conditions, is roughly proportional to the quantity of water present in the seeds and in the surrounding atmosphere, although exceptions are known. It is highly desirable, therefore, to control the respiratory activity of the seeds to a maximum degree during storage and thus prevent a loss of available food supplies, which normally should be utilized by the embryo in the development of a strong and vigorous seedling.

In the intelligent production and care of seeds it is possible to insure

the presence of the necessary organic and inorganic nutrient materials, and in quantities sufficient for satisfactory seedling growth. It is thought by some that large seeds, owing to the greater quantity of nutrient material, give rise to greater crop yields than do small seeds. This may be true in the case of a rapidly maturing crop but does not appear to hold true in the majority of other cases, especially where the environmental conditions are suitable otherwise for normal plant development.

TABLE 15

AVERAGE AIR-DRY WEIGHTS, WITH COMPARATIVE RATIOS, OF TOPS OF SOYBEAN PLANTS GROWN FROM SEEDS OF DIFFERENT WEIGHTS UNDER FIELD CONDITIONS

(*Data of Schmidt, New Jersey Station Bulletin* 404, 1924)

Seed weight, grades	Average air-dry weights	Ratios
mgm	gm	per cent
250 to 300	81	108
206 to 249	117	156
152 to 200	103	138
108 to 150	75	100

It has been fully established that some of the elements essential to plant growth may occur in the seed in quantities sufficient not only for seedling production but also for the production of a fully matured plant. Although this is not usually the case, the possibility remains, however, that traces of elements normally found in seeds but not considered necessary for plant growth are really essential, but that no more is required than the traces present to carry the resulting plant to complete maturity.

Factors Influencing the Process of Seed Germination. — We are told that rapid germination and vigorous growth of the seedling are conducive to maximum plant production. It is necessary, therefore, to take into consideration a number of factors which may directly or indirectly affect this process. Some of the most important of these will now be considered.

Maturity of the Seed. — Complete maturity before harvesting insures, among other things, the deposition in the seed of a maximum of both organic and inorganic materials. Immature seeds, however, are generally found to germinate much more quickly than well-matured seeds. This is probably due, in a measure at least, to the fact that water is taken up more readily by the former. This advantage, if it may be termed an advantage, is offset by the fact that seedlings produced from

immature seeds are usually less vigorous than those obtained from well-matured seeds.

Seeds harvested in an immature condition are found to contain more *free water* than fully matured seeds. This is an important factor in the case of seeds that are to be used for crop production. It has been proved that the resistance of seeds to low temperatures depends almost entirely on the quantity of water found in their tissues. If a seed of high water content is exposed to low temperatures, a more or less complete disorganization of the structure of the protoplasm and nucleus results, tending to lower its vitality. Such a seed also suffers a loss in vitality when subjected to relatively high temperatures, as these conditions aid in the development of molds and increase the rate of respiration, both of which are undesirable. It is possible, however, to remove the excess water under appropriate conditions, but it is far more desirable to choose well-matured seeds for germination purposes.

Age of the Seed. — Seeds have been known to retain their vitality for a long period of years, if kept under the proper conditions. Eventually, however, they lose their vitality, regardless of the conditions under which they are preserved. This loss of vitality is sometimes designated as *age-degeneration* and is thought to be due to a time-temperature denaturing of certain colloids of the protoplasm. If this is true, there is, simultaneously, a time-temperature denaturing of the enzymes of the seeds which also are of a colloidal nature.

A number of years ago, Beal, of the Michigan Agricultural College, buried twenty lots of seeds, each including fifty seeds of several species of plants. The seeds were placed in moist sand in pint bottles, and the uncorked bottles were buried on the campus, twenty inches below the surface of the soil, with the nozzle of the bottle pointing downward. Every five years one bottle was taken up and the seeds were tested for germination. The fortieth year test gave the following results:

TABLE 16

GERMINATION TEST OF BURIED SEEDS

(*Data of Crocker, Journal of the New York Botanical Garden*, 1925)	Percentage germination after being buried 40 years
Amaranthus retroflexus (pigweed)	2
Ambrosia elatior (ragweed)	4
Brassica nigra (black mustard)	18
Bromus secalinus (brome grass)	0
Bursa bursa-pastoris (shepherd's purse)	0
Erechtites hieracifolia	0
Euphorbia maculata	0
Lepidium virginicum (pepper grass)	2
Lychnis githago	0
Anthemis cotula (dog fennel)	0
Malva rotundifolia (dwarf mallow)	0
Oenothera biennis (evening primrose)	38
Plantago major (plantain)	10
Polygonum hydropiper (smartweed)	0
Portulaca oleracea (purslane)	2
Rumex crispus (yellow dock)	18
Chaetochloa lutescens	0
Alsine media	0
Trifolium repens (white clover)	0
Amaranthus graecizans (tumble weed)	66
(Chenopodium album) (lamb's quarter)	2*

* Germinated though not recorded as buried.

Practically all seeds require a certain amount of aging before they will germinate properly. This is commonly known as the *rest period*, or a period of *after-ripening*. During this period various desirable physical and chemical changes are said to take place within the tissues

It is fortunate that seeds do require a rest period before they germinate normally, otherwise germination may be initiated before the seed is removed from the parent plant, as in the case of the so-called "germinating seeds." Were it not for this rest period, plant propagation would be extremely difficult to control. This is true especially of seeds peculiar to the temperate zones, as it is necessary to carry seeds over to the following year, and thus escape the rigors of winter.

During the resting period, seeds respire. That is to say, oxygen is consumed and carbon dioxide and water are given off, a process common to all living organisms. The respiratory activities may be controlled to a considerable degree, not only by lowering the moisture content, as previously stated, but by maintaining a low temperature throughout this period.

Antiseptic Treatment of Seeds. — In order to control many seed-transmitted diseases, it is customary, at the present time, to treat seeds with antiseptic materials before they are used for plant propagation. Sterilized seeds, after planting, behave in much the same manner as unsterilized seeds, but the time for complete germination is uniformly longer. This is thought to be due to chemical changes brought about within the seed itself by the antiseptic material used, rather than to the destruction of desirable bacteria. Formaldehyde, which is sometimes employed either as a gas or in solution form, has a decided effect on the organized structure of the embryo, if used in too high a concentration. This deleterious effect is due, partly, to the precipitation of colloidal protein materials. Mercury and silver salts exert a similar effect in this respect. Moreover, if used carelessly, these compounds have a tendency to inhibit normal enzyme activity.

The deleterious effects of antiseptic materials may be lessened if due regard is given to their concentration. It is highly desirable, therefore, to use the smallest quantities sufficient for complete sterilization. If the materials used have no effect on the organized structure of the seed and yet are capable of controlling seed-borne disease organisms, so much the better. Certain chlorphenol-mercury compounds are ideal in this respect, penetrating seed-coats easily and destroying the parasites without impairing the germinating qualities of the seeds.

Water. — The rate of germination after planting is affected materially by the water content of the seeds and of their environment, and there are a number of reasons why this is true. Some of these will now be considered. All biochemical changes normally take place in aqueous solution. Water exerts a profound solvent action on many of the seed constituents, thus aiding chemical reactions between these and other compounds. Although a number of the seed constituents are looked upon as being more or less insoluble, some, however, are quite soluble in water.

Water readily penetrates all parts of the seed, forming physical or chemical unions with many of the constituents present. As a result, the seed swells and this facilitates the entrance of oxygen. In this way, water plays an indirect rôle in the oxidative changes taking place during the germination process. In addition, it plays a direct rôle, since no biological oxidation can take place in its absence. Moreover, as water is a good ionizing agent, it hastens the rate of many of the reactions taking place by facilitating ion combination.

As a rule, both living and non-living seeds absorb water if placed in contact with it, but there are striking variations between seeds of different species, in this respect. This has been attributed, in part, to

the relative permeability of the seed-coats involved. Normally, water, in dissolving the soluble seed constituents, increases the osmotic concentration within the seed-coat. The result of this osmotic change is to increase the rate of water absorbed. Also, the marked molecular attraction between many of the seed constituents and water must be considered. This attraction is modified to a considerable extent by the surface tension of the water itself; the lower the surface tension, the more readily it is attracted by the seed constituents.

In order that seeds may germinate quite rapidly after planting, considerable attention has been given to increasing their power of water absorption. Certain materials, such as concentrated sulfuric acid, dilute acids and bases, acting on the seed-coats, have proved beneficial in a number of cases. It is thought by some that these acids and bases change the character of the seed-coat, through chemical combination and subsequent hydrolysis, to simpler and more soluble compounds, which have greater affinity for water than do the original compounds themselves.

Seeds which germinate quite readily are the most readily influenced by acids; those that germinate less readily are more resistant. (See Table 17.)

TABLE 17

RESULTS OF TREATING SEEDS OF SMALL FRUITS WITH CONCENTRATED SULFURIC ACID

(*Data of Tukey, New York Station Bulletin* 509, 1924)

Variety	Number of seeds used	Check	15 minutes	1 hour	2 hours	4 hours	5½ hours
		Per cent		Per cent	Per cent	Per cent	
Ancient Briton blackberry	50	0		0	0	32	Dead
Lucretia dewberry	50	0		0	12	12	Dead
Wineberry	50	0	.	0	12	Dead	Dead
Newman No. 23 red raspberry	50	0		0	0	34	Dead
Buckeye red raspberry	50	0		4	4	10	Dead
Purple raspberry	50	0		78	52	Rotted	.
Van Fleet gooseberry	50	10		36	Rotted	Rotted	..
Poorman gooseberry	50	82	Rotted	Rotted	Rotted		...
Houghton gooseberry.	50	48	Rotted	Rotted	Rotted	..	..

As stated before, the quantity of water absorbed varies widely with different seeds. Seeds of the clover and alfalfa plants under appropriate environmental conditions absorb quantities of water equal to the weights of the seeds themselves. Other seeds, such as those of wheat and corn, absorb a quantity equal to about one-half their total weight under like conditions. The attractive power of seeds for water is best exemplified by the fact that it is sufficient to overcome, in a large measure, the attractive force of soil colloids for this substance and may bring the soil to the point of air-dryness.

Under normal conditions, a large portion of the water absorbed is found in physical or chemical combinations with cell colloids. Water in this condition will not freeze; it is no longer *free* water. The quantity of water so combined varies considerably for different seeds, but in some instances more than 75 per cent of the total water absorbed enters into such colloidal combinations. Any factor, therefore, which tends to modify the colloidal condition of the seed constituents must likewise affect the quantity of water held in colloidal combination.

FIG. 22. *Rosa Rubiginosa seeds:* Check stored dry; the others in moist sand for 6 months at the temperature designated and then planted in a flat in the greenhouse. The picture shows the effective temperature for stratification, that is, 5° C., or 41° F. (Courtesy of the Boyce Thompson Institute for Plant Research.)

Temperature. — Relatively high temperatures generally increase the rapidity of seed germination, but when the temperature is too high, the seedlings produced make but little growth. This is probably due to the

incompleteness of the germination process which is said to be a result of temperatures somewhat above the normal. This effect is more pronounced in the case of large seeds, since the size of the seedlings is proportional to the quantity of food material present and to the completeness of the germination process.

That the temperature under which many seeds are held, previous to planting, is one of the factors influencing the germination process is shown by Fig. 22.

It is of interest to note that the acceleration of chemical reactions normal to the germination process, as brought about by increased temperatures, is quite similar to that observed for chemical reactions in general. This is true especially for temperatures close to the optimum. Higher temperatures generally cause fluctuations in the rates of the reactions involved and the correlation between temperature rise and rate of reaction comes to an end. In general, however, rises in temperature accelerate all biological reactions to a greater or less degree, until death ensues.

One of the many reasons given for the effect of temperature on the rate of reactions normal to the germination process is its relation to the surface tension of water. The surface tension of water is considerably lowered by increases in temperature, and this results in its being more readily absorbed by the cell colloids, which increases the rate of germination for reasons given in a preceding paragraph.

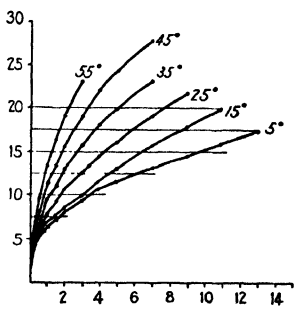

FIG 23. Absorption curves for corn at various temperatures: abscissae, time in hours; ordinates, percentage of water intake. (Courtesy of the Boyce Thompson Institute for Plant Research.)

The temperature at which a seed germinates best is known as the *optimum* temperature, and is intermediate to the *maximum* and *minimum* temperatures for the same process. The minimum temperature is that below which germination will not take place; the maximum temperature is that above which seeds will not germinate. It is possible for a seed to have its vitality preserved below the minimum temperature, but not above the maximum.

Oxygen. — Any biological process, such as seed germination, is accompanied by a transfer of energy. It is possible for the chemical and other forms of potential energy of seed constituents to be transformed into various forms of kinetic energy. That part of the kinetic energy which appears as heat, however, is considered to be of the most impor-

tance and has been studied in greater detail than any other form of energy change involved.

All forms of living matter are exothermic in character, that is, they are constantly producing heat. In the case of germinating seeds the quantity of heat liberated may be extremely great; some of this heat may be lost, but a considerable quantity of it may be used in promoting the

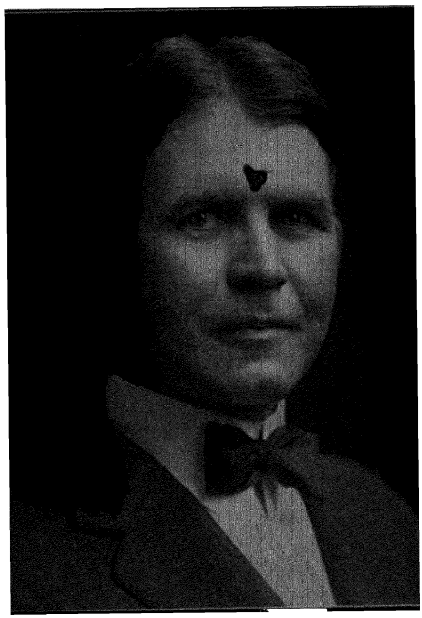

FIG. 24. Dr. William Crocker of the Boyce Thompson Institute for Plant Research, whose investigations on seed germination are of outstanding importance.

chemical reactivity of the seed constituents, especially those concerned with the mechanism of oxidation, and thus materially aid in hastening the rate of germination.

The quantity of oxygen required for normal germination varies for different seeds. White clover seeds, for example, will germinate in boiled distilled water, even if the surface of the water is covered by a layer of paraffin oil. These seeds will not germinate, however, if previously subjected to a vacuum treatment. This indicates that the quantity of oxygen normally found in the tissues of the seed is sufficient for their germination. Although germination has been observed where minimum quantities of oxygen were present, it may be stated that the

heat produced is very much less than where larger quantities of oxygen are present, as should normally be the case.

During the period of germination any condition leading to a dearth of oxygen, or to an oversupply of carbon dioxide, should be avoided. If only a few hundredths of a part of carbon dioxide are present to one part of oxygen, the germination process is hindered to a marked degree. A thick soil covering may bring about such a condition. There is, however, no fixed rule regarding the thickness of the soil cover. It depends entirely upon the size of the seed, the porosity of the soil and the supply of available soil moisture.

The rôle of oxygen in seed germination is quite involved and may vary for different seeds. It has been shown that this element influences the formation of protein from carbohydrates and helps, either directly or indirectly, to transform zymogens into active enzymes. Fats may be changed to carbohydrates through the process of oxidation. Moreover, many *oxygen-carriers* are present in all viable seeds, and obviously these require oxygen for their normal functions. These oxygen carriers have to do with the process of respiration, where oxygen plays the predominating rôle. This relationship, however, will be considered more in detail in Chapter XX.

Fertilizers. — The effect of fertilizers on the germination of seeds depends largely upon the soil type, the kind of seed, the method used in application, and the quantity of fertilizers used. In general, however, it is not desirable to have fertilizers in immediate contact with the seeds because they may retard or inhibit germination. This injury, however, is governed largely by the quantity of fertilizers used.

An application of fertilizer always increases the osmotic concentration of the soil solution. Owing to this condition, considerable resistance is offered to the rate at which water is absorbed by the seeds. If seeds are previously soaked in water, however, these injurious effects of fertilizers may be offset to a considerable degree.

The quantity of water present in the soil naturally modifies the action of fertilizers on the germination process. The greater the quantity of water present, the more fertilizer is required to impede the rate of germination. For this reason rainfall, during the germination period, would have a beneficial effect by lowering the osmotic concentration of the soil solution.

The more clay present in soils, the larger the quantity of fertilizers required to hinder germination. This is due to the adsorption of the dissolved constituents by soil colloids thereby preventing an unduly high concentration of the soil solution.

The harmful effects of fertilizer applications are sometimes apparent

in the growth of seedlings, for the reasons which have been given above. It was found by Truog and his students, for example, that corn grains require considerable quantities of water for germination and that the effect of fertilizer applications is quite marked if the grains are placed in close proximity to the fertilizers, as should normally be the case. If, however, the seeds germinated eventually, the fertilizer was found to have but little harmful effect on the growth of the seedlings. This resistant action exhibited by the seedlings is due to the remarkably high osmotic concentration of their cells. A seedling, such as the potato, having cells of very low osmotic concentration, was found to be less resistant to the action of fertilizer applications.

TABLE 18

EFFECT OF FERTILIZER ON GERMINATION OF SEEDS WHEN APPLIED IN WAYS INDICATED

(*Data of Truog and co-workers. Wisconsin Station Research Bulletin* 65, 1925)

Kind of seed	Retarding effect of fertilizer on germination		
	With the seed	Above the seed	Below the seed
Field pea	Very marked	Very slight	Slight
Cowpea	Marked	Very slight	Very slight
Navy bean	Very marked	Slight	Very slight
Soybean	Very marked	Slight	Slight
Lettuce	Slight	Marked	Very slight
Carrot	Slight	Marked	Very slight
Squash	Very marked	Slight	Marked
Cucumber	Marked	Slight	Marked
Muskmelon	Very marked	Slight	Very marked
Watermelon	Very marked	Slight	Marked
Potato	Marked	Marked	Very slight
Corn	Marked	Very slight	Very slight

Hydrogen- and Hydroxyl-ion Concentration of the Medium. — The effect of the hydrogen- and hydroxyl-ion concentration of the environment in relation to seed germination has been the subject of many researches. As a result of such studies, it appears that, on the whole, an acid reaction is more favorable than an alkaline reaction. A very high acidity or alkalinity, however, completely prevents seed germination.

The depressing effect of a relatively high acidity is probably greater during the period of germination than during the subsequent growth of

the seedling. This has been explained by the fact that the enzymes concerned with the process, and particularly with the process of oxidation, are highly susceptible to acid conditions. The majority of investigators are of the opinion that the beneficial or deleterious effects of acids and bases are directly or indirectly related to their favorable or unfavorable influence on the enzymatic processes involved.

We have already learned that the chemical action of acids and bases on certain constituents of the seed-coats modified their water relations. Furthermore, bases and acids may increase the rate and quantity of water absorbed by cell colloids. If they are used in too high concentrations, a precipitating action on the cell colloids may be brought about which lowers their imbibitional powers.

The Metabolism of Germinating Seeds

Viable seeds constantly lose weight during storage as a result of respiration. If this process becomes very intense, the vital processes may come to an end, owing to a dearth of respirable material. The greatest of care, therefore, should be taken in the treatment they receive previous to planting.

The respiratory changes of most seeds should be kept at a minimum during storage; this may be effected by reducing the moisture content of the seeds, and placing them in an environment where the air is cold and dry but relatively pure. In other words, the optimum conditions for normal germination of seeds are almost diametrically opposed to the conditions involved in their care and preservation.

The environmental conditions surrounding a seed during germination should be of such a nature that the respiration processes are not impeded. Therefore, water and oxygen should be available and the proper temperature should be maintained. Under these conditions, an intense metabolism is brought about within the seeds themselves during which time compounds are hydrolyzed and oxidized, or otherwise changed in such a way that they may be utilized by the embryo with the subsequent production of a normal seedling. These chemical changes are brought about, mainly, by the action of enzymes which should be present in all seeds used for plant propagation. Additional quantities of enzymes are produced during the period of germination.

Carbohydrate Metabolism. — The main storage form of carbohydrates, in practically all seeds, is starch. In order that this compound may be utilized for energy by the embryo, it must first be rendered soluble. This is effected by a vegetable amylase called *diastase.* Under its influence, the starch is hydrolyzed into a number of simpler compounds which are available for metabolic purposes.

Some authors believe that there are two diastases concerned with the hydrolysis of starch: one that resolves the molecule into dextrin; the other that resolves the dextrin into glucose. As a matter of fact, several different dextrins are formed during this process, and, since enzymes are more or less specific in their action, it is quite probable that several diastases may be concerned in starch metabolism.

Starch is present in the form of granules enclosed within rather resistant cell walls. Before these granules can be acted upon by diastase, the membranes must be disintegrated. This is brought about by the enzyme *cytase*, an enzyme having the power of changing the insoluble hemicelluloses of the cell walls to monosaccharides. The starch grains are subsequently acted upon by diastase, layer by layer, the grains retaining their original shape but diminishing gradually in size until they are no longer visible.

Several chemical changes are brought about by the action of diastase on starch. This compound is first hydrolyzed successively to amylo-dextrin, erythro-dextrin, malto-dextrin and eventually to maltose. An enzyme, maltase, resolves maltose into two molecules of glucose. As is true of all chemical reactions, the rate and extent of these changes are facilitated by the removal of the products formed. Under ordinary conditions of germination, this removal is accomplished by the embryo and seedling. If diastase is destroyed, starch degradation comes to an end, because no other enzyme present possesses the power of hydrolyzing starch. Some of the inorganic constituents of seeds appear to be necessary for the maximum efficiency of diastase. In addition, this enzyme seems to function best under fairly acid conditions. It is quite susceptible to alkalinity.

In addition to diastase, seeds also contain many other carbohydrate-splitting enzymes, the main group being the so-called *invertases*, which have the power of resolving disaccharides into monosaccharides, one of which, maltase, has already been mentioned.

Following the hydrolytic processes brought about by enzymes, come those of oxidation. These reactions are of extreme importance in that they are responsible for the production of heat and all other forms of energy, and are brought about through the agency of a system made up of organic compounds which are auto-oxidizable, giving rise to unstable peroxides, which are either activated by a peroxidase enzyme or decomposed into harmless constituents by catalase. This system of a peroxide and peroxidase, commonly called an *oxidase*, plays the major rôle in biological oxidations. The mechanism of these reactions will be considered in Chapter XX.

Oxidases, to use the generic term, are widely distributed in the vege-

table kingdom and are concerned primarily with aerobic respiration. They are present in all viable seeds and increase during the period of germination. They are quite susceptible to changes in hydrogen-ion concentration, their greatest activity being at or near the neutral point.

The products of oxidation in the germinating seed are many and varied, depending to a certain extent upon the characteristics of the combustible material present but more so upon the environmental conditions. Under any condition, however, carbon dioxide and water are the ultimate oxidation products.

Attention has already been called to the fact that the catalase enzyme is concerned in biological oxidations and that its rôle was to render the peroxide harmless through bringing about its decomposition. Through the decomposition of hydrogen peroxide, or other peroxides, formed in biological oxidations, it is thought that catalase protects the peroxidase, since peroxidase is quite susceptible to the action of peroxides. Although that rôle has not been established definitely, it is recognized that a parallelism exists between the catalase activity of a germinating seed and its rate of respiration, unless that relationship is modified, for the time being, by other factors. In a general way, experiments have brought out the fact that there is a close relationship between oxidase activity and catalase activity. At the very beginning of the process of germination the catalase content falls, but this fall is quickly followed by a sharp rise. At no time, however, does the evolution of carbon dioxide decrease. It is entirely possible that at the beginning of germination there is such an outburst of chemical activity that the catalase may be destroyed through oxidation or an over-production of organic acids, since catalase is quite susceptible to changes in hydrogen-ion concentration.

Desiccation to the extent of retarding respiration reduces catalase activity, as would be expected. On the other hand, submergence of the seeds in water lowers the catalase activity and reduces respiratory activities. It is likewise true that dead seeds show a lowered catalase activity after submergence in water, compared to viable seeds. This test serves, in a general way, as a means for determining the relative viability of seeds.

Protein Metabolism. — The nitrogen of seeds occurs mainly in proteins, although some protein degradation products are always present. The proteins are extremely insoluble, and, like starch, must be reduced to more soluble forms before they can be utilized by the embryo and seedling. Certain enzymes which are capable of effecting protein hydrolysis are present in seeds. These are called *proteases*. The prod-

TABLE 19

RELATION OF VIABILITY OF LETTUCE SEEDS (LACTUCA SATIVA) TO THE CHANGE IN CATALASE PRODUCED BY SOAKING IN WATER

(*Data of Davis, The Boyce Thompson Institute of Plant Research*, 1926)

Kind of seed	Sample number	Per-centage of germi-nation	cc of O_2 released in 10 minutes		Catalase ratio of soaked seed to dry seed
			Dry seed	Seed soaked over-night	
Lettuce of black-seeded varieties from 8 different sources; 50 seeds used	1	50	34 5	25 2	0 72
	2	97	40 0	41 0	1 03
	3	94	34 0	35 8	1 05
	4	95	34 0	35 0	1 03
	5	94	28 5	28 5	1 00
	6	99	27 0	27 5	1 02
	7	22	27 3	18 7	0 68
	8	0	25 0	9 6	0 38
Lettuce of white-seeded varieties from 15 different sources; 50 seeds used	9	33	31 5	15 1	0 48
	10	98	44 5	47 5	1 07
	11	0	34 0	18 0	0 53
	12	0	20 3	6 4	0 32
	13	99	44 0	45 0	1 02
	14	97	33 5	33 8	1 01
	15	95	26 0	26 5	1 02
	16	0	10 2	4 2	0 40
	17	0	16 3	10 0	0 61
	18	97	31 0	32 5	1 05
	19	96	29 7	31 0	1 04
	20	96	25 5	27 5	1 00
	21	0	9 6	1 3	0 14
	22	87	28 0	25 0	0 89
	23	0	33 5	9 3	0 28

[illegible] complete hydrolysis, namely, the amino acids, are quite soluble and readily available as nutrient material.

Normally, protease enzymes occur in zymogen form, as do other enzymes, although some of the active enzymes may be present. During the first stages of germination there is no marked production of proteases, but the opposite is true during the later stages of germination. When such a change is effected, the rate of protein hydrolysis is hastened until the process is more or less complete. Proteins are hydrolyzed by proteases, successively, to proteoses, peptones, peptides, and amino acids. The peptides are hydrolyzed into amino acids by erepsin-like enzymes. As the germination process proceeds, a marked increase is observed in the quantity of amide nitrogen produced. This indicates that, possibly, some of the nitrogen of the amino acids is changed to ammonia, part of which unites with some of the other organic acids forming ammonium salts, which on dehydration form amides. In all probability, amide nitrogen serves as nutrient material for the embryo and seedling. The optimum hydrogen-ion concentration for proteolytic activity is approximately the same as for diastase.

Fat Metabolism. — The fatty materials of mature seeds are stored in relatively large globules, which become reduced in size during the resting stage until they are of microscopical dimensions. It is possible that this dispersion reaches such a degree that direct translocation of such material can take place. In the germination process, however, it is thought that all fats undergo hydrolysis into glycerol and fatty acids and that this is brought about by specific enzymes called *lipases*. Moreover, it is thought that these hydrolytic products may be transformed into carbohydrates and other material as germination proceeds. However that may be, the fats are rendered available as sources of energy for the living organism.

The enzyme lipase requires a certain degree of acidity for maximum activity. It has been shown, however, that the acidity does not necessarily function by converting an inactive zymogen into an active enzyme, as is evidenced by the fact that seeds treated with acids have exhibited no lipase activity after the acid has been removed.

The transformation of carbohydrates into fats and of fats into carbohydrates has been observed frequently in germinating and maturing seeds. In fact, examination of the stem parts during the development of oil-containing seeds has shown clearly that the nutrients entering the seed are quite similar to those entering starchy seeds, namely, soluble carbohydrates, so that fats must be synthesized from carbohydrate material within the seed itself. The reverse process takes place just as readily during germination.

It is probable that the character of the carbohydrates formed from fats depends upon the kind of fatty material originally present. It has been postulated that fats, made up of saturated fatty acids, contribute in a much less degree to the formation of carbohydrates than do those synthesized from unsaturated fatty acids, and that the former are mainly used for respiration purposes by the germinating seed.

Résumé. — Seeds are somewhat analogous to milk or eggs, that is, they contain the necessary material for the growth and development of a living organism. The materials found in seeds, eggs and milk are likewise the materials found in protoplasm. Attention has been called to the chemical and physical changes taking place during germination which resulted in rendering the constituents of the seed more available as nutrients. Eventually, all the organic and inorganic material is used by the embryo in the change to a seedling, and further growth and development ceases if it does not receive additional material from outside sources. In succeeding chapters it will be shown how the environmental conditions affect the extent of plant growth directly or indirectly. Of these environmental factors, the *soil* is by far the most important. For that reason we shall consider, in the following chapter, the chemistry of soil, in relation to plant response.

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CHAPTER IX

THE SOIL

When the seedling has exhausted the available food contained in the seed, it normally requires further supplies of inorganic materials for its complete growth and development. It is, however, almost completely independent of further supplies of organic materials since under favorable soil and other environmental conditions, it will now be able to synthesize these compounds.

The soil, according to Coffey, may be defined as "the superficial unconsolidated mantle of disintegrated and more or less decomposed rock material, which, acted upon by organic agencies and mixed with varying amounts of organic matter, may furnish conditions necessary for the growth of plants."

A soil should contain all the necessary chemical elements in quantities sufficient for plant growth. A fertile soil meets these requirements. In addition, many organic materials derived from plants and animals are always present in all stages of decay; various microorganisms and their metabolic products; atmospheric and other gases; enzymes and water. Although these are present in all soils, some soils are better adapted to plant growth than others, for reasons which will be considered later.

The character of the soil exerts a profound influence on plant growth in general, and on the growth of agricultural crop plants in particular. At all times, certain physical, chemical and biological changes are taking place, some of which are beneficial, others of which may be harmful, depending, to a considerable extent, upon the physical and chemical condition of the soil itself. All changes involved in the formation of soils, or within the soil itself, are directly or indirectly related to plant growth, and it is on this account that a knowledge of soil conditions is of extreme importance to those who are interested in the production of plants.

THE FORMATION OF SOILS

There are many natural agencies which play a part in soil formation and soil changes. Among the most important of these are atmospheric oxygen and carbon dioxide, water, temperature, and plant and animal organisms.

Oxidation. — Under proper conditions, oxygen is capable of acting upon rock constituents, bringing about many physical and chemical changes through the process of oxidation. A rock is made up of an aggregation of minerals, some of which are more readily oxidized than others. Among these may be mentioned some of the iron-bearing min-

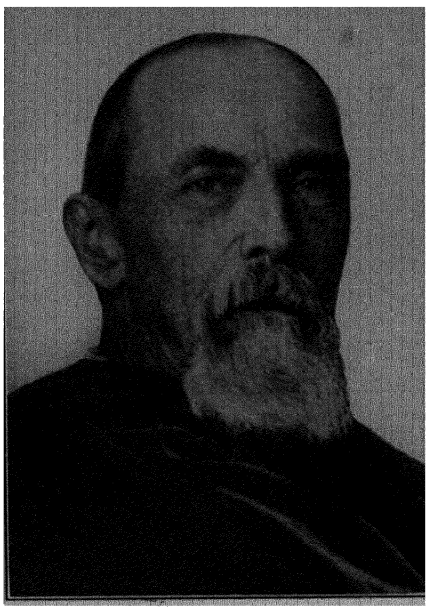


FIG. 25. Dr. E. W. Hilgard (1833–1916), prominent agricultural chemist and geologist, whose writings and researches contributed much to soil science. (Courtesy of his daughters — Alice R. and Louise Hilgard.)

erals. Upon oxidation it is possible for iron to be oxidized to a higher state of valence, forming an oxide, hydrated oxide or hydroxide as the case may be. These are reddish-colored materials. Such changes generally take place gradually and always lead to an increase in the volume of the original mineral. Thus there may be set up pressure sufficient to cause the rock to split, which materially hastens its decomposition and disintegration through the exposure of additional surfaces to be acted upon by the different soil-forming agencies.

Oxidative changes are taking place in soils continuously and are highly beneficial from the standpoint of plant growth. Highly oxidized compounds appear, as a rule, to be more readily assimilated by plants than

are the less completely oxidized compounds. These oxidative changes in the soil, however, are biochemical, rather than non-biochemical in nature, which does not necessarily hold true in the case of soil formation from the parent rocks.

Oxidation under natural conditions is a relatively slow process. Bouyoucos found, however, that certain fertilizer materials exerted a catalytic effect when used on soil materials in the presence of oxygen. Calcium nitrate was found to be quite efficient in this respect. Such an effect, however, is not unusual, as nitrate-containing materials, in general, are looked upon as oxidizing agents. On the other hand, Bouyoucos found that monocalcium phosphate, alone, had an inhibitory effect upon oxidation and, when used with calcium nitrate, masked the catalytic effect of the latter compound.

Sulfur-bearing minerals and compounds, as well as the element itself, yield sulfuric acid upon oxidation. The amount of sulfuric acid formed may be sufficient to exert a marked solubility-effect on the soil-forming rocks and minerals. Similar effects may be obtained, to a certain degree, by other acid-forming elements.

Carbonation. — Carbon dioxide is appreciably soluble in water, forming carbonic acid, and this acid possesses a solvent action on rocks and minerals. Its action is so pronounced in this respect that it is sometimes alluded to as the " universal soil solvent."

Most rocks, including the silicates, are vulnerable to the action of carbonic acid. Of these, the limestone rocks are most readily attacked by it on account of the fact that calcium, the predominating base, is changed to the water-soluble bicarbonate. Feldspars are somewhat less easily attacked than limestones.

The action of carbonic acid upon soil-forming minerals, or what is known as the *process of carbonation*, is quite important, and probably the most important of the inorganic soil constituents are formed in this way. During this process, the various bases present in the minerals are liberated in soluble forms which may become available for plant growth.

Not all the carbon dioxide in the soil comes from the atmosphere. The major portion probably comes from the decomposition of organic matter under the influence of microörganisms present. Moreover, crop plants, through their root systems, contribute considerable carbon dioxide to the soil.

The extent of the effect of carbonic acid on mineral decomposition in the soil is determined largely by the character of the soil. A porous condition would aid such a reaction, since it would permit ready penetration of oxygen which is necessary for carbon dioxide production.

In fact, a more or less definite relationship exists between the absorption of oxygen by soils and the production of carbon dioxide.

Temperature. — The soil-forming minerals differ from one another in physical and chemical properties. It is not to be expected, therefore, that any of these minerals would behave in the same manner under like temperature conditions. For example, it has been found that, in the parent rocks, these minerals have different coefficients of expansion and contraction, and under the influence of temperature changes these differences are made manifest. Under these conditions, therefore, there is a tendency for the rocks to split, thus increasing their surface area. This is true, particularly, when sudden temperature changes obtain.

The solvent action of soil water is rendered more efficient when the temperature is relatively high. This has the tendency to give to the soils of warm humid regions a larger amount of clay than the soils of colder regions, on account of a more thorough decomposition of the soil-forming minerals. When decomposition and leaching take place to an excessive degree, there is left but little more than ordinary sand, rather than clay, which is not so desirable from the standpoint of soil fertility.

Water. — All the soil-forming rocks and minerals are at least slightly soluble in water. Moreover, under the influence of water, various chemical and physical changes take place, accompanied by decomposition. The mechanical effects of water on rock degradation, through freezing in crevices, can hardly be over-emphasized. In soils, however, this mechanical effect is not so pronounced. The action of water upon the process of oxidation is of great importance because such reactions are not possible unless water is present. It also aids in a number of other chemical reactions, some of which are biological in character. As a result of oxidation, various inorganic and organic acids are produced, which may act on soil minerals, increasing their solubility. In dissolving basic and acidic elements, which become dissociated, water aids in bringing about the formation of new compounds, by facilitating chemical reactions. Some minerals take up water, forming hydrates. Hydrated silicates are constantly being formed in this way as a result of the decomposition of soil silicates.

The Action of Plant and Animal Life. — If a rootlet of a growing plant gains entrance to a rock crevice, continued growth may set up a pressure sufficient to split the rock, thus exposing more surface to the weathering forces. Higher plants may thus aid in soil formation by mechanical means.

Certain of the lower plants have a tendency to aid in the formation of soils in a different way. Lichens, for example, attach themselves to rock

surfaces and corrode these surfaces by the excretion of acids through their absorbing systems. The inorganic materials which are thus made soluble and, later, absorbed by these organisms, may become available, subsequently, for the growth of higher plants after the death and subsequent decomposition of the organisms.

THE CHEMICAL COMPOSITION OF SOILS

We have learned, in a general way, that soils consist of inorganic and organic materials. This subject will now be considered in more or less detail, and special stress will be laid on the more important constituents and the part they play in soil fertility and plant growth.

Calcium

Occurrence of Calcium in Soils. — Calcium occurs in soils to a considerable extent, in silicate form. Most of these so-called silicates, however, contain other bases. Calcium occurs also as the carbonate, either alone, or in combination with magnesium. A relatively large quantity is generally present as bicarbonate, nitrate, sulfate, phosphate, chloride and fluoride. Part of the calcium fluoride or chloride is usually found in combination with phosphorus, forming the mineral *apatite*. Calcium is found to occur in organic as well as inorganic combinations. It is a well-known fact that calcium unites readily with the acids produced in the decomposition of organic matter, and this is true especially of the so-called *humic acids* which will be discussed in greater detail under Soil Organic Matter.

The total quantity of calcium found in soils is subject to wide variations. In some cases less than 1 per cent of the soil consists of calcium, whereas in others it may exceed 10 per cent. Under average conditions, however, more than 1 per cent of the soil consists of calcium. The subsoil, or that part of the soil lying just below the point of maximum plant activity, contains less calcium, as a rule, than does the upper layer.

Factors Influencing the Availability of Calcium in Soils. — Relatively large quantities of this element are continually being made soluble and available for absorption by living plants. Ordinarily, a soil loses considerable calcium by the removal of crops. The quantity lost in this way, however, is relatively small, compared with the amount lost by leaching.

Studies of the factors influencing the loss of calcium and other inorganic soil constituents are made at a number of institutions by means of lysimeters. The lysimeters are tanks built of concrete. At the Cornel Station there are twenty-four tanks arranged in two rows of twelve each Between the rows is a tunnel six feet wide, the bottom of which is ter

feet below the top of the tanks. From the lowest point in the bottom of each tank, a brass drainpipe lined with tin leads into the tunnel, where receptacles are placed to catch the drainage water.

The surface of the soil in the tanks is level with the surrounding soil of the field. The soils in the tanks may be fertilized as desired and used

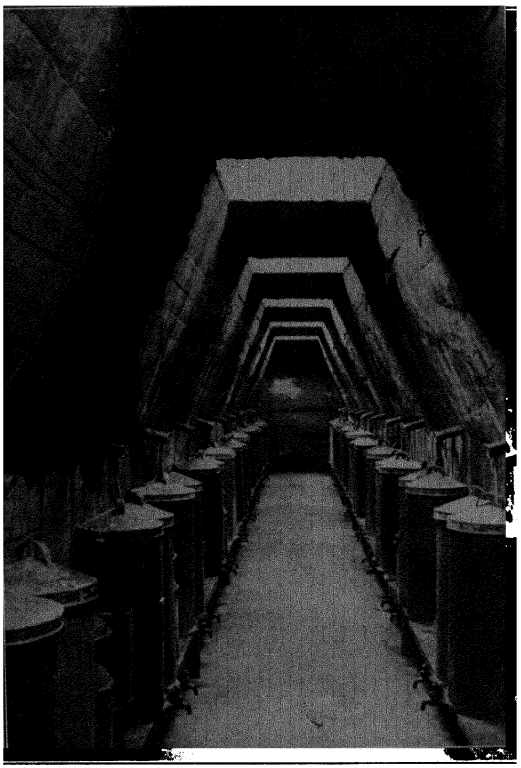


FIG. 26. Lysimeter tunnel between tanks with cans for collecting drainage water. (Courtesy of the Cornell Station.)

for the growing of crops. Very important data on leaching have been obtained as a result of lysimeter experiments.

There is at all times a loss of calcium from the soil and this is true, especially, during the summer months. This is due, primarily, to the fact that considerable calcium nitrate and calcium acid carbonate are formed by the activity of soil microörganisms, which reaches a maximum during the summer months. The loss of calcium by leaching may

be intensified through the direct or indirect effects of many fertilizers which tend to modify its solubility. Especially is this true in the case of sulfate of ammonia, which, when oxidized by soil bacteria, gives rise to both nitric and sulfuric acids. These acids then react with the calcium compounds present, forming salts which may have greater solubility than the naturally occurring forms.

The physical condition of the soil has a great deal to do with the amount of calcium removed by leaching. Experimental evidence has shown that calcium salts diffuse more rapidly through a sandy soil, less rapidly through a loam and only slightly through a clay soil.

So many factors play a part in facilitating the loss of calcium from soils by increased solubility that it is not surprising to find that calcium-deficient soils are quite common in practically all parts of the world, and the question of calcium economy in soils is one that has long occupied the attention of soil investigators.

The Influence of Calcium on Soil Constituents. — All agricultural soils, sooner or later, become deficient in calcium, which must be restored in order to preserve the fertility of the soil. A very common form of calcium used in this way is ordinary *lime*, CaO, although the carbonate, hydroxide and silicate are used frequently. These are also designated as *lime compounds*. The chemical and physical effects of these various soil additions, and the changes they undergo, have been the subject of numerous investigations, and a number of facts concerning the same have been definitely established For example, it has been shown that calcium oxide changes readily to the hydroxide and, through carbonation, eventually forms the carbonate, although the latter change takes place much more slowly than the former. The reason for this slow carbonation is due, in all probability, to a layer of carbonate formed around a particle of the hydroxide, thereby tending to retard the process.

In addition to reacting with carbonic acid, these compounds may react with other soil constituents For example, considerable quantities of calcium hydroxide may unite directly with organic and inorganic soil colloids, thereby becoming quite insoluble.

The chemical effect of calcium compounds on the reaction of the soil is quite important. It has been pointed out that, when lime, in excess, is added to a soil, there is produced a relatively high alkalinity, extreme applications rendering the soil too alkaline for maximum plant growth. It has been shown, however, that this alkalinity decreases markedly within a period of even a few days and continues to decrease over a considerable period of time, but that the alkaline reaction is still apparent even after a period of several months. (See Table 20.) This is probably due to carbonation and subsequent production of calcium carbonate.

TABLE 20

EFFECT OF DIFFERENT FORMS OF LIME ON SOIL REACTION

(Data of Hoagland and Christie, Soil Science, 1918)

Number of soil	Description of soil	Treatment	p_H immediately after treatment	p_H after 4 days	p_H after 7 days	p_H after 18 days	p_H after 26 days	p_H after 46 days	p_H after 80 days	p_H after 187 days	p_H after growing barley 10 months after treatment	p_H soil + 0 10 per cent $CaCO_3$	p_H soil + 0.40 per cent $CaCO_3$
1	Clay adobe	None	7 56									7 39	7.39
1	Clay adobe.	0 07 per cent CaO	8 56	8 36	8 56	8 00	8 50	8 05	8 74	8 00	8 22		
1	Clay adobe.	0 28 per cent CaO	10 16	9 26	9 43	9 08	8 74	8 94	9 00	8 70	8 84		
2	Sandy loam	None	7 56									7 87	7.80
2	Sandy loam..... ..	0 07 per cent CaO	9 26	8 18	8 50	8 25	8 25	8 11	8 56	8 29	7 87		
2	Sandy loam..	0 28 per cent CaO	10 96	10 00	9 85	9 05	9 05	8 94	9 00	8.67	8.43		
3	Silty clay loam... .	None . . .	7 77									7 80	7.81
3	Silty clay loam.....	0 07 per cent CaO .	9 05	8 43	8 63	8 36	8 29	8 15	8 15	8 15	7 80		
3	Silty clay loam.....	0 28 per cent CaO	10 30	9 43	9 47	8 84	8 94	8 88	8 91	8 50	8 32		
4	Beach sand.....	None	7 35									8.08	8.18
4	Beach sand.	0 07 per cent CaO	10 82	9 00	9 43	8 53	8 53	8 32	8 29	7 92			
4	Beach sand	0 28 per cent CaO	12 45	11 68	11 51	10 89	10 65	9 61	9 05	8 39			

Therefore, one would expect an application of calcium carbonate to be less intense in this respect than ordinary lime, and actual experience has shown this to be true. However, it has been suggested that the oxide rather than the carbonate should be used on highly adsorptive soils, since a more rapid reaction between calcium and the soil colloids would be effected thereby.

4. Calcium oxide, when added to soils high in organic matter, is found to accelerate the absorption of oxygen and the production of carbon dioxide. It has been shown also that this compound is quite effective in neutralization of organic acids. The chief function of calcium carbonate appears to be the neutralization of organic and inorganic soil acids and, in this way, plays an important rôle in the decomposition of organic matter.

The relation of calcium compounds to the base-exchange phenomena of soils has naturally received considerable attention in the past. As in the case of any other adsorbed base, calcium has been found to be replaced readily by other bases, if the soil be treated under the proper conditions, the extent of the exchange being roughly proportional to the concentration of the solution used. This would indicate that the base-exchange reaction is influenced not only by the principle of chemical equilibrium but by mass action as well. If a soil is extracted with a sufficient quantity of a solution of potassium chloride, potassium will be practically the only base left in replaceable form. If the sample, so treated, is subsequently brought in contact with a solution of calcium chloride, calcium is found to replace potassium.

TABLE 21

REPLACEABLE CALCIUM AS DETERMINED WITH ALCOHOLIC 0.2 *N* KCl (63 PER CENT C_2H_5OH)

(*Data of Chapman and Kelley, Soil Science*, 1930)

Sample	Milliequivalents Ca per 100 gm. soil	
	First 400 cc	Second 400 cc
Soil alone	8.30	0.00
	8.20	0.10
Soil + 2½ per cent $CaCO_3$	9.40	1.07
	9.50	0.99

It was the consensus of opinion at one time that calcium compounds, if added to soils, made potassium more available. It is evident from

what has been said that this will probably hold true under certain conditions. There is a tendency, however, for some calcium compounds to render potassium less soluble. For example, an application of calcium hydroxide may lead to a greater dispersion of the negatively charged colloidal particles through the introduction of hydroxyl ions, thereby increasing the surface for the adsorption of positively charged potassium. This has been demonstrated in many instances. Adsorption of a base, however, may be either physical or chemical in character.

Adsorbed calcium, and likewise any other adsorbed base, is available for plant growth, provided that the absorbing system of the plant is in direct contact with the adsorbed material, although bases, so combined, are not looked upon as being water-soluble.

6. Calcium aids in the absorption of mineral nutrients by plants by promoting the development of root-hairs and thus increasing the feeding capacity of the plant. The relation of calcium to the availability of phosphorus-bearing compounds has been an important soil study in the past. The decomposition of organic matter is extremely slow in soils which lack calcium. Since considerable phosphorus is organically combined, it is not available for plant growth unless decomposition is effected. The effect of calcium in this regard is due partly to the betterment of soil conditions, so far as bacterial activity is concerned, and partly to its more or less direct effects on the decomposition process through the neutralization of the acids produced.

On inorganic phosphates, calcium compounds may lead to an opposite effect, phosphorus being rendered more unavailable by being converted to insoluble calcium phosphate, as indicated by the following equation:

$$CaH_4(PO_4)_2 + 2\,CaCO_3 \leftrightharpoons Ca_3(PO_4)_2 + 2\,H_2CO_3$$

The effect of calcium on the availability of soil sulfur and nitrogen is not to be disregarded. If calcium is not lacking, there generally prevails a good physical condition of the soil, rendering better conditions for oxidation. Under such conditions, the sulfur and nitrogen present in organic combinations are oxidized to acids. The acids so produced are neutralized by basic calcium compounds, and, therefore, the reaction may proceed to completion.

The Relation of Calcium to Biological Soil Processes. — Soil bacteria produce acids which are toxic to themselves, and, unless these acids are neutralized, bacterial activity is impeded. It is mainly for this reason that calcium additions have such a pronounced effect on the activity and development of soil microörganisms. When the by-products produced by soil organisms are alkaline in nature rather than acidic, these salts are not as beneficial.

That the application of calcium as the carbonate to soils is beneficial in respect to bacterial development is indicated in Table 22. Two soils were used, each of which was treated with varying quantities of calcium carbonate. These were mixed thoroughly each week for 12 weeks and proper moisture conditions maintained. Plate counts of the number of microorganisms were made at the end of the 12-week period. At this time the soils were treated with monocalcium phosphate, equal to 0.2 per cent the weight of the soil, and handled in like manner for another 12-week period, plate counts again being made at the end of this period. The greatest change occurred after the neutral point was reached.

TABLE 22

THE EFFECT OF CALCIUM CARBONATE ON THE NUMBER OF BACTERIA IN SOILS
(*Data of Bear, Soil Science*, 1917)

Calcium carbonate per 2,000,000 pounds of soil	Bacteria per gram of soil			
	Soil I without phosphorus	Soil I with phosphorus	Soil II without phosphorus	Soil II with phosphorus
pounds				
0	3,341,000	4,150,000	3,503,000	3,438,000
250	4,127,000	4,320,000	3,418,000	3,536,000
500	3,537,000	3,540,000	4,614,000	4,121,000
1,000	3,439,000	2,750,000	3,781,000	5,207,000
2,000	3,930,000	2,520,000	4,472,000	5,781,000
3,000	4,127,000	4,090,000	4,919,000	5,683,000
Neutral point (Veitch method)				
4,000	4,422,000	5,820,000	7,348,000	10,005,000
5,000	5,306,000	7,700,000	9,741,000	17,392,000
7,500	5,601,000	7,070,000	15,827,000	14,297,000
10,000	3,341,000	6,680,000	14,973,000	7,959,000
20,000	6,682,000	10,750,000	14,892,000	3,635,000
40,000	9,335,000	13,050,000	18,199,000	9,826,000

Magnesium

Occurrence of Magnesium in Soils. — Magnesium is found in soils in variable quantities and in combinations similar to those of calcium. Some soils contain very little, however, and magnesium additions must

TABLE 23

Decomposition of Magnesite by Contact with Moist Soils Under Laboratory Conditions*

(Data of MacIntire, Willis and Hardy, Tennessee Station, Bulletin 107)

Soil type	Total grams of air-dried soil	$MgCO_3$ indicated by Veitch method to neutralize soil acidity, m.f basis		$MgCO_3$ added, grams	Decomposition of $MgCO_3$ — determined by analyses of residual carbonates, m.f basis CO_2 liberated from 30-gm charges by boiling 30 minutes with 1-15 H_3PO_4											
					After 30 days' contact				After 65 days' contact				After 138 days' contact			
					Grams	Excess of Veitch			Grams	Excess of Veitch			Grams	Excess of Veitch		
		Per cent	Grams			Grams	Per cent	Lb per 2,000,000 of soil		Grams	Per cent	Lb per 2,000,000 of soil		Grams	Per cent	Lb per 2,000,000 of soil
Sandy clay loam	(*A*) 400	0 0454	0 1736	9 424	0 5755	0 4019	0 105	2100	0 6538	0 4702	0 123	2460	1 4288	1 2552	0 328	6560
	(*B*) 400	0 0454	0 1736	9 424	0 4979	0 3243	0 085	1700	0 7070	0 5334	0 139	2780	1 3978	1 2244	0 320	6400
	Av. 400	0 0454	0 1736	9 424	0 5367	0 3631	0 095	1900	0 6804	0 5018	0 131	2620	1 4133	1 2398	0 324	6480
Silty loam	(*A*) 400	0 0564	0 2100	9 424	1 4082	1 1982	0 322	6400	1 5313	1 3213	0 355	7100	2 2449	2 0349	0 547	10940
	(*B*) 400	0 0564	0 2100	9 424	1 5229	1 3129	0 352	7040	1 5789	1 3693	0 368	7360	2 3625	2 1525	0 578	11560
	Av 400	0 0564	0 2100	9 424	1 4656	1 2556	0 337	6740	1 5551	1 3453	0 362	7230	2 3037	2 0937	0 563	11250
Loam	(*A*) 400	0 1633	0 5939	9 424	0 9143	0 3205	0 088	1760	0 9666	0 3728	0 102	2040	1 5802	0 9863	0 271	5420
	(*B*) 400	0 1633	0 5939	9 424	0 9396	0 3458	0 095	1900	0 9982	0 4344	0 119	2380	1 4822	0 8884	0 244	4800
	Av 400	0 1633	0 5939	9 424	0 9270	0 3332	0 092	1830	0 9824	0 4036	0 111	2210	1 5312	0 9374	0 258	5150

* The flasks were shaken during the second and third contact periods.

be made to these from time to time, to insure vigorous plant growth. Soils derived from calcareous materials which have been but partly decomposed may contain large quantities of calcium and magnesium. In general, the percentages of magnesium and calcium in agricultural soils are practically the same, although pronounced exceptions to this rule are known.

Factors Influencing the Availability of Magnesium in Soils. — The solubility of magnesium carbonate in carbonated water exceeds the solubility of calcium carbonate under the same conditions. Under soil conditions, therefore, decomposing organic matter, through the production of carbon dioxide, should readily facilitate the solubility and loss of magnesium. As a rule, however, the loss of magnesium rarely, if ever, exceeds the loss of calcium. It is thought by some investigators that only very small quantities of magnesium are present as magnesium carbonate in agricultural soils at any one time, on account of its chemical reactivity.

The Influence of Magnesium on Soil Constituents. — The addition of magnesium oxide, MgO, to soil, results in the formation of magnesium hydrate, which, through the carbonation process, changes to the carbonate and bicarbonate. These compounds may react with some of the soil silicates, forming magnesium silicates. Considerable amounts of *magnesium humates*, which will be discussed later in greater detail, are formed as a result of neutralization of organic acids by basic magnesium compounds and have been identified in soils.

The Relation of Magnesium to Biological Soil Processes. — Magnesium compounds, as a rule, are said to stimulate bacterial activity to a much greater degree than do equal quantities of calcium compounds. This is probably due to the pronounced basicity of the magnesium compounds, which are more reactive than similar calcium compounds. Magnesium carbonate, for instance, neutralizes the acid by-products of the soil microorganisms much more readily than does calcium carbonate. On the other hand, owing to the pronounced change brought about in the soil solution, excessive quantities of magnesium compounds may limit the activities of these soil microorganisms to a much greater degree than do calcium compounds.

Laboratory studies have shown that the magnesium ion is decidedly toxic to the growth of higher and lower plant organisms when used in relatively large quantities. Magnesium poisoning, however, under soil conditions, is rarely, if ever, noted. The reason for this may be due, in part, to the fact that small quantities of calcium are able to overcome the toxic effect of magnesium. Some investigators maintain that there must be, for normal plant growth, a more or less definite relationship,

other conditions being equal, between the available magnesium and calcium. This relationship is known as the *calcium-magnesium ratio.* Although this ratio may hold within certain limits for sand-and-solution-cultures, it does not appear to be true of soil cultures.

Potassium

The Occurrence of Potassium in Soils. — Potassium occurs largely as silicate minerals, which contain other elements as well. The most important potassium-bearing minerals, in practically all soils, are *orthoclase* and *microcline*, both having the empirical formula $KAlSi_3O_8$. A great deal of potassium is sometimes found as *muscovite* a mineral having the empirical formula $H_2KAl_3Si_3O_{12}$, and as other alumino-silicates, which may be either hydrated or non-hydrated. These minerals decompose slowly under ordinary soil conditions, leading eventually to the formation of *kaolinite*, $Al_2(OH)_4Si_2O_5$, or ordinary clay. Potassium also occurs in various organic combinations, and, as in the case of other soil bases, it forms adsorption compounds with organic and inorganic soil colloids.

The quantity of potassium found in soils varies to a greater extent than any other element necessary for the growth of plants. Certain agricultural soils, such as mucks, peats, and light sandy soils, are sometimes markedly deficient in available supplies of this element The majority of soils, however, contain relatively large quantities of potassium, the average content lying between 1 75 and 2.50 per cent of the total soil mass. Most of the potassium, however, generally occurs in very insoluble compounds and hence is not readily available for plant growth.

Factors Influencing the Availability of Soil Potassium. — The potassium-bearing minerals found in soils have a very limited solubility in water, although there is some evidence to show that they may be very slightly hydrolyzed. When powdered orthoclase is treated with water, hydrolysis may be shown by the color change of phenolphthalein, or of a similar indicator. The reaction is thought to take place, in a general way, according to the following equation:

$$\underset{\text{orthoclase}}{KAlSi_3O_8} + HOH \rightleftarrows KOH + \underset{\text{alumino-silicic acid}}{HAlSi_3O_8}$$

Such a reaction may be materially assisted by further reactions of the KOH with carbon dioxide:

$$KOH + CO_2 \leftrightharpoons KHCO_3$$

This removal of potassium hydroxide from the sphere of the reaction allows hydrolysis to proceed to a much greater extent. Under soil

conditions, however, such a reaction does not necessarily take place to any great degree. The reason for this is that the unchanged particles of the orthoclase become coated with a layer of the colloidal alumino-silicic acid, a product of hydrolysis, which prevents further intimate contact between the mineral and water.

It is thought that a part of the potassium liberated in the hydrolysis of orthoclase is adsorbed by the colloidal alumino-silicic acid. If, however, the concentration of hydrogen ions present is sufficient, absorption of potassium may be lessened. Actual practice has shown that a slightly acid reaction of the soil is desirable from the standpoint of the absorption of potassium by plants. During the growing period of a plant, sufficient carbon dioxide is given off through the root system to increase the concentration of hydrogen ions through the formation of carbonic acid, which is sufficient to replace potassium from such a colloidal combination. Obviously, therefore, an intimate contact between root-hairs and soil minerals is desirable for maximum absorption of soil potassium by the plant.

Laboratory studies have shown that some salt solutions apparently increase the hydrolysis of potassium-containing minerals. This is probably due, however, to the replacement of adsorbed potassium by the action of the dissociated salt. The addition of a hydroxide, rather than a salt, would probably not be as effective in this connection, owing to the dispersing effect it would have on the negatively charged alumino-silicic acid, as more surface would be exposed for the absorption of potassium. On the other hand, there may be a tendency for the base to further the hydrolysis of the unchanged mineral particles through the removal of the colloidal layer which, as stated before, may prevent continued hydrolysis of the mineral.

The Influence of Potassium on Soil Constituents. — The addition of potassium to the soil results in a number of changes. It is generally added as the chloride, sulfate, nitrate or carbonate. If the sulfate, chloride, or nitrate of potassium is used, there ordinarily results a displacement of adsorbed hydrogen, if present, with the formation of sulfuric, hydrochloric, or nitric acid as the case may be. These acids, as stated before, have a pronounced effect on the solubility of many of the soil constituents. If the carbonate is used, the reaction is more intense, owing to its greater chemical activity. The more active the form of potash, the more readily it is retained by the upper layers of the soil; the less active the form, the greater the depth to which it is capable of penetrating. Instead of increasing the acidity of the soil, the result of the addition of the carbonate is to lower the acidity. In many cases, this would be very desirable. Under other conditions, where the soil is alka-

line, this would not be true. Through its marked effect on increasing the concentration of hydroxyl ions a greater dispersion of soil colloids is brought about which is commonly known as a "puddling effect." When a soil becomes "puddled," the productivity of that soil is decreased, because it becomes less penetrable by atmospheric gases and, moreover, will not permit a ready penetration of the roots of growing plants. On account of the effect on the physical property of soils, the frequent use of the carbonate is not to be recommended.

TABLE 24

THE CONCENTRATION OF WATER-EXTRACTABLE POTASSIUM IN SOILS AS AFFECTED BY FIELD TREATMENT. FIVE-YEAR ROTATION FERTILITY PLOTS

(*Data of Ames, Ohio, Station Bimonthly Bulletin*, 1927)

Plot	Fertilizer treatment, 5-year period, pounds per acre	Potassium, pounds per acre	
		Limed	Unlimed
1	None	46	68
2	Acid phosphate, 320	34	74
3	Muriate potash, 260	72	154
4	None	46	74
5	Nitrate soda, 480	46	62
6	Acid phosphate, 320, nitrate soda, 480	34	64
7	None	56	72
8	Acid phosphate, 320, muriate potash, 260	60	120
9	Muriate potash, 260, nitrate soda, 480	64	152
10	None	46	68
11	Acid phosphate, 320; muriate potash, 260, nitrate soda, 480	62	122
18	Manure, 18 tons ..	68	104
19	None	54	62
24	Acid phosphate, 480; muriate potash, 260; ammonium sulfate, 165	68	102
25	None	52	74

The Relation of Potassium to Biological Soil Processes. — Potassium salts are known to have specific effects on certain of the common biological soil processes. Under many conditions, potassium salts are thought to stimulate the process of nitrification, but under other conditions, their effects have been deleterious. According to Smith, it appears that the application of potassium compounds, under field conditions, commonly exerts a depressing action on nitrification, but that

lime is able to overcome this effect, at least to a certain degree. This could be explained in the case of the chloride, sulfate and nitrate as due to the neutralization of the acids formed by the reaction of these salts with soil colloids. According to Mack, carbonate of potash aids the process of nitrification, probably through the neutralization of the by-products, i.e., nitrous and nitric acids.

Iron

The Occurrence of Iron in Soils. — Iron occurs in soils in varying quantities and in diversified chemical combinations. According to Robinson and Fry, the analyses of many different American soils show the iron content calculated as Fe_2O_3 to vary from 0.33 to 16.23 per cent. Some soils, however, are known to contain Fe_2O_3 in excess of 30 per cent. These soils are worthless for plant production because iron in such excessive quantities is toxic to most plants. It is thought by some that, where the Fe_2O_3 content of the soil is below 0.5 per cent, the application of iron salts would be at least beneficial, if not necessary, for plant growth. Iron occurs as the carbonate, as various oxides, hydrated oxides, and hydroxides. In addition to these it occurs in silicates, and many other inorganic as well as organic forms of combination.

Hematite, Fe_2O_3, and *limonite*, $2\,Fe_2O_3 \cdot 3\,H_2O$, are decomposition products of the minerals *biotite*, $KHMgFeAl_2Si_3O_{12}$, *hornblende* and *augite*. The two last-mentioned minerals are of variable composition, containing calcium, magnesium, iron and aluminum in combination with silicic acid. These minerals impart to certain soils their characteristic red and yellow colors.

Factors Influencing the Availability of Soil Iron. — The consistent use of *physiologically acid fertilizers*, or those which leave an acid residue in the soil, results in making iron more soluble. In addition, certain hydroxy organic acids in combination with alkalies tend to react with iron, forming soluble compounds, for example, potassium-iron-citrate. On the other hand, it is entirely possible for free citric acid, where present, to act as a peptizing agent upon colloidal iron compounds, which would have a tendency to increase their solubility through better contact with the soil water. It has been shown that, the lower the hydrogen-ion concentration, the lower the solubility of soil iron. For this reason, therefore, the addition of lime compounds in excessive quantities, tends to decrease the solubility of iron. (See Table 25.)

The Influence of Iron on Soil Constituents. — According to Spurway, iron, like aluminum, does not appear to be present in soils in exchangeable form, at least to any great extent. Lipman, however, is of the opinion that iron substitutes itself quite readily for the adsorbed bases

normally present. There is, however, a pronounced tendency for iron, like aluminum, to react with phosphorus to form insoluble compounds.

TABLE 25

ADSORPTION OF IRON BY SOILS AT DIFFERENT DEPTHS AS RELATED TO THE CALCIUM CONTENT

(*Data of Doyne and Morison, Soil Science*, 1926)

	$CaCO_3$, per cent	Fe removed from 100 cc. 0 1 *N* $FeCl_3$ by 100 gm. soil
First inch*	0 2508	56 3
Second inch	0 1672	54 2
Third inch	0 2090	54 2
Fourth inch	0 2132	54 2
Fifth inch	0 1923	53 5
Sixth inch	0 1044	50 0
Seventh inch	0 1754	50 0
Eighth inch	0 0334	43 6
Ninth inch	0 0417	40 2

* Top

The action of water on the iron-bearing minerals and compounds of the soil brings about a colloidal condition of the iron. Not only is colloidal iron important as a soil constituent in adsorbing certain ions, and thereby preventing their loss from the soil; it is likewise capable of acting as a catalyst in soil oxidation processes. In order that this may be accomplished it is necessary for iron to be present, at least for a period

TABLE 26

THE EFFECT OF ADDITIONS OF IRON AS FERROUS SULFATE ON THE SOLUBILITY OF CERTAIN SOIL CONSTITUENTS

(*Data of Lipman, Soil Science*, 1922)

Treatment	Total solids, grams	Non-volatile solids, grams	Ca, grams	K, grams	P, grams
$FeSO_4$*	0 0585	0 0420	0 0044	0 0049	0 0027
Untreated	0 0431	0 0266	0 0023	0 0034	0 0018

* $FeSO_4$ added to soil at the rate of approximately 7.5 lb. per area, 15 ft. in diameter. Soil solution obtained by pressure method.

of time, in divalent form. This form appears to be quite distinct from trivalent iron with regard to catalytic properties. It is able, for example, to absorb oxygen from the soil atmosphere, forming a more or less unstable higher oxide. It is possible that the oxygen, so combined, may act as a dehydrogenation agent toward the organic compounds present. On the other hand, it is likewise possible for colloidal iron compounds to adsorb molecular hydrogen, which may be released from the surface in nascent form, and which is then capable of uniting with molecular oxygen to form hydrogen peroxide which likewise functions as an oxidizing agent. In either case, according to Baudisch, the inert atmospheric oxygen becomes activated by iron.

The Relation of Iron to Biological Soil Processes. — Some soil bacteria have the power of oxidizing ferrous to ferric compounds:

$$4\ FeCO_3 + 6\ H_2O + O_2 \rightleftarrows 4\ Fe(OH)_3 + 4\ CO_2$$

The energy so liberated is utilized in the growth of the organisms involved, although it is possible for the latter to thrive in nutrient media containing no iron. Other soil organisms, including some of the molds, have a tendency to liberate iron in inorganic form through the oxidation of iron-bearing organic compounds.

The addition of soluble iron compounds to the soil has been known to produce a reduction in the number of organisms normally present. For this reason, it is thought that any soil process involving the formation of considerable quantities of soluble iron compounds may limit the number and activity of the soil microorganisms. On the other hand, there is considerable experimental evidence to show that some biological processes are favorably influenced by the additions of iron compounds This favorable effect may be due, in part, to the fact that iron serves as a food for the organisms involved According to Greaves, both organic and inorganic colloidal soil substances exert a favorable action on *Azotobacter* (nitrogen-fixing bacteria), although the action of the inorganic colloids is fully manifest only in the presence of organic colloids. The favorable influence of iron on the activity of Azotobacter organisms has been attributed to the fact that, in addition to oxygen, it may also adsorb nitrogen, thus bringing these elements into immediate contact with the organisms, and in forms which are more chemically active than ordinary atmospheric oxygen and nitrogen

Aluminum

The Occurrence of Aluminum in Soils. — Aluminum is the third most abundant element found in soils, exceeded only by oxygen and silicon. In general, the percentage of aluminum calculated as Al_2O_3 varies

between 1.19 and 27.58 per cent. The aluminum-bearing minerals, through decomposition, become the basic materials of clay soils. Aluminum is generally present as the oxide, hydrated oxide, hydroxide, phosphate and silicate, and it is altogether possible that some is present in organic forms. Aluminum, like iron, forms complex soluble compounds with alkali salts of hydroxy-organic acids. More aluminum is found in the subsoil than in the uppersoil layers.

Factors Influencing the Availability of Soil Aluminum. — In order to understand the deportment of aluminum salts in the soil, their behavior has been studied under laboratory conditions. It has been found, for example, that a 1 per cent solution of aluminum sulfate has a *p*H value of 2.2, and that this value is not altered to any great extent over a considerable range of dilutions. The acidity developed is caused, of course, by the partial hydrolysis of the salt. If this solution is treated subsequently with an aqueous solution of calcium hydroxide, there results, naturally, the precipitation of aluminum hydroxide which occurs at a *p*H of 4. Further additions of calcium hydroxide result in the complete neutralization of the solution and the formation of calcium aluminate.

If this titration is performed electrometrically, a typical titration curve of buffered solutions is obtained, the buffering action of the salt ceasing, however, between *p*H 4 2 and *p*H 4 5. It has been suggested that at this point the precipitation of colloidal aluminum hydroxide takes place.

If, instead of calcium hydroxide, a solution of monocalcium phosphate is mixed in equimolecular proportions with the aluminum sulfate, no precipitation occurs. On the addition of larger quantities of the phosphate, however, precipitation usually takes place. These reactions, according to Line, may be shown by the following equation:

$$Al_2(SO_4)_3 + CaH_4(PO_4)_2 \rightleftharpoons 2\ AlPO_4 + CaSO_4 + 2\ H_2SO_4$$

If the titration is carried through electrometrically it is found that the addition of monocalcium phosphate results in a slight lowering of the acidity. At *p*H 2.7 there is an incipient precipitation of aluminum phosphate, and at *p*H 3 to *p*H 4 complete precipitation takes place.

These observations serve to show that aluminum, contrary to popular belief, may not be very soluble under acid soil conditions. (See Table 27.) Until recently, it was thought that the addition of lime resulted in the precipitation of aluminum hydroxide from soluble compounds of aluminum. From what has been said, it is doubtful if much aluminum occurs in soluble form in soils of ordinary acidity.

We have just learned that the addition of lime to a solution of aluminum sulfate results eventually in the formation of calcium aluminate, a soluble compound. In order to ascertain whether an alkaline soil may

TABLE 27

ALUMINUM PRESENT IN THE SOIL SOLUTION OF NATURAL SOILS AT THE INDICATED *p*H VALUES

(*Data of Magistad, Soil Science*, 1925)

*p*H value	Al_2O_3
	p p m
4 87	1 2
5 14	2 0
5 30	0 7
5 50	0 3
6 90	0 7
9 01	31 0

contain measurable quantities of this compound, experiments have been carried out on the behavior of calcium aluminate, in the presence of water. On standing, it was found that a deposition of gelatinous aluminum hydroxide always occurred. From this experiment, it seems evident, therefore, that very small quantities of aluminum could occur as calcium aluminate under alkaline soil conditions

The Influence of Aluminum on Soil Constituents. — Owing to the relative insolubility of most of the compounds of aluminum found in the soil, their chemical activity is not so pronounced as in the case of many compounds of other metals The reactivity of aluminum compounds of most interest at the present time lies in the fact that they form colloidal complexes which aid in the retention of many other elements.

The effect of the chloride, acetate and some other ions is to peptize the naturally occurring hydrated aluminum complexes. This effect is not so pronounced in the case of the sulfate, phosphate and silicate ions As these ions normally occur in practically all soils, their effects on the colloidal condition of the aluminum combinations must be given due consideration.

Hydrated alumina, on the alkaline side of its isoelectric point, forms a series of complex anions which only approximate to the simple aluminate ion at hydroxyl-ion concentrations higher than are normally found in agricultural soils. Aluminate anions may form adsorption complexes with various cations present in soils which may exhibit different degrees of peptization, depending on the degree of hydration of the adsorbed cation.

The fact that aluminum forms soluble organic compounds with such substances as sugars, certain proteins, starches, and glycerin probably accounts for the large quantities of aluminum absorbed by plants from

highly organic soils. Attention must be drawn to the fact, however, that the complex colloidal electrolytes and coördinated complex ions may be absorbed directly by plants.

The Relation of Aluminum to Biological Soil Processes. — Aluminum, through the dissociation and hydrolysis of its inorganic salts, may affect, to some extent, the reaction and character of the soil solution, and modify in a material manner the biological reactions of the soil. This would be true, especially, in the case of a poorly buffered soil.

Through the precipitation of phosphates, aluminum may seriously affect the nutrition of soil organisms. It may be said, however, that, even in the case of aluminum phosphate, incipient hydrolysis occurs which may liberate sufficient phosphorus for the bacteria.

Soil bacteria are electrically charged, and therefore aluminum, through the formation of colloidal complexes which bear either positive or negative charges, may exert a precipitating action on these organisms, which on the whole is undesirable.

Silicon

Occurrence of Silicon in Soils. — Next to oxygen, silicon is the most common element found in soils. Sandy soils are made up almost wholly of silicon in the form of silica, SiO_2. In highly organic soils, however, there are very small quantities of this element.

Silicon commonly occurs as crystallized silica or quartz; as feldspars, which are generally looked upon as double silicates of alumina, with potassium, sodium or calcium; as micas, which are primary or parent minerals of varying composition, being silicates of magnesium; as iron or manganese; and as hornblendes and pyroxenes, which include a number of silicates of magnesium, aluminum, calcium and iron. Silicon is never found in soils in the free state.

The minerals obtained by the decomposition of the primary minerals of silicon are known as secondary minerals; they are of great importance in soil fertility. Of these, kaolinite, a hydrated mineral, is probably one of the most important, and is found in relatively large quantities in clay soils.

Water reacts with both primary and secondary silicate minerals, producing a series of hydrates. The hydrated silicates are readily decomposed and are more chemically reactive than the original silicates from which they were derived. They are colloidal in nature. In addition to these, hydrated silicas, formed by the action of water on silica are probably present to a limited extent.

Factors Influencing the Availability of Soil Silicon. — As is true of all minerals, silicon-bearing minerals of the soil are dissolved by soil water,

and the action is intensified by the presence of carbonic acid. That soluble silicon is present in the soil solution can be proved by chemical tests and also by the fact that relatively large quantities are laid down in the cells of a large number of plants, although we have already learned that silicon in the colloidal state may be absorbed by plants.

It has been found that organic and inorganic soil acids increase the solubility of all silicates over that produced by water alone. A striking example of such a reaction is quoted by Lohnis and Fred, who say that the gradual disintegration of a siliceous mountain peak has actually been observed, caused by the formation of acids of nitrogen as a result of excessive nitrification.

The addition of alkaline salts to soils results in an increased solubility of the silicon present The long-continued use of potassium carbonate is quite pronounced in this respect, for this substance reacts with silicon-bearing material which in turn produces in the soil a condition we have already referred to as puddling.

The Influence of Silicon on Soil Constituents. — An aqueous solution of silicic acid reacts with a solution of calcium hydroxide, forming the comparatively insoluble compound known as calcium silicate:

$$H_2SiO_3 + Ca(OH)_2 \leftrightharpoons CaSiO_3 + 2\,H_2O$$

It is thought that reactions similar to that illustrated in the above equation take place under soil conditions. The main reactions of soil silica are, however, physico-chemical rather than purely chemical in nature. Through their highly adsorptive capacity, colloidal-silica-complexes adsorb water and all the elements necessary for plant growth. The quantity of material held in this way is supposed to vary in proportion to the amount of leaching undergone by the soil and by the kinds of plants grown upon the soil. As in the case of other colloidal soil materials, these adsorbed bases are easily replaced by other ions, especially by hydrogen.

An interesting experiment, showing the adsorptive power of colloidal silica as influenced by the hydrogen-ion concentration, is shown by Starkey and Gordon. Two 50-gm samples of silica hydrogel containing 43.7 per cent of water, were freed of impurities. One of the samples was mixed with 200 cc. of a dilute solution of sulfate of potassium and the other with 200 cc. of potassium nitrate solution having the same concentration of potassium as the solution of potassium sulfate. In another test, two 50-gm. samples of the air-dry colloidal material were treated in exactly the same way as the first two samples. Both sets of solutions were brought to varying hydrogen-ion concentrations through additions of sodium hydroxide or hydrochloric acid. The solutions were shaken

for a period of 72 hours and filtered; aliquots were then taken for analysis. The results obtained are given in Table 28:

TABLE 28

ADSORPTION OF POTASSIUM BY THE HYDROGEL OF SILICON

(*Data of Starkey and Gordon, Soil Science*, 1922)

No. of solution	Reaction *p*H	KNO_3		Reaction *p*H	K_2SO_4	
		K adsorbed per gm. gel			K adsorbed per gm. gel	
		dry, mgm	wet, mgm.		dry, mgm	wet, mgm
1	9 873	11 2	11 45	9 890	10 06	10 38
2	8 554	9 86	10 22	8 706	9 93	10 19
3	6 830	2 60	3 68	7 337	4 70	5 37
4	6 390	−1 07	0 37	7 117	1 91	2 79
5	3 360	−1 332	0 114	5 275	1 16	2 36

This table shows very clearly that the adsorptive power of colloidal silica for the potassium ion is markedly influenced by the hydrogen-ion concentration of the medium

The Relation of Silicon to Biological Soil Processes. — Very little work has been done on the relation of silicon-bearing soil materials to biological soil processes. Through their buffering action and other properties, they must have, if present in reasonable quantities, a desirable effect. Excessively large quantities of colloidal silicon materials would have a deleterious effect because the poor physical structure of such a soil would interfere with the gas-exchange (absorption of oxygen and evolution of carbon dioxide), which is so important for biological soil reactions in general.

Phosphorus

Occurrence of Phosphorus in Soils. — Some soils contain no more than a mere trace of phosphorus. Other soils, however, according to Robinson and his co-workers, contain as high as 8 per cent, calculated as P_2O_5. As a rule, however, the average content lies between 0.15 and 0.20 per cent. Phosphorus is found in soils in both organic and inorganic combinations but is never present in the free state. Soil phosphorus has been derived, to a considerable extent at least, from the

mineral *apatite,* having the empirical formula $Ca_5(PO_4)_3(Cl \cdot F)$. It is found in igneous rocks and in practically all soils. Phosphorus is also derived from the mineral *hydroxy-apatite,* $Ca_3P_2O_8 \cdot Ca(OH)_2$. According to Russell, this mineral is quite stable over a fairly wide range of hydrogen-ion concentrations. Soluble phosphorus compounds, when

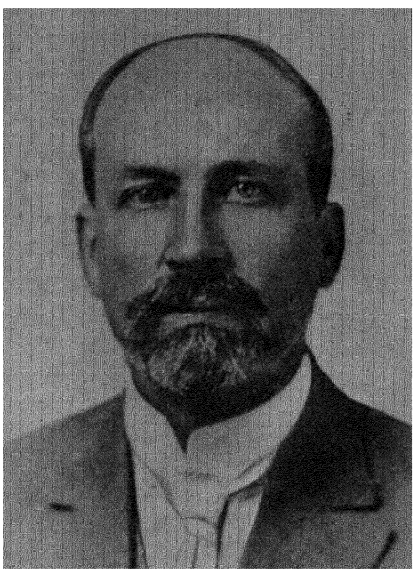


FIG. 27. Dr. Cyril G. Hopkins (1866-1919), prominent agricu..... .....st and agronomist whose researches and teachings played a most important rôle in the promotion of scientific agriculture in the United States. He was an eminent authority on soils and fertilizers. (Courtesy of Dr. H. W. Mumford.)

added to soils, may possibly be changed to this substance. Phosphorus is also thought to occur in soils as tri-calcium phosphate, although the latter has not been isolated. *Vivianite* $(Fe_3(PO_4)_2 \cdot H_2O)$, the ferrous phosphate, and *wavellite* $(3\ Al_2O_3 \cdot 2\ P_2O_5 \cdot 12\ H_2O)$, the basic aluminum phosphate, are fairly widely distributed soil minerals. According to Lyon and Buckman, phosphorus may also occur as aluminum and iron phosphates.

It is universally recognized that some soil phosphorus is combined with the organic matter present, but very little is known at the present time as to the nature of these so-called organo-phosphorus compounds. It

is known, however, that certain definite phosphorus-bearing compounds normally occur in both plant and animal organisms, and these are supposed to be present, for a time at least, in the organic matter that becomes incorporated with the soil. For example, *phytin*, a typical representative of these compounds, is found in the seeds of many cereals and in other plant products. It is also found in many of the soil organisms. Moreover, the so-called *nucleo-proteins* and related compounds are always present in both plant and animal organisms.

According to some investigators, nucleo-proteins and phytin probably make up most of the organo-phosphorus compounds of the soil, with phosphatids next, and a very minute quantity of phospho-proteins. Schollenberger found that, of the total phosphorus present in certain cultivated Ohio soils, 34 per cent (in the upper soil layers) and 20 per cent (in the subsoil) were present in organic form. On the other hand, the figures obtained for virgin soils were 24 and 21 per cent, respectively.

Factors Influencing the Availability of Soil Phosphorus. — The solubility of *tricalcium phosphate*, $Ca_3(PO_4)_2$, in water is no more than one part in 50,000, measured under standard conditions. If the soil phosphorus is held in this form it should be quite unavailable so far as the nutrition of plants is concerned. By the action of carbonic acid, however, the solubility, and therefore the availability of the phosphorus of this mineral, is greatly increased. *Dicalcium phosphate*, $CaH_2(PO_4)_2$, one of the derivatives of tricalcium phosphate, is soluble in water to the extent of one part in 7500. *Monocalcium phosphate*, $CaH_4(PO_4)_2$, another derivative, commonly called superphosphate, is soluble to the extent of one part in 500. Under laboratory conditions, tricalcium phosphate is found to react readily with various organic and inorganic acids, forming soluble products. Similar reactions are thought to take place under soil conditions, but, in view of the fact that the solubility of phosphorus is much more difficult to measure under field conditions as compared with those of the laboratory, conflicting results have been obtained in comparative studies of this kind. As stated elsewhere, an acid condition of the soil may lead to decreased solubility of phosphorus, as for example, by the formation of iron phosphate.

Decomposing organic matter, through the evolution of carbon dioxide, is thought to increase the solubility of soil phosphorus when it is inorganically combined. Notwithstanding the vast amount of investigational work that has been conducted on this subject, however, it has not yet been established as a fact. On the other hand, the presence of decomposing organic matter, in contact with phosphorus-containing minerals, appears to depress their normal solubility. According to

Tottingham and Hoffman, this may be attributed to the assimilation of phosphorus by the soil microorganisms.

TABLE 29

SOLUBILITY OF FLOATS* IN THE PRESENCE OF FERRIC HYDROXIDE, CALCIUM CARBONATE, AND CARBON DIOXIDE

(*Data of Breazeale and Burgess, Arizona Technical Bulletin* 231)

No.	Treatment	Parts per million of phosphorus in solution	
		After 18 hours	After 12 days
1	Floats in solution saturated with carbon dioxide	2 03	2 70
2	Floats with ferric hydroxide in solution saturated with carbon dioxide	1 28	88
3	Floats with calcium carbonate in solution saturated with carbon dioxide	Trace	00

* Finely ground phosphate rock

Hopkins and Whiting have shown that it is possible to increase the solubility of phosphorus-containing minerals by the action of nitrite-forming bacteria They found that these organisms oxidize ammonium sulfate in solution and in the presence of tricalcium phosphate, with the production of nitrous and sulfuric acids. These acids, reacting with the tricalcium phosphate present, caused a considerable quantity of calcium and phosphorus to go into solution. As a result of their work, these investigators believe that the nitrite organisms of the soil have much to do with the availability of phosphorus in inorganic form.

The Influence of Phosphorus on Soil Constituents. — Through the formation of phosphates of iron and aluminum, phosphorus tends on the whole to render these elements less available for plant growth. This effect, however, may be more beneficial than detrimental.

The Relation of Phosphorus to Biological Soil Processes. — The presence of relatively large quantities of phosphorus tends to insure good growth and development of soil organisms. It is believed that phosphorus is associated with the nuclear cellular material, and that the increased production of soluble phosphorus in the soil solution and subsequent assimilation results in increased cell division of these organisms. In general, phosphorus exerts a beneficial effect on biological soil reactions.

Sulfur

Occurrence of Sulfur in Soils. — The sulfur content of soils, as a rule, is quite low, rarely exceeding 0.1 per cent calculated as SO_3. It generally occurs in the form of sulfates and sulfides of the common bases, and in a few instances as sulfites. The latter compounds, however, are fairly unstable, and oxidize to the sulfate form. The presence of sulfates, sulfites and sulfides signifies the occurrence of the corresponding acids.

Sulfur is sometimes present in the free state, where it may undergo reduction or oxidative changes, as the case may be. Under normal conditions, however, oxidative changes predominate, and these may be either biological or non-biological in character.

Some of the sulfur found in soils comes from the atmosphere. The Rothamsted Station reports certain cases where as much as 7.4 pounds per acre were added annually from this source. The quantity of sulfur washed down from the atmosphere is found to vary considerably in different localities, being greater in industrial centers and large cities than elsewhere. Much of the soil sulfur comes from fertilizing materials such as superphosphate, sulfate of calcium, sulfate of potash and similar materials. Sometimes this element is added in the uncombined state. For utilization by plants, free sulfur must undergo oxidation, with the subsequent production of sulfates.

Factors Influencing the Availability of Soil Sulfur. — Where the soil has the proper physical structure, and where it contains the proper biological organisms, the sulfur present is rendered available for plant growth. Under poor conditions of aeration hydrogen sulfide is likely to be formed, which is toxic to plants. Sulfur does not appear to be retained very readily by soils, although some of its compounds are more insoluble than others. Sufficient water is generally present in all soils to dissolve practically all its compounds.

The Influence of Sulfur on Soil Constituents. — The sulfuric acid formed in the oxidation of sulfur, or certain sulfur-bearing compounds, is a powerful chemical solvent and reacts with both organic and inorganic soil constituents. Duley states that the lime requirements of a sulfur-treated soil may be correlated with the sulfates produced. Several investigators have found that sulfuric acid formed in this way has the ability to make insoluble phosphates available to plants.

In all probability, sulfuric acid reacts with tricalcium phosphate, with the formation of monocalcium phosphate:

$$Ca_3(PO_4)_2 + 2\,H_2SO_4 + 4\,H_2O \rightleftharpoons CaH_4(PO_4)_2 + 2\,CaSO_4 \cdot 2\,H_2O$$

Several investigators have shown that it is possible to inoculate a

mixture of tricalcium phosphate and sulfur with a sulfur-oxidizing organism thereby rendering the phosphorus soluble. (See Table 30.)

TABLE 30

COURSE OF REACTION AND TRANSFORMATION OF INSOLUBLE PHOSPHATES IN DILUTE SULFUR–FLOATS–SOIL MIXTURES

[75 gm greenhouse soil, 15 gm floats and 10 gm sulfur]
(*Data of Joffe, Soil Science*, 1923)

Age of culture, weeks	Culture 1		Culture 2	
	Reaction, *p*H	Available phosphorus per 100 gm of mixture, mgm	Reaction, *p*H	Available phosphorus per 100 gm. of mixture, mgm
1	5 0	31 6	5 0	32 7
2	3 4	41 8	3 4	43 9
3	3 0	72 7	3 0	73 4
4	2 8	99 4	2 8	101 3
5	2 8	129 7	2 8	128 9
6	2 6	179 9	2 6	183 6
7*	2 2	186 7	2 2	187 4
8	1 6	183 4	1 6	182 7

* After the seventh week a 1 per cent HCl extract showed that 6 47 gm of sulfur had been oxidized

Through the ionization of sulfuric acid in the soil, it is possible that the hydrogen ions may replace adsorbed bases from colloidal combinations, which would be desirable if the acidity were not too pronounced. If, however, the concentration of the sulfuric acid in the soil becomes high, its deleterious effects may overshadow any beneficial effects it might possibly have.

The Relation of Sulfur to Biological Soil Processes. — *Sulfofication*, or sulfur oxidation, is an important process occurring in all soils; in general, it is brought about by certain bacteria known as *sulfofying bacteria*. Numerous soil bacteria are instrumental in bringing about the decomposition of organic matter, which generally contains sulfur in a form more or less unavailable for plant growth. Hydrogen sulfide, formed in some cases by decomposition, is oxidized to free sulfur:

$$2\,H_2S + O_2 = 2\,H_2O + S_2$$

and the free sulfur may be subsequently oxidized to sulfuric acid:

$$S_2 + 3\,O_2 + 2\,H_2O = 2\,H_2SO_4$$

Although a number of organisms can play a part in the breaking down of organo-sulfur compounds, the main organism bringing about the final oxidation to sulfuric acid is *Thiobacillus thiooxidans*; it is found in all soils. This organism is able to withstand high concentrations of acidity. The optimum reaction for its growth is, according to Joffe, somewhere between *p*H 2.7 and *p*H 4.0. It functions best in fertile soils owing to the presence of sufficient quantities of both organic and inorganic materials to meet its requirements. Such a soil is usually well-buffered thus preventing excessive acid accumulation as a result of sulfur oxidation.

The production of sulfuric acid should affect the main biological reactions of the soil. Theoretically, it should aid the process of ammonification, since it would tend to neutralize the ammonia thus formed. In the case of nitrification, however, the presence of sulfuric acid might be detrimental to the reaction, and careful attention should be given to the lime content of the soil.

Manganese

Occurrence of Manganese in Soils. — On the average, about 0.1 per cent of the soil mass is made up of manganese, calculated as MnO. In some cases, the manganese content is so high that such soils are designated as *manganiferous soils*. Manganese forms many different oxides and hydroxides, a few of which are generally present in soils. Manganese is also present, to a certain extent, as salts of organic and inorganic acids. As a rule, inorganic manganese compounds which occur in soils are quite insoluble.

Factors Influencing the Availability of Manganese in Soils. — The production of organic and inorganic acids by soil bacteria and fungi may lead to increased solubility of manganese. Moreover, the addition of fertilizer materials, such as potash and ammonium salts, has been observed to increase its solubility. On the other hand, its solubility may be limited by the presence of such basic materials as lime.

The Influence of Manganese on Soil Constituents. — References to the reactions of manganese compounds with various soil constituents are numerous. In certain forms, manganese is adsorbed by soil colloids, or it may unite with other organic and inorganic compounds in the soil to form products of varying solubilities. It is quite possible for manganese to function as an acid and as a base.

As a catalyst in the oxidation processes of the soil it plays a very important rôle. Its power as a catalyst, however, is modified to a considerable extent by the form in which it is present. Interesting results have been reported on the effect of some of the organic acids of the soil, especially the hydroxy organic acids, on the activity of this element. In this

TABLE 31

OXIDATION IN DUNKIRK SILT LOAM TREATED WITH MANGANESE SALTS

(Tests made seven months after treatment)

(*Data of Deatrick, Cornell Memoir* 19)

Parts per million of manganese added	Relative oxidation	
	Aloin	Phenolphthalein
0	100	100
Potassium permanganate		
10	101	100
100	136	120
1000	444	200
Manganese dioxide		
10	93	100
100	94	100
1000	95	100
Manganese chloride		
10	105	100
100	113	125
1000	171	167
Manganese carbonate		
10	105	100
100	117	100
1000	128	142
Manganese sulfate		
10	105	100
100	136	130
1000	233	172

respect, manganese appears to have properties similar to those of iron. Through the stimulation of soil oxidation, manganese plays a part in increasing the nutrient concentration of the soil solution.

The Relation of Manganese to Biological Soil Processes. — Soil bacteria respond in much the same way to manganese as do higher plants; a low concentration accelerates their growth and activities, but relatively high concentrations are generally quite toxic. The degree of stimulation or poisoning is determined largely by the hydrogen-ion concentration.

Nitrogen

Occurrence of Nitrogen in Soils. — Nitrogen occurs in relatively large quantities in the atmosphere as an inert gas, and it is this nitrogen that constitutes the ultimate source of soil nitrogen. The various agencies having to do with the conversion of atmospheric nitrogen into forms available for plant growth and the transformation of organic nitrogen to inorganic forms have been studied to a considerable extent. The results of these and related studies have led to efficient agricultural practices from the standpoint of soil productivity.

The nitrogen content of agricultural soils is approximately 0 15 per cent, and it is rarely found in larger quantities. It is very closely associated with soil organic matter, so closely in fact that for one part of nitrogen in soils there are approximately 20 parts of organic matter. An extremely close relationship exists, therefore, between the carbon and nitrogen. This relationship is usually spoken of as the N/C or nitrogen-carbon ratio.

Nitrogen is found in soils in many organic and inorganic combinations. Ammonia, nitrites and nitrates constitute the important types of inorganic nitrogen compounds, but these rarely account for more than 1 per cent of the total amount. The greater quantity of this element is found in the form of proteins and related materials. The various processes having to do with the conversion of the organic nitrogen into plant nutrients, and the processes having to do with the conversion of atmospheric nitrogen into various combinations, are principally of a biochemical nature. We will study the processes in detail from this viewpoint.

The Biochemistry of Soil Nitrogen

The different processes, which are bacterial in character and which have to do with nitrogen changes in soils, are designated by the terms *ammonification*, *nitrification*, *denitrification* and *nitrogen fixation*. These processes will now be considered.

Ammonification. — Ammonification, or the production of ammonia from organic nitrogenous materials, represents an intermediate stage in the production of nitric acid and nitrates. The organisms concerned with this process are bacteria, molds and related forms of plant life.

The power to split off ammonia from proteins is a characteristic of the majority of soil bacteria. Of these, *Bacillus mycoides* is probably the most outstanding. This particular organism is widely distributed in nature and is always found in the upper layers of cultivated soils. It has also been identified in stable manure and similar materials.

Laboratory experiments have shown that *B. mycoides* have been known to ammonify as high as 46 per cent of the nitrogen of a solution containing as much as 1.365 gm. of organic nitrogen per liter in 20 days' time; similar experiments with other ammonia-formers show considerably less activity. Furthermore, cultures of *B. mycoides*, derived from different sources, exhibit pronounced differences in ammonifying powers.

A neutral solution of albumin, if inoculated with *B. mycoides*, becomes alkaline in a very short time. This is due to the accumulation of ammonium carbonate in the solution. While this reaction is taking place, oxygen is absorbed. At the same time, proteoses, peptones, peptides, amino and fatty acids are formed, and the albumin content correspondingly decreases

Under ordinary agricultural practice, protein materials are constantly being added to the soil. Therefore, it becomes desirable to obtain a knowledge of the intermediate changes these materials undergo under soil conditions This is of special significance in studies dealing with the availability of commercial organic fertilizers and manures. Apparently, the first step in the degradation of protein materials in the soil is proteolytic hydrolysis, brought about by active proteases secreted by the microorganisms themselves. In general, the degradation of proteins under soil conditions proceeds along the same general lines as that produced by enzymes and acids under laboratory conditions. In the soil, however, further changes take place after hydrolysis has been effected. Among some of the compounds formed in addition to ammonia may be mentioned carbon dioxide, methane, amines, organic acids, aldehydes, phenol, scatole, indole and, in some cases, hydrogen sulfide.

In all probability, there are two ways in which ammonia is produced: first, by direct hydrolysis, with subsequent destruction of the amide group, $CO–NH_2$, in the protein; and, second, by the destruction of other nitrogenous groups of the protein molecule, for example, the amino group, NH_2. The nature of the chemical changes involved by the formation of ammonia from amino groups by microorganisms depends largely upon the nature of the organisms themselves. In general, however, the

anaerobic organisms reduce amino acids with the liberation of ammonia and formation of fatty acids with the same number of carbon atoms as the original molecule, whereas aerobic organisms oxidize the amino acids to ammonia and organic acids containing one less carbon atom, carbon dioxide being formed in this process.

Factors Influencing the Process of Ammonification

The ammonification process is controlled to a marked extent by a number of different factors, several of which we will now consider:

Moisture. — The moisture content of the soil has a great deal to do with the rate of ammonification. According to Greaves and Carter, ammonification is at a maximum in a soil containing 60 per cent of its total moisture-holding capacity. A higher or lower moisture content has the effect of appreciably slowing up the process. Variations in moisture content of the soil cause a change in the character of the microflora present. A high moisture content leads to a suppression of aerobic bacteria and a stimulation in the growth and reproduction of anaerobic organisms.

Temperature. — Up to the present time, the study of ammonification in its relation to temperature has been limited. Marchal, one of the first workers in this particular field of research, found that between 0° C. and 5° C. very little ammonia was produced in cultures impregnated with ammonifying organisms, but at 20° C. the production was quite marked Further studies showed that 30° C was the optimum temperature for ammonia production.

Aeration. — A plentiful supply of oxygen is necessary for normal ammonification. Because different organisms take part in the ammonification process proper, it is difficult to ascertain just what the optimum conditions are in this respect. It has been found that, where oxygen is deficient, less ammonia is produced than where this element is present in relatively large quantities, but nevertheless even under the former conditions appreciable quantities of ammonia are produced.

Reaction of the Nutrient Medium. — The ammonifying organism (*B. mycoides*) appears to function best in a slightly alkaline medium.

Nature of the Substrate. — The nature of the substrate has a great deal to do with the rate of ammonification. It has been found that concentrated tankage, a highly nitrogenous material, undergoes rapid ammonification when added to the soil. On the other hand, cottonseed meal, which is lower in nitrogen, will not ammonify as readily under the same conditions. Lipman and Brown are of the opinion that the N/C ratio is important in determining the rate of ammonification of nitrogenous

materials, and that the modification of this ratio by organic additions may lead to changes in number and species of soil organisms with subsequent depressed or intensified ammonification.

TABLE 32

THE AMMONIFICATION OF EQUAL AMOUNTS OF NITROGEN OF DIFFERENT MATERIALS IN 100 GRAMS OF SOIL

(Average of 2 samples)

(*Data of Kelley, Hawaii Station Bulletin* 39)

Period of incubation	Nitrogen as ammonia from				
	Casein, 1 072 gm.	Dry blood, 1 000 gm	Soybean cake meal, 1 605 gm	Cotton-seed meal, 2 606 gm	Linseed meal, 2 658 gm
	mg	mg	mg	mg	mg
2 days	58 3	4 0	14 9	17 3	3 5
4 days	74 9	38 5	17 1	37 9	32 9
6 days	75 7	59 6	64 8	42 6	39 9
9 days	75 2	65 6	58 6	40 3	46 0
Per cent of total N converted into NH_3	56 9	49 3	18 7	32 0	34 6

Organic Materials. — The addition of soluble and of insoluble carbohydrate materials such as starch, cellulose and related compounds usually decreases the accumulation of ammonia in soils. The ammonia produced in soils is used to some extent by the organisms themselves for the synthesis of vital compounds, so that, when an excess of carbohydrate material is present, the speed with which ammonia is transformed into protein material may be accelerated. Insoluble materials, on the other hand, may indirectly affect the process of ammonification, in that they may make some of the soil moisture unavailable to the microorganisms. The addition of fresh stable manures greatly enhances the production of ammonia in soils, but this is due, in a degree, to the presence of urea in the manure.

Inorganic Materials. — The presence of inorganic materials does not modify the activities of the various groups of ammonifying organisms to the same extent as does the presence of carbohydrate and protein materials. The addition of lime to an acid soil, however, results in a corre-

sponding increase in the number and activities of actinomyces and certain other soil organisms, but the number of fungi is decreased.

Greaves has found that many salts, when added to a soil in minute quantities, increase the ammonifying power of that soil, but if added in larger quantities they exert a depressing effect, owing to their toxic action on the particular organisms involved. Greaves finds that there is a close correlation between the toxicity of these salts and their effect on the osmotic concentration of the soil solution, showing that, in part at least, the effect is due to osmotic disturbances. Greaves also found that certain ions produced physiological disturbances within the cells of the microorganisms. He found that the relative toxicity of the anions could be written $Cl > NO_3 > SO_4 > CO_3$, indicating that the monovalent anions were more toxic than those of higher valences. With the cations he obtained the series $Mn > Mg > Fe > Ca > Na > K$, thus showing that the divalent cations were more toxic than the monovalent.

In addition to lime, other inorganic materials may aid or hinder ammonification by their effect on the reaction of the soil solution. For example, sulfur additions may be effectual in increasing the acidity to a pronounced degree which would not be desirable. At the same time, however, there is a possibility that the acids produced may react with the ammonia, thus tending, for a time at least, to preserve the original reaction of the soil solution.

Nitrification. — The ammonia formed by ammonifying organisms from nitrogenous organic materials is rapidly converted by nitrite-forming bacteria into nitrous acid. This acid is subsequently changed to nitric acid by nitrate-forming bacteria. These changes take place quite rapidly, and, as a result, but little ammonia or nitrite nitrogen is present in soils at any one time Of the various processes involved in the *mineralization* of organic soil nitrogen, Russell is of the opinion that the formation of nitrous acid takes place more rapidly than either of the other processes, namely, ammonification and nitrification proper. Russell states that ammonification, under any set of conditions, takes place very slowly, and therefore sets a limit to the quantity of nitrites and nitrates produced in a given period of time. Therefore, the measure of the speed at which nitrites are formed in soils does not necessarily state the rate of nitrite formation, as is generally assumed, but rather the rate of ammonification.

The organisms concerned with the formation of nitrites are known as *Nitrosomonas*; the nitrate-formers are designated as *Nitrobacter*. Their work is oxidative in character.

A number of investigators have reported that, in some instances, the oxidative changes in the mineralization of nitrogen may lead to a loss

of this element. It is altogether possible that nitrous acid reacts with ammonia according to the following equation:

$$HONO + NH_3 \rightarrow N_2 + 2\,H_2O$$

The entire subject of nitrification in soils is at present indefinite in character, but it is established that the entire process involves the production of ammonia and nitrous and nitric acids, in the order named. However, Kaserer states that one organism, *B. nitrator*, can oxidize ammonia directly to nitric acid:

$$NH_3 + H_2CO_3 + O_2 \rightleftarrows HNO_3 + H_2O + H \cdot CHO$$
$$H \cdot CHO + O_2 \rightleftarrows H_2O + CO_2$$

Factors Influencing the Process of Nitrification

Moisture. — It is generally known that, other things being equal, maximum production of nitrates occurs only at an optimum moisture content of the soil. According to Greaves, nitrification in 22 different soils, of different physical properties, yielded maximum results when the moisture was between 50 and 56 per cent of the total moisture-holding capacity.

Temperature. — According to Russell and his co-workers, nitrification is relatively slow at 5° C. As the temperature is raised, the rate is found to increase rapidly until at a temperature of 35° C. a maximum production occurs. Above 35° C. the rate falls off gradually, and at 55° C. it ceases altogether.

Aeration. — The nitrifying bacteria, being aerobic in nature, naturally function best in a well-aerated soil. This is well illustrated by the work of Schloesing, who exposed soils for four months to an atmosphere containing varying quantities of oxygen. He found that the production of nitrate nitrogen was closely correlated with the oxygen content of the air. Much of this oxygen, however, was required for the decomposition of organic matter in addition to that required for oxidation of the nitrogen.

Reaction of the Nutrient Medium. — The optimum reaction for the nitrite bacteria lies in the neighborhood of *p*H 8.8. Meyerhof found that nitrate-formers can act, however, in solutions at reactions ranging between *p*H 5.6 and 10.3 with an optimum at *p*H 8.3 to 9.3. The average hydrogen-ion concentration of soils, which is somewhat below *p*H 7, is still sufficient, and, as a matter of fact, nitrification has been observed in very acid soils. Both the nitrate- and nitrite-forming organisms are more resistant to acid conditions in the soil than to the same degree of acidity in solution.

Nature of the Substrate. — Under natural conditions practically all organic materials in the course of time tend to stimulate nitrification.

Lyon and Buckman report that applications of farm manure totalling 20 tons per acre per year for three years resulted in a larger accumulation and probably a larger production of nitrates than was true of untreated soil in close proximity. Gowda, however, found that better results were obtained with 12- rather than 20-ton applications, although even as large a quantity as 36 tons per acre definitely increased nitrate production.

Various green manures, in their relation to nitrification, have been studied. On the whole, it has been found that legumes accelerate the rate of nitrate production, and more so than equal quantities of rye and similar plants. Several investigators have shown that applications of straw prevent, for a time at least, the production of nitrates. In other words, the "N/C ratio" of the substrate has a great deal to do with influencing the rate of nitrate production.

White showed that nitrification of green materials took place at a more rapid rate than equal quantities of the same materials after they had been dried. This is due, in all probability, to a number of reasons among which may be mentioned loss of enzymes and bacteria, and a change in the colloidal condition as a result of drying.

TABLE 33

PERCENTAGE OF NITRIC NITROGEN FORMED IN 100 GRAMS OF SOIL CONTAINING 2 GRAMS OF DRIED BLOOD AND VARYING AMOUNTS AND FORMS OF IRON SALTS

(The untreated soil is taken as 100 per cent)

(*Data of Carter and Goldthorpe, Journal of Agricultural Research*, 1919)

Amount of iron, p p m	Percentage of nitric-nitrogen formed in presence of			
	ferric chloride	ferric sulfate	ferric nitrate	ferric carbonate
None	100 0	100 0	100 0	100 0
2 9	103 4	102 0	95 6	102 5
5 8	101 0	94 2	95 4	104 1
11 6	115 5	67 0	92 4	102 9
23 2 .	116 5	82 8	90 1	96 0
46 5	128 4	97 1	89 7	99 4
93 0	103 4	97 5	86 2	100 7
186 0	102 6	98 9	89 2	105 6
372 3 .	78 5	100 0	53 4	110 7
744 6 ..	32 6	84 3	12 4	117 4
1,116 9... . .	10 9	87 9	7 9	104 5

Inorganic Materials. — A close relationship appears to exist between nitrification and the crop-producing power of soils, and this has not been attributed altogether to the organic matter present. In a fertile soil relatively large quantities of basic elements are always present, and these are instrumental in furthering the activity of the nitro-bacteria in a number of ways. In addition to furnishing nutrient material for the nitrifying organism, they have the power of neutralizing the acids produced in the process.

As in the case of the ammonifiers, certain concentrations of inorganic salts are stimulating, whereas greater concentrations may prove toxic. The toxicity, according to Greaves, varies with the anion and cation and is approximately the reverse of the order we have already learned for the ammonifiers. The toxicity of the anions varies in the following order: $CO_3 > NO_3 > SO_4 > Cl$; the order for the cation is: $K > Mg > Fe > Mn > Ca$. Exceptions to this rule, however, are known. (See Table 33.)

Denitrification. — The term *denitrification* is used to denote the complete reduction of nitrates to elemental nitrogen. It is applied, however, to all processes whereby nitrates are partly or wholly reduced. The organisms bringing about these changes work best under abnormal soil conditions, as, for example, in poorly drained soils, or when the temperature is abnormally low. Hence, it usually plays a minor rôle in ordinary soil practices. The partial reduction of nitrates to nitrites and ammonia does not involve a loss of nitrogen, but when reduction is brought to completion all the nitrogen is lost.

Nitrogen Fixation. — So far, the biological activities considered in relation to soil nitrogen have had to do with the transformation and loss of this element. The organisms involved have no power of adding to the store of combined nitrogen already present. Fortunately, however, just as the nitrogen atoms of the nitrogenous soil material may be released by some organisms, as in the process of denitrification, and allowed to return to the atmosphere in elemental form, so other organisms have the power of converting the elemental nitrogen into combined forms with the final result of increasing the total supply of nitrogen in the soil. Some of these organisms are capable of exercising their functions independently of higher plants and others are not. The first process is known as *non-symbiotic nitrogen fixation*; the latter is referred to as *symbiotic nitrogen fixation.*

Non-symbiotic Nitrogen Fixation. — The most important of the non-symbiotic nitrogen-fixing organisms found in soils is the aerobic bacillus, *Azotobacter.* A great number of other soil organisms, however, including fungi, have the property of fixing nitrogen, but only to a limited extent.

According to Lyon and Buckman, the amount of nitrogen fixed by the non-symbiotic organisms, though subject to wide variations, averages about 25 pounds per acre annually, or about five times the amount added by rainfall.

The fixation of nitrogen leads eventually to the formation of protein. It is quite possible and highly probable, therefore, that various intermediate compounds used in the synthesis of proteins are first synthesized by these organisms. According to Lohnis and Fred, some investigators are of the opinion that certain bacteria are able to unite nitrogen to hydrogen, forming ammonia, whereas others oxidize nitrogen directly to nitrous and nitric acids, but their opinions have not been fully substantiated.

The quantity of atmospheric nitrogen assimilated by Azotobacter is materially affected by the following factors:

Moisture. — Azotobacter are extremely resistant to dry conditions, under which they assume an inactive form. They require considerable quantities of water for good growth and development, the amount required depending on the kind of soil but averaging approximately 20 per cent of the total moisture-holding capacity of the soil.

Temperature — Azotobacter functions best at temperatures between 20° C. and 30° C., although some nitrogen is fixed at lower and some at higher temperatures.

Aeration. — Naturally, as the process of nitrogen-fixation involves the process of oxidation, the soil must permit free circulation of atmospheric gases. The deleterious effects of a high water content may be attributed in a large measure to a lack of oxygen.

Reaction of the Nutrient Medium — The optimum reaction for Azotobacter lies between *p*H 7.0 and *p*H 7.4.

Organic Materials. — Organic matter is one of the principal factors affecting the extent of the activities of the Azotobacter. Its beneficial influence is due, in part at least, to the better circulation of the air its presence insures, and to its moisture-holding capacity. The inorganic materials making up part of the organic matter may likewise prove beneficial.

Probably the main reason why organic matter is so desirable from the standpoint of Azotobacter is that it may be used as a source of energy. These organisms require considerable quantities of energy-producing materials for their activities. It is not surprising, therefore, to find that the addition of simple carbohydrates, under otherwise optimum conditions, generally stimulates the Azotobacter to greater activity.

The N/C ratio, so far as is known, plays no part in its relation to these organisms. Investigational work has shown that non-legumes and

straws which are high in carbon and low in nitrogen are just as satisfactory as are the legumes which are high in nitrogen and low in carbon. It has been stated that these organisms require 90 parts of carbon for the fixation of one part of nitrogen. Moreover, it has been found that the

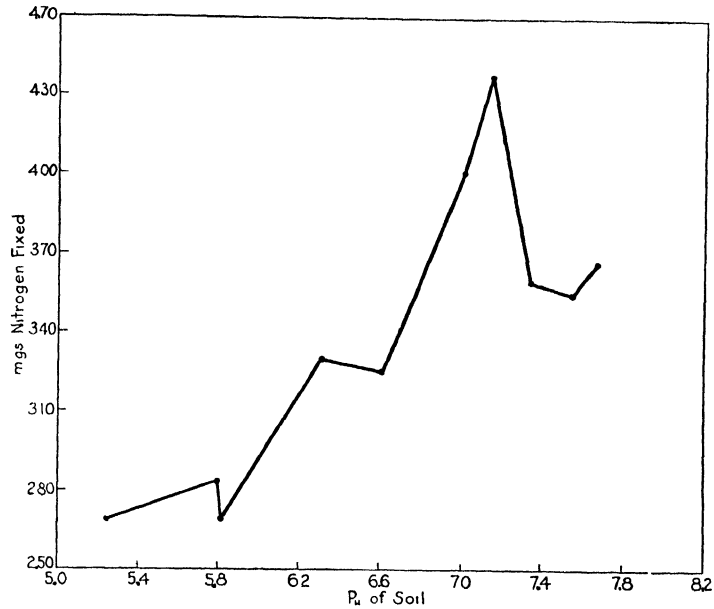


FIG 28 Influence of soil reaction on nitrogen fixation

amount of available phosphorus present has a tendency to increase their rate of carbon assimilation

Humus has a very favorable influence on *azofication*, as this process is sometimes called. It appears that a better utilization of organic matter takes place after it has been humified Rapidly decaying organic matter and stable manure, however, exert a remarkably stimulating effect on azofication.

Inorganic Materials. — Nitrogenous compounds in small quantities do not appear to interfere with non-symbiotic nitrogen fixation. In fact, nitrate additions, and to a certain extent the additions of ammonium salts as well, have been known to stimulate this process. The results obtained by the use of large quantities of these salts, however, invariably show less nitrogen fixed from the air. The presence of nitrates in normal quantities appears to favor multiplication of the organisms involved, but in larger quantities seriously reduces their physiological

efficiency. An abundance of nitrate nitrogen in the soil, therefore, is not conducive to efficient non-symbiotic nitrogen fixation.

Azotobacter respond to additions of phosphorus and sulfur in practically all cases. This is probably due to the effect of these elements on protein formation. In general, however, these organisms require the same mineral nutrients for their growth as do the higher plants. For this reason, a soil should be fertile in respect to available mineral nutrients as well as organic materials for the process of azofication.

Symbiotic Nitrogen Fixation. — It has been known for a great many years that legumes possess the power of enriching the soil with nitrogen, and that this nitrogen is obtained from the atmosphere through the agency of bacteria living with them symbiotically. The organisms involved are found within the nodules which are generally present in greater or less numbers on the rootlets of the legumes, and, although the organisms obtained from the nodules of one plant may differ from those in the nodules of others, certain characteristics are peculiar to all of them. The main organisms involved have been known for many years as *B. radicicola* and *Rhiz leguminosarium.* At present, the term *B. rhizobium* is used to include both these organisms *B. rhizobium* is capable of fixing nitrogen non-symbiotically as well as symbiotically, although the latter process is by far the most important.

Some legumes are readily infected with *B. rhizobium,* whereas others are not. In any case, however, infection is made possible more readily if the plants are in a weakened condition, as for example, in nitrogen starvation.

TABLE 34

THE EFFECT OF NITRATES ON GROWTH AND NODULE FORMATION IN SAND CULTURES

(*Data of Fred, Whiting and Hastings, Wisconsin Station Research Bulletin* 72)

No	Nitrogen as NO_3 in 100 cc of culture solution, mg	Alfalfa nodules	Vetch nodules
1	None	Numerous	Numerous
2	1	Numerous	Numerous
3	5	Numerous	Few
4	10	Few	Very few
5	15	None	None

Under optimum conditions, a legume is found to thrive in the absence of *B. rhizobium.* On the other hand, a culture of these organisms may

be developed in the absence of legumes. It has been found, however, that symbiosis or *parasitism*, as it is sometimes called, is beneficial for both the legumes and the bacteria, the legumes gaining nitrogen, while the bacteria gain organic energy-producing materials. The biochemistry of the process of symbiotic nitrogen fixation is not well understood. All theories thus far proposed appear to be purely speculative in character. It is recognized, however, that the final products of the process include proteins, asparagin, and various aliphatic and carbocyclic amino acids.

The quantity of nitrogen actually fixed by symbiotic legume organisms is subject to great fluctuations, owing to a number of external factors, a few of which will be mentioned later. According to Lipman, however, the total annual addition of nitrogen to the soil from the growth of legumes in the United States is equivalent to 1,750,000 tons of nitrogen.

Factors Influencing Symbiotic Nitrogen Fixation

Moisture. — A relatively high moisture content of the soil is necessary for efficient symbiotic nitrogen fixation. When legumes are grown in moist soils, a good development of the root system generally results, presenting a larger surface of young tissue to the action of the infecting organism. Not only is a less extensive root system developed under dry conditions, but the tissues are not as tender, since they become covered over with a thickened cuticle and are consequently more resistant to the entrance of the organisms. The optimum moisture content for the activity of *B. rhizobium* is apparently the total moisture-holding capacity of the soil itself. Larger quantities of water may exclude the necessary nitrogen and oxygen from the roots.

Temperature. — The optimum temperature for the metabolism of the legume organisms, according to Greaves, lies between 18° C. and 20° C.

Aeration. — The legume bacteria are aerobic in character, and the exclusion of air, therefore, inhibits nodule formation. More nodules are found near the surface than at lower depths, mainly on account of the differences in the oxygen content of the soil atmosphere

Reaction of the Nutrient Medium. — The optimum hydrogen-ion concentration for the growth and development of the legume organisms is at or near the neutral point. Nodules are formed, however, under any condition of the soil in which the legumes may grow.

Nature of the Substrate. — Under optimum conditions the legume bacteria obtain their energy-producing substances from certain of the plant compounds present, particularly from the soluble carbohydrates. Wilson, however, has shown that the addition of soluble carbohydrates or their derivatives stimulates nodule production in soil cultures.

On the whole, however, only these substances aiding in the growth of the host plants will favorably influence nodule formation.

Inorganic Materials. — Inasmuch as the legume bacteria require the same compounds for their growth and activity as the host plants, any addition stimulating the growth of the latter should likewise stimulate the growth and development of *B. rhizobium.* An outstanding exception to this rule, however, is that an application of nitrate nitrogen, though probably increasing the growth of the legumes, has a tendency to inhibit nodule formation, although the organisms retain their vitality. It is the opinion of some authorities that, as in the case of non-symbiotic organisms, the addition of nitrates decreases their nitrogen-fixing powers by a sort of compensatory action. It is not altogether impossible for legumes grown in a very fertile soil to be immune to the attack of nodule-forming organisms because of the greater vitality of the plants so grown. At any rate, the chance of infection of such plants is reduced to a minimum.

The Relation of Nitrogen to the Availability of Soil Constituents. — It has been shown that the principal nitrogenous compounds found in soils are proteinaceous in nature and that the nitrogen of these compounds is slowly changed to nitrous and nitric acids. Both nitrous and nitric acids are highly dissociated and react with many of the soil constituents, especially with those of inorganic origin forming soluble compounds. Nitrous acid, being formed before nitric, exerts a greater solvent action. As a matter of fact, calcium carbonate and nitrous acid react to form calcium nitrite, and this compound is later oxidized to the nitrate form by the nitrifying bacteria. Moreover, nitrous acid reacts with tricalcium phosphate, if present, forming soluble monocalcium phosphate

Organic Matter

Attention has already been called to the fact that ordinary agricultural soils are composed of both organic and inorganic substances. Although in most cases the inorganic substances predominate, this is certainly not true of others. Some soils, such as peats, are made up almost wholly of organic substances. On the average, however, the amount of organic matter in the soils of the United States, according to Schreiner and Shorey, is practically 2 03 per cent.

Organic matter, as it occurs in soils, is derived from the tissues of both plants and animals. Most of it, however, is of plant origin and has been derived from green or stable manures or from the residual tissues left in the soil after the removal of the principal plant parts. Soil organisms, however, contribute to the total supply. According to Lyon and Buck-

man, the bacteria of one acre of soil weigh between 400 and 800 pounds. It is apparent, therefore, that the organic matter of soils must be of a heterogeneous nature. Moreover, it is constantly undergoing chemical changes, eventually resulting in the formation of a fairly stable material, usually alluded to as *humus*, which in the course of time becomes almost wholly incorporated with the soil

Numerous investigators have been conducting experiments on soil organic matter from the very inception of soil science up to the present time. As a result of these investigations, several facts have become well established. On the whole, however, our knowledge of the subject is still far from being satisfactory.

The Origin and Classification of Soil Organic Matter. — Since soil organic matter comes largely from higher plants, it seems advisable to consider the chemical nature of such materials Plant materials are looked upon as consisting largely of fats, proteins and carbohydrates. In addition to these compounds, there are, however, a number of other important ones, such as hydrocarbons, glucosides, alkaloids, phenols, and their derivatives

The complex organic compounds are changed into simple compounds in the soil, and the products of the chemical reactions involved are practically the same as those obtained under laboratory conditions. Of the reactions involved, those effected by enzymes are among the first to take place, and are to a considerable extent hydrolytic in character. As a result of these changes, various chemical compounds are formed; they include practically all compounds intermediate between the original complex molecules and the final gaseous products. For example, proteins break down into proteoses, peptones, peptids and amino acids. On oxidation, the amino acids lose nitrogen and become ordinary fatty acids On further oxidation, these fatty acids give rise to water and carbon dioxide. In a similar manner the complex monosaccharides, on hydrolysis, give rise to simple sugars which are subsequently oxidized first to organic acids and finally to carbon dioxide and water. Less is known regarding the degradation of fats in the soil, but it is assumed that they undergo a slow hydrolysis and on subsequent oxidation form carbon dioxide and water.

In addition to the compounds mentioned above, a number are synthesized by various soil organisms which are quite similar to those found in higher plants. These undergo decomposition after the death of the organisms.

Plant compounds are quite similar to animal compounds, and they form practically the same substances on decomposition. The character of the residual material, therefore, is influenced to a far greater degree

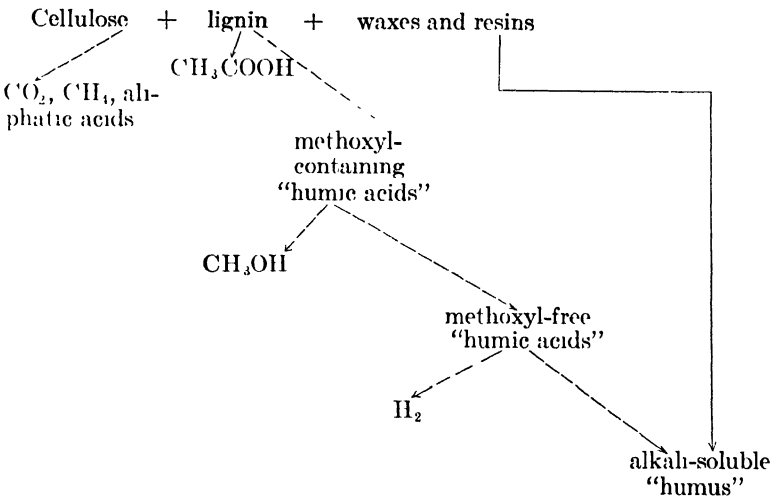
by the environmental conditions than by the source of material. From what has been said, it may appear that the decomposition of organic matter in soils is a relatively simple process, but this is not entirely true. Some compounds are very resistant and persist in the soil practically unchanged for a long period of time. Moreover, because of oxidations, reductions, condensations and other reactions, many materials which are normally susceptible to rapid decomposition may give rise to material extremely resistant to further chemical change. On the whole, the study of soil organic matter is one of our most complex soil problems, especially those studies dealing with its formation. It is known, however, that any kind of organic matter, when incorporated with the soil, eventually gives rise to a complex mass of dark-colored substances which have practically no relation to the original materials, and which in the free state are soluble in dilute alkalies. According to Waksman, Achard was the first investigator to show that alkalies dissolved these dark-colored substances.

How the residual soil organic matter was derived was given considerable attention by de Saussure, who stated that these materials originated from vegetable matter after subjection to the combined action of air and water. Still later, the term *humic acid* was first used by Dobereiner for the residual material, regardless of its origin, and a chemical study was first made of this so-called humic acid by Sprengel in 1826. Sprengel stated that free humic acid occurs in a soil low in bases, giving to the soil an acid reaction. He was also of the opinion that the free humic acid was more resistant to biochemical changes than the combined humic acid of fertile soils.

Various studies similar to those of Sprengel's were soon carried out, with the result that the so-called humic acid could be resolved into a number of different substances, hence proving that it was not a definite compound. In 1919 Oden separated the soil organic matter into four complex groups based primarily on solubilities. In the case of that portion insoluble in dilute alkalies, the name *humus coal, ulmin* or *humin* was given. That portion soluble in dilute alkalies and precipitated by acids Oden designated as humic acid. If precipitated by alcohol rather than acids, it was found that the properties of the material were quite different from the properties of humic acid. Hence, the term *ulmic acid* was employed for this group of substances. The remainder of the original organic residual material, namely, that portion soluble in both dilute alkalies and acids, was designated as *fulvic acid.* This classification as submitted by Oden is quite similar to the classification submitted by a number of investigators who worked in this field of research almost a hundred years ago.

Many fundamental studies have aided very materially in obtaining information relative to the composition of soil organic matter. The work of Schreiner and Shorey is of special interest. They have proved quite definitely that the organic matter is composed of an extremely large number of individual compounds. Propionic, malic, levulinic, oxalic, succinic, hydroxy-stearic and other acids were isolated. In addition to many other compounds, phytosterol, agosterol, cholesterol, some pectins and pentosans were isolated and identified.

The Decomposition of Organic Matter and the Formation of Humus. — Some of the early workers are of the opinion that cellulose, being relatively stable as compared to other organic compounds, was the precursor of humus. Bacteria were believed to oxidize cellulose to oxycellulose, which later changed to humic acid or humus. Later, however, it was demonstrated that cellulose, as such, did not play a predominating rôle in this process.



(*Data of Waksman, Soil Science*, 1926)

On the whole, lignin is more resistant to the decomposition action of soil bacteria than cellulose. In 1889, Hoppe-Seyler called attention to the rôle played by lignin in the formation of humus, and it is now recognized that lignin decomposes at a very slow rate under soil conditions.

That proteins play a part in the formation of humus has also been suggested by various investigators. Studies of the behavior of proteins in the laboratory, when subjected to the action of acids or alkalies, showed that various colored products were obtained. This has been attributed,

in a measure, to a series of reactions between the carbohydrates present in some proteins and amino acids such as tryptophane or tyrosine. It now appears that the aromatic groups of the original protein molecule, as a result of the activity of certain soil organisms, contribute to humus formation. According to Waksman, homogentisic acid is formed from tyrosine by the action of soil fungi. Other substances, also similar to humus, but containing no nitrogen, have been obtained directly from carbohydrate material by condensation or oxidation. It is a well-known fact that a carbohydrate such as glucose may react with acids forming hydroxy-methyl-furfural. This compound, upon condensation, tends to form a humus-like substance. Inasmuch as this material has been isolated from the soil, the same kind of change is thought to have taken place in its formation.

A number of investigators are inclined to the opinion that filamentous fungi play a very important part in humus formation. Wehmer found that higher fungi were effective in decomposing wood, completely breaking down the cellulose portion of the molecule, and converting the remainder of the lignocellulose, namely, the lignin, into humus.

Lignin, on treatment with alkalies, yields dark-colored compounds and finally humic acids. It has also been shown that these acids are closely related to the so-called lignic acids. Although lignin itself is free from nitrogen, this element is quite likely to be found in the resulting humus and apparently comes from the nitrogenous products of the soil organisms as they undergo decomposition If this is true, humus may be looked upon as a mixture of partially changed lignin and proteins, or their cleavage products.

At the present time, no theory fully explains the nature of humus, or even that part soluble in dilute acids, namely, humic acids. It is not altogether impossible that all the above-mentioned reactions play a part in its formation.

Factors Influencing the Decomposition of Soil Organic Matter. — Of the factors involved in the decomposition of soil organic matter, among the most important are the type of the organic matter, the nature and number of soil organisms, the character of the soil and the kind and quantity of inorganic soil constituents present.

Nature of the Organic Matter. — The rate of decomposition of the organic matter depends largely on the quantity and quality of the material and on its physical state. It has been shown that organic matter, which normally decomposes quite slowly, will decompose much more readily if finely pulverized. Moreover, green plants decompose more quickly before drying than after drying and subsequent remoistening. This is due partly to a greater exposure of surface for chemical action and

partly to the presence of more enzymes, the drying process being more or less destructive to the enzymes normally present.

If the material is rather rich in sugars (hemicelluloses and celluloses) and poor in lignin, the rate of decomposition is more rapid than when the reverse is true. The presence of lignin tends to prevent, in a measure, the rapid decomposition of the cellulose material with which it is chemically combined. Lignin bodies have a tendency to accumulate in the soil and especially in soils which are not conducive to good bacterial development. Organic matter of a fatty nature does not decompose very readily under soil conditions. As a matter of fact, almost all lipid materials are quite resistant to the action of soil bacteria.

The quantity of nitrogen present, relative to carbon, profoundly affects the rate of decomposition of the organic material. As a matter of fact, quite a definite relationship seems to exist between the amount of nitrogen and the carbon present. Materials having a wide N/C ratio will not decompose as readily as those having a narrower ratio unless, possibly, the material used is quite soluble. According to Waksman, this is specially true for decomposition by soil fungi, and the reason is

TABLE 35

RATES (I) AND COURSES (II) OF DECOMPOSITION OF SOME ORGANIC MATERIALS

(*Data of Starkey, Soil Science*, 1924)

Organic matter used	After different periods of incubation (days)										Totals
	1	2	3	4	5	6	7	8	9	10	
I Daily production of CO_2 in mgm											
Cellulose	1 83	1 84	1 83	7 14	6 87	28 05	28 04	27 00	27 00	33 46	163 06
Rye straw	74 29	29 31	37 83	22 68	16 65	12 59	13 08	12 93	9 77	9 77	239 20
Alfalfa meal	106 63	67 83	68 24	37 12	27 70	15 18	14 58	13 76	8 33	8 32	368 38
Fungous material	79 80	44 44	63 70	62 46	37 92	41 34	33 44	27 80	16 58	16 58	444 06
Dried blood	7 23	41 49	68 30	82 33	82 32	56 72	48 61	31 10	26 05	23 15	467 60
Dextrose	84 98	156 52									
II Percentage decomposed at daily intervals											
Cellulose	0 23	0 45	0 68	1 55	2 39	5 84	9 28	12 59	15 90	20 01	
Rye straw	11 13	15 52	21 19	24 64	27 13	29 02	30 98	32 91	34 38	35 84	
Alfalfa meal	15 98	26 14	36 37	41 98	46 14	48 46	50 64	52 71	53 95	55 20	
Fungous material	11 96	18 62	28 16	38 20	46 20	52 40	57 41	61 75	64 06	66 54	
Dried blood	1 05	7 08	17 01	28 98	40 95	49 19	56 26	60 83	64 61	67 98	
Dextrose	12 37	35 16									

partly, if not wholly, due to the fact that considerable quantities of nitrogen are necessary for the nutrient requirements of the fungi, as well as of other soil organisms. Soil fungi decompose approximately 30 parts of cellulose to every part of nitrogen assimilated. For this reason, a material high in nitrogen, such as alfalfa, will naturally decompose much more rapidly than cereal straws, which are low in nitrogen. (See Table 35.)

A factor of considerable importance in the decomposition of organic matter is the relative bacterial content of the material. Stable manure, for instance, carries to the soil myriads of bacteria. This increase in the number of soil organisms materially hastens these decompositions.

All organic materials added to the soil tend to form humus on decomposition. White and Holben have shown that even pure sucrose, when added to an inoculated soil devoid of nitrogen and organic matter, yielded humus in the course of time. Humus itself is slowly decomposable under soil conditions, otherwise there would be a far greater accumulation than occurs normally. A soil of known composition has been known to lose much of its humus because of continuous cropping. A loss has also been observed in the case of summer fallowing.

Nature and Number of Soil Organisms. — Naturally, the number and kind of soil organisms would have much to do with the rate of organic matter decompositions. Several factors influence the number and activity of the organisms which are concerned with the process of decomposition. The optimum temperature is from 15° C. to 25° C., although they still function above and below these temperatures. Moisture is also a predominating factor. The optimum quantity of moisture for sandy soils is about 10 per cent, and considerably larger quantities should be present in a heavy soil. Excess water is undesirable because it would interfere with the quantity of oxygen available for the aerobic organisms, the predominating organisms of decay.

The acidity of the soil affects the multiplication of all organisms. On the whole, a neutral or slightly acid reaction is more favorable for decay-organisms than extreme conditions of either acidity or alkalinity. It is a matter of common knowledge that additions of organic matter to the soil modify the character of the microflora as well as the number of organisms present, depending upon its chemical composition. On the whole, however, all organic additions which may be used as sources of energy result in an increase in the number of organisms present.

Owing to the fact that considerable organic and inorganic nutrients are required by the decay-bacteria, fertile soils contain more of these organisms than non-fertile soils. Even in such soils, however, only the upper layers offer them a suitable environment. The number in the

TABLE 36

INFLUENCE OF 0.5 PER CENT GLUCOSE UPON THE DEVELOPMENT OF MICRO-ORGANISMS IN THE SOIL AFTER 2 DAYS

(Data of Waksman and Starkey, Soil Science, 1924)

Soil number	Annual treatment	pH	Fertility	Fungi		Bacteria + Actinomycetes	
				Start	End	Start	End
5A	Manure, minerals*	5 5	Very fertile	87,300	109,400	6,500	43,000
5B	Manure, lime, minerals*	6 7	Very fertile	19,700	24,000	9,360	103,000
7A	Untreated	5 1	Very poor	115,700	82,000	3,860	22,200
7B	Lime alone	6 5	Fair	20,000	25,400	7,760	22,000
9A	$NaNO_3$, minerals	5 8	Good	73,300	102,000	8,000	47,400
11B	$(NH_4)_2SO_4$, minerals, lime	6 0	Good	25,700	36,000	8,400	30,880

* Minerals = potassium salt and phosphate.

subsoil is quite low, and less are found in light than in heavy soils, owing partly to the better aeration of the former. As the sunlight is very deleterious to these organisms, few, if any, are present on the outer surface of the soil

Nature of the Soil — From what has already been said, it is apparent that the kind of soil affects the decay of organic matter either advantageously or disadvantageously. The easy permeability of sandy soils might be expected to favor the decay of organic matter. Its relatively low moisture-holding capacity, however, is a retarding factor. It is apparent, therefore, that the various soil factors involved in the decomposition of organic matter may help or counteract one another, and the general effect may be due to the predominating quantitative relations In the above case, the supply of moisture is the controlling factor of organic decomposition in a soil of this character. In humid regions, on the other hand, the decomposition may proceed so readily in sandy soils that very little residual organic matter remains.

Heavy soils retain moisture to a relatively great extent, but there is generally a lack of air and, moreover, the temperature is relatively low. According to Russell and Appleyard, temperature is the chief factor in determining the extent of biochemical changes in these soils. However, their permeability to air is a factor of importance

Russell and Appleyard are of the opinion that the beneficial effects of a rain on moist heavy clay soils are due in a measure to the oxygen dis-

solved in the rain, thus enriching the soil atmosphere in respect to this element.

Inorganic Constituents. — Attention has already been called to the fact that relatively large quantities of nitrogen increase the rate of organic matter decay. Various other compounds occurring naturally in soils, or added as manures, influence the number, species, and activities of soil organisms. Some may serve as food; others may change the physical characteristics of the soil. Such changes brought about in this environment must necessarily influence the growth and development of the microorganisms. Greaves has shown that in manures, certain materials are beneficial whereas others are not, and usually those that are more toxic in lower concentrations are the greatest bacterial stimulants.

Any inorganic material which changes the hydrogen-ion concentration in a material way will also tend to modify bacterial activity. According to Lohnis and Fred, when humic acids are present in considerable quantities they play a prominent rôle in organic matter decomposition. Unless these acids are neutralized by inorganic bases, the normal humus material will not accumulate to any considerable extent, because of the inactivation of the majority of soil organisms. When the reaction is adjusted by liming, a vigorous bacterial activity becomes evident immediately, even in peat soils. For this reason, excessive liming may be detrimental because of too rapid decomposition of the organic materials.

The Relation of Organic Matter to Soil Fertility and Plant Growth. — Organic matter plays a number of different rôles in soil fertility, a few of which will now be considered.

Relation to the Physical Condition of the Soil. — The important physical conditions of a soil from the standpoint of fertility generally include its moisture-holding capacity, absorption, heat conductivity and granulation. Ordinarily, the average moisture content of a soil is found to parallel very closely the amount of organic matter present. For example, loam soils have a tendency to hold more moisture than sandy soils, and it is a well-known fact that loam soils contain more organic matter than sandy soils. Water, so held, is almost wholly available for plant nutrition.

Soils having large quantities of organic matter generally possess a dark color, much darker than those of a low organic content. This constitutes a factor which influences soil temperature, the dark soils being warmer than those lighter in color, other conditions being equal. It has been found that a highly organic soil warms up much more readily when dry than when moist. Moreover, a soil high in organic matter does not freeze readily as compared with a soil low in organic matter. It may be seen, therefore, that the organic content of a soil may control,

to a certain extent, radical temperature changes. Humus improves the condition of a soil by making a sandy soil more compact and heavy soils more mellow. In other words, humus forms loams, for a sandy loam is simply a sandy soil rich in humus; a clay loam is a clay soil rich in humus.

Relation to the Supply of Available Nutrients. — Through the decay of organic matter, certain mineral substances are liberated which become available to plants. Moreover, gases given off during this process may react with other materials in the soil liberating further supplies of nutrient materials. For example, insoluble phosphates are acted upon by carbon dioxide, with the formation of available phosphates:

$$Ca_3(PO_4)_2 + 4\ H_2CO_3 \leftrightharpoons CaH_4(PO_4)_2 + 2\ Ca(HCO_3)_2$$

Attention has already been called to the fact that the adsorption of soil constituents by colloidal substances may be lowered somewhat by an increase in the concentration of hydrogen ions. It is mainly for this reason that decomposing organic matter, through the formation of organic and inorganic acids, is able to increase the availability of adsorbed material. On the other hand, it is possible for the availability of some materials to be lowered through precipitation, as for example, the formation of calcium humate from calcium and acid humic materials. It is through such chemical and physical unions that the leaching of soluble nutrients is prevented to a considerable extent.

Relation to the Reaction of the Soil. — Through the formation of acid decomposition products, especially where sufficient basic materials are lacking, an increase of soil acidity results, and such soils as those containing large quantities of organic matter invariably yield water extracts which are acid in reaction. White has found, in the decomposition of green manure under soil conditions, that there was a tendency towards a reduction in acidity. He attributes this to the liberation of basic material on the decay of green manures. However, it was found that, at the end of the experiment, there was a measurable increase in the acidity as compared with soils where green manures were not added. His experiment was carried out on an acid soil, and it is probably for this reason that the results obtained were so striking. In the mineralization of organic nitrogen, nitrates are formed, some of which may hydrolyze, thus tending to increase the acidity, even if no other factor was taken into consideration. On the whole, therefore, organic matter, on decomposition, tends to make a soil acid.

Relation of Organic Matter to Biological Soil Processes. — Organic matter aids the various biological processes of the soil by furnishing energy-producing materials for the bacteria and other organisms involved. Wollny, however, has found that not all organic materials are

helpful so far as bacterial activity is concerned. The presence of resins, waxes, lipids and tannins, frequently occurring in peats, is partly responsible, for the slow decomposition normally taking place. For example, sawdust, a tannin-containing material, may exert a toxic effect in respect to ammonification and nitrification, and these effects may persist in the soil for considerable periods of time. It must not be forgotten, however, that sawdust has a very wide N/C ratio. This, as well as the tannin content, should be given due consideration in accounting for its toxicity.

Relation to Soil Sanitation. — One of the principal explanations advanced for the fact that certain soils become infertile is the *soil-toxin* theory, which assumes that this condition is due to the presence of toxic organic matter in the soil. According to Schreiner and Reed, many amino acids obtained as cleavage products of proteins are not toxic, but others, such as tyrosin and products resulting from it, are toxic. Moreover, if the soil conditions are extremely unfavorable for normal biological processes, proteins may give rise to cresols, phenols, aromatic acids, cholin, neurin and other substances harmful to plant growth.

Higher plants may contain chemical compounds other than protein derivatives which in certain concentrations are toxic to other plants. Coumarin may be cited as an example. This compound may, however, be synthesized from carbohydrates by means of molds. Another example is quinone, which normally occurs in measurable quantities in many plants. This compound may also be synthesized from proteins by means of fungi.

A number of toxic compounds, in addition to those mentioned above, have been isolated from soils, and their general effect on the growth of plants has been studied under controlled conditions. It has been found that many of these compounds lose their toxicity when partially oxidized. It is evident, therefore, that imperfect soil oxidation may lead to an accumulation of these toxic organic compounds. Under proper conditions of oxidation all these compounds may be sufficiently changed to become beneficial, rather than detrimental, to plant growth.

Relation to Plant Growth. — Organic matter may be an aid or a detriment to the growth of plants. On the whole, however, its effect on plant growth is decidedly beneficial, and this beneficial effect is not due to humus alone, but may be attributed to the soluble decomposed products as well as the products given off by the organisms producing the decomposition. These soluble substances are present in all soils in variable concentrations. There is no apparent reason why higher plants should not absorb and utilize some of the soil organic matter. As a matter of fact, plants have been known to utilize organic nutrients advantageously. For example, fungi and bacteria certainly can utilize soluble

organic compounds directly, and there should be no reason for assuming that the permeability and metabolism of these lower forms of plant life are fundamentally different from those of a higher order.

When a virgin soil is cleared of its native vegetation and prepared for the cultivation of plants it is generally found to be quite fertile. One of the reasons for this is attributed to the relatively large quantity of organic matter usually present. When plants are allowed to grow under natural conditions, they assimilate certain of the inorganic constituents of the soil. After they die and become incorporated in the soil, this inorganic material becomes available once more for plant growth, otherwise it is held in physical and chemical combinations until complete decay has taken place. Moreover, in addition to the prevention of leaching and the holding of inorganic materials available for plants, the organic matter may yield the elements in a form which is beneficial from the standpoint of normal plant growth. For example, the potassium absorbed under these conditions may be in basic form, in which it acts best in plant metabolism.

The soil solution as it normally occurs in a virgin soil possesses, as a rule, all the necessary inorganic materials for plant growth, but the concentration of these materials is never very high. If, however, the solubility equilibrium is disturbed through the withdrawal of some of these nutrients, the normal conditions of equilibrium are again soon re-established. This is due, primarily, to the presence of organic matter, which controls the solubility by means of adsorption. The tendency, therefore, for a soil rich in organic matter is to possess a soil solution which is of the proper *physiological balance* so far as the growth of crop plants is concerned. Furthermore, no great concentration of unnecessary mineral substances is present as there would possibly be under more artificial conditions, or in other words, where the mineral nutrients are added to the soil in the form of commercial fertilizers

When a virgin soil is placed under cultivation, the organic matter has a chance to become oxidized very rapidly. The organic matter so lost should be replaced by additions of other organic materials. In this practice, ordinary stable manure is sometimes used. Such a material, however, is unbalanced in respect to the necessary mineral nutrients, and such applications may seriously interfere with plant growth. On decay, the residual organic material is found to be similar to that present in the original soil.

Soil Moisture

A fertile soil is one which possesses, among other properties, the power of holding sufficient amounts of water to insure good plant growth and development. In a general way, a soil shows a tendency to maintain a

water supply which is fairly constant within definite seasonal limits. Excess quantities are, as a rule, lost rapidly through drainage, and the remainder disappears rather slowly under any given set of conditions. On the whole, a fertile soil retains moisture much more efficiently than a non-fertile soil. Certain fluctuations in soil moisture invariably take place during practically every growing season, and these fluctuations are of paramount importance from the standpoint of plant growth. A knowledge, therefore, of the factors tending to prevent too high or too low a moisture content of the soil is of considerable importance from an agricultural point of view.

The water supply of a soil is derived from precipitation, either as rain, snow or dew. The water derived from any one of these sources exhibits a tendency to penetrate the upper soil layers, and in some instances at least may penetrate the subsoil and the layers of rocks beneath. In the course of time the excess quantities are drained away. The water supply of a soil represents, in a general way, the difference between the quantities supplied and the quantities lost.

The Classification of Soil Water. — The ordinary classification of soil water is as follows:

Hygroscopic Water. — If a moisture-free soil sample is placed in contact with moist air, a certain quantity of moisture is absorbed. The quantity held in this way after an equilibrium has become established is known as *hygroscopic water.* It is sometimes referred to as the water of condensation. The percentage of water held in this way, by a given weight of dry soil after exposure to an atmosphere saturated with moisture, is designated as the *hygroscopic coefficient* of that particular soil.

Capillary Water. — The amount of water held in such a way that it is not free to respond to gravity, minus the hygroscopic water, is designated as *capillary water.* However, no sharp line of demarcation between these two classes exists. The total amount of hygroscopic and capillary water held by a soil is sometimes referred to as its *maximum water-holding capacity,* or its *saturation point.*

Gravitational Water. — The water found in the soil which responds to gravity and thus drains away is designated as *gravitational water.*

Bouyoucos has found that the lowering of the freezing point of a soil increased in approximately a geometric progression as the moisture content increased in an arithmetic progression, indicating that the concentration of the soil solution is not necessarily inversely proportional to the moisture content. Apparently, therefore, part of the water present is not able to function as a solvent. Possibly it is adsorbed by the soil particles, or forms solid solutions, and in this way is removed from its active liquid phase. Having found that the freezing-point method was

not satisfactory for determining the quantity of *combined water* present, Bouyoucos employed the *dilatometer method*, instead. This method involves the principle of the expansion of water on freezing, the amount of water frozen being indicated by the amount of expansion. It was found that not all the water in soils could be frozen. Experiments on silica gels have shown that, of the water that it is possible for them to hold, 29.6 per cent to 44.6 per cent of the total could not be frozen at −78° C. According to Fisher, if these gels were subjected to heavy pressures, considerable quantities of this so-called *unfree* water could be squeezed out

In these studies, Parker has shown that, although colloidal particles have an extremely small or even negligible effect on the freezing point of a liquid, very different results are obtained if the amount of water is reduced until it is entirely in the film condition In this connection, Fisher calls attention to the fact that water present in very fine capillary tubes or pores must be under considerable tension and hence the density of such water is not necessarily the same as that of water in bulk, and that this point should be taken into consideration before accepting conclusions based solely on dilatometric studies.

A physiological classification of soil water is accepted by a large number of investigators According to this classification, soil water may be looked upon as either *available*, *non-available* or *superfluous*. Accordingly, the gravitation water and, in certain cases, part of the capillary water as well, may be considered as *superfluous* The hygroscopic water and that part of the capillary water which is tightly held are accepted as *unavailable*, and the remainder is considered *available*; certain cases are known where plants have reduced the capillary water almost to that which is held hygroscopically.

Factors Influencing the Water-holding Capacity of Soils. — One of the characteristic properties of colloids is their high adsorptive power for water, whether the water is presented to it in the liquid or vapor state. It is readily understood, therefore, that soil colloids have considerable to do with the retention of water This is especially true for hygroscopic water. A highly colloidal soil always possesses a high hygroscopic coefficient. Both inorganic and organic colloids function in this way. Soils high in organic colloids, such as mucks and peats, have a greater hygroscopicity than soils high in inorganic colloids, such as clays.

Soil colloids play an important part in determining the quantity of water held by capillarity. The portion of the soil water thus held is capable of but slight diffusion, if any, and is mainly held by colloidal material. It may be looked upon as transitional between the true hygroscopic water and the more active capillary portion, or that which lies

more or less beyond the realm of colloidal influence and which therefore may move from place to place as equilibrium stresses may demand.

The innermost portions of the capillary water are held by the adsorptive powers of the colloidal surfaces present, either physically or chemically combined, but the outer portion is retained by the surface tension of the water film. The distinctive characteristics of these two portions, therefore, are colloidal in one case and surface tensional in the other.

TABLE 37

PERCENTAGE OF MOISTURE EQUIVALENT, COLLOIDAL CONTENT, AND RELATIONSHIP BETWEEN THESE TWO SETS OF RESULTS FOR THE VARIOUS KINDS OF SOIL

(*Data of Bouyoucos, Soil Science*, 1929)

	Percentage of colloids	Percentage of water	Ratio $\frac{\text{water}}{\text{colloids}}$
Strongs sandy loam A_1	10 7	5 8	0 557
Strongs sandy loam B_1	27 00	14 02	0 519
Calumet sand	8 70	5 60	0 644
Michigan sand C	8 5	5 74	0 675
Michigan sand C	14 0	9 27	0 662
Fox loam B_2	18 08	11 95	0 657
Onaway loam A_1	11 69	7 42	0 634
Stalwart loam A_1	24 22	12 62	0 521
Ontonagon silt loam A_2	63 82	32 70	0 512
California Yolo clay loam	53 94	35 10	0 651
California Yolo clay	56 80	35 16	0 618
Brookston clay loam A_2	38 90	22 35	0 573
Greenville clay loam B	46 30	23 63	0 510
Cecil clay loam B	61 50	36 60	0 595
Manor schist loam B_1	46 00	31 75	0 689
Manor schist loam B_2	32 4	22 45	0 691
Michigan silt loam	36 00	19 00	0 528
Wabash clay	54 20	38 50	0 710
Cass clay	44 00	29 00	0 659
Minnesota Clyde silt loam	36 00	26 15	0 713
Bremer clay	55 40	38 20	0 689
Minnesota Carrington silt loam	51 00	35 00	0 686
Average			0 6224

In addition to soil colloids, many other factors play a part in the retention of water by soils. It has been found that farm manure has a considerable influence in this respect, and the effect has been noticed by Whitney to a depth of four feet. It has been shown that manurial addi-

tions tend to lower the surface tension of the soil water in the upper layers. The moisture of the upper layers, therefore, tends to move to the subsoil layers where the surface tension is greater. However, it appears that the urine of the manure has a tendency to deflocculate the soil to a certain extent, and this would tend to prevent the downward movement of the water.

Soil water is lost to a considerable extent by evaporation. Under this condition, the surface tension of the water in the upper soil layers is increased, and this has a tendency to cause the water of the lower soil layers to move upward. Enormous quantities of water may be lost from the soil in this way and should be controlled, if possible, during the growing season. Tillage, by producing a layer of dry loose soil, tends to prevent such losses by disturbing the normal capillary movements of the water. This layer, sometimes called a *soil mulch*, resists wetting because of its dryness and at the same time affords but little surface for active capillary pull.

The retention of water by a soil is influenced by dissolved material. Ordinarily, however, the concentration of dissolved materials is so low that this condition is of no great importance. Applications of commercial fertilizers to soils do not, as a rule, affect the concentration of the soil water materially, except in certain isolated cases. Sometimes, however, they have the effect of modifying the moisture-holding capacity of a soil through changes in its colloidal condition.

Relation of Moisture to Biological Soil Processes. — Bacterial life and activities are not possible without water. As a matter of fact, bacterial cells contain as much as 75 to 98 per cent of water. On the other hand, molds generally prefer a relatively dry, but not moisture-free, environment. Frequently, therefore, it depends solely on the water content of a soil whether the molds or bacteria predominate.

Soils containing 10 per cent of moisture appear to be dry, but this quantity is sometimes sufficient for bacterial activity. According to Lohnis and Fred, the optimum moisture content of a soil for normal bacterial activity should range from 60 to 80 per cent of its total moisture-holding capacity. At a moisture content below 50 per cent there is a tendency for the bacteria to lose water; on the other hand, if excessive quantities are present, only certain of the soil bacteria will thrive. Moreover, unless a soil is well drained, certain substances, such as acids and other materials, have a tendency to accumulate, which may exert a toxic effect on the bacteria normally present. Lack of available water, however, does not necessarily lead to a destruction of the bacteria, since, under these conditions, there is a tendency for the active forms to assume a state of inactivity in which they are extremely resistant to dry conditions.

This is also true to a certain extent of the active or vegetative organisms on account of an enveloping slime which affords considerable protection.

Factors Influencing the Composition and Concentration of the Soil Solution. — No one factor is of greater importance in determining the kind of plants to be grown on a given soil than the available water supply of that soil. The relation of water to the quantity and quality production of plants, however, will be considered elsewhere. The soil solution represents that part of the soil water which is available for plant growth and which is, partly at least, in equilibrium with the solid and gaseous components of the soil. It is this solution that constitutes the nutrient medium for plant growth. The composition and concentration of the soil solution are influenced by a number of factors. Among these may be mentioned soil colloids.

The Colloidal Matter of the Soil

Soil colloids, with their large capacity for the adsorption of water, their power of adsorbing certain mineral and organic bodies, constitute a great reservoir for holding in reserve the materials necessary for the growth of plants. It is this so-called *colloidal* fraction or *colloidal complex* which chiefly characterizes soils.

Origin of Soil Colloids. — Soil colloids are derived from the weathering of soil-forming materials such as minerals, rocks, and organic matter. The weathering processes, which involve decomposition, are most active near the surface, and it is in the upper surface that organic matter tends to accumulate. For these reasons, therefore, most of the colloidal material of the soil is found there. As a result of the main soil-forming processes, considerable basic materials have been leached away, leaving a comparatively insoluble material, which is usually colloidal in nature. This colloidal residue is composed almost wholly of complex aluminosilicates, acid silicates, and the hydrates of iron, aluminum, titanium, silicon, and humus. Moreover, the alkali metals, alkaline earths and other elements are usually present in loose chemical combinations but in smaller amounts. Because the colloids of the soil have been derived from both organic and inorganic materials, their composition shows wide variation. Obviously, therefore, no theory of the nature of soil colloids based on chemical composition can be applied to soil colloids in general. The organic soil colloids ordinarily represent but a small fraction of the total colloids, except in soils very high in organic matter, such as peats. In addition to humus, the microflora of the soil may be looked upon as organic colloidal material, but it constitutes but a mere fraction of the total colloidal material normally present.

Until quite recently it was thought that the colloidal content of agri-

cultural soils was quite low and that the colloidal matter occurred as a gelatinous coating only on the surfaces of unchanged soil particles. At the present time, however, it is generally recognized that the quantity normally present is much in excess of that required for covering the coarser particles. Recent studies indicate that the total colloid content of a soil may exceed, in certain cases, 70 per cent of the total soil mass. This new conception has been brought about by modern methods of soil-colloid investigations and involves studies dealing with the water-absorptive power, heat of wetting, base exchange and specific gravity of soil suspensions as well as by many other methods

Electrical Properties of Soil Colloids. — Colloidal clay when dispersed in water is found to be negatively charged. Bradfield, who has given considerable time to the study of clay colloids, found that the addition of hydrogen ions to such systems reduced the velocity of the particles which tended to move toward the positive pole, after a potential difference was set up between two electrodes dipping in the liquid, and eventually rendered the particles electrically neutral. Bradfield was not able, however, to reverse the charge by adding a large excess of hydrogen ions. In a similar experiment, Dayhuff and Hoagland were able to show that clay colloids, after being separated from adsorbed materials, retained their negative charge when the hydrogen-ion concentration varied from *p*H 2 1 to *p*H 12 7. Using a cataphoresis apparatus, Dayhuff and Hoagland found that, under the same electrical conditions, the purified clay particles had a greater speed of migration than ordinary clay suspensions. This is taken to indicate that ordinary salts such as those found in soils have a tendency to reduce the potential difference. Clay colloids with a high silicic acid and a low iron and aluminum oxide content are always found to be strongly electronegative, but where the reverse is true, the particles are less strongly negative, whether the particles are saturated with exchangeable bases or whether these adsorbed bases have been completely removed by means of electrodialysis.

These facts have led to more fundamental studies, namely, studies on the electrical properties, or the kinetic behavior of the individual components of such a colloidal system. Silicic acid has always been found to be electronegative in aqueous solutions and does not have an iso-electric point in acid solutions. According to Michaelis, such a colloid may be termed an *acidoid.* On the other hand, colloidal oxides of both iron and aluminum may be either electronegative or electropositive, depending on the hydrogen-ion concentration of the medium. Their iso-electric points lie very near the point of neutrality. According to Michaelis, these colloids may be termed *ampholytoids.*

Humus is also an electronegative colloidal material. It would appear,

therefore, that the principal inorganic and organic soil colloids are electronegatively charged, which has proved to be the case.

Flocculation of Soil Colloids. — It is extremely difficult to correlate the *flocculation* or *coagulation* of soil particles with the established facts of physical chemistry, because the soil system is exceedingly complicated. For example, soil particles may be covered with organic and inorganic colloidal material, and, moreover, the soil is a system composed of particles of all sizes. Studies on such a heterogeneous material may yield results which are not strictly analogous to those obtained with a simple suspensoid colloidal system. Various studies have been conducted in the past relative to the flocculation of soil colloids by means of electrolytes. Such studies have shown that under these conditions the larger particles are the first to precipitate and at a lesser electrolyte concentration than the smaller particles. In the course of time, however, complete precipitation ordinarily takes place. The flocculating power of cations increases with their valency in all but a few isolated cases. Not all the equivalent ions have equal flocculating power, some being more efficient in this respect than others. The flocculating power of the alkalies increases in the series Li, Na, K, and Rb; the flocculating power of divalent ions increases in the series Mg, Ca, Ti, and Ba. The hydrogen ion, as a flocculating ion, is comparable to a divalent rather than to a monovalent ion. All these differences may be due to the relative degree of hydration of the various ions.

Electronegative colloids dispersed in water are flocculated when their charge is removed by neutralization and brought to their iso-electric point. Calcium ions, therefore, being divalent and bearing positive charges, flocculate these colloids quite readily. If, however, the hydroxyl concentration of the solution is increased, flocculation does not necessarily take place. As a matter of fact, a higher dispersion of the colloid may be brought about. This is due to the fact that OH-ions increase the negative charge carried by the electronegative colloid. The calcium and hydroxyl ions, as they exist in a solution of calcium hydroxide, will work in opposition, calcium tending to bring about flocculation and the hydroxyl ion tending to bring about deflocculation, or dispersion. Under ordinary conditions, therefore, calcium chloride should be a more efficient flocculant of ordinary electronegative suspensoid colloids than calcium hydroxide. If, however, the concentration of calcium hydroxide exceeds a certain point, there is a tendency for the flocculating power of calcium to overcome the deflocculating power of the hydroxyl ion, and flocculation occurs. This is sometimes referred to as *abnormal flocculation.*

In studies pertaining to the flocculation of soil colloids, attention should

be given to the effect of changes in the reaction of the medium. When the soil suspension is rendered alkaline, a precipitation of calcium, magnesium, iron and aluminum, as the hydroxides, carbonates, silicates, or phosphates, tends to take place, and the presence of these compounds may further the flocculation process. In very low concentrations, however, the hydroxyl ion appears to behave normally and to militate against the flocculating power of neutral salts. The hydroxyl ion may, therefore, function in different ways. When added after calcium salt additions, for example, it may produce the desired alkalinity for the precipitation of such salts as those mentioned above. On the other hand, if added before the addition of calcium salts, it peptizes the electronegative colloidal particles, producing a greater amount of emulsoid surface, with the consequence that there is produced, on the addition of calcium salts, an increase in the rate of flocculation and likewise an increase in the volume of the coagulum. According to Mattson, the hydroxyl ion is adsorbed by the soil colloids, and two electronegative particles may, perhaps, be held together by a positive ion such as calcium, and in this way intensify flocculation reactions.

Neutral salts are found to be much better than hydroxides as flocculants of silt. From what has already been mentioned, it is apparent that the efficiency of neutral salt additions on clay is not as great as on silt, but that the previous addition of an alkali tends to better the conditions for flocculation. In explaining this, Comber calls attention to the fact that silt is not an emulsoid colloid, whereas clay, being *protected* by silicic acid, an emulsoid colloid, behaves as an emulsoid. If this emulsoid layer is removed by dispersion, the action between the *inner core* and the salt will be quite similar to the action on silt. It must be remembered, however, that the inorganic colloids of the soil do not necessarily possess what may be termed an *inner core*, or unchanged particle, but in the majority of cases it is quite probable that they do.

Calcium hydroxide and calcium acid carbonate are regarded as good flocculants, and their actions have been considered anomalous. If the emulsoid character of clay be admitted, however, calcium hydroxide would appear to be more nearly ideal, inasmuch as the calcium and hydroxyl ions would not necessarily be working in opposition but in collaboration. On the whole, Comber and many others have found that clays are flocculated by calcium salts very quickly in alkaline solutions and slowly in a neutral solution.

When clay suspensions are treated with varying quantities of colloidal aluminum or iron hydroxide, flocculation takes place at a more or less definite point. Beyond this point, the colloidal material added is found to protect the particles and produce a stable, positively charged system.

It appears, therefore, that the flocculation of clay by iron or aluminum falls into line with the iso-electric point theory. According to Comber, the beneficial effects of acids on the flocculation of soils may be due in part at least to the bringing of aluminum and other bases into solution, and these bases may play a part in flocculation. Under certain conditions, the addition of neutral salts to colloidal soils may result in the formation of acids with the same result as may be obtained by the addition of acids. Mixtures of humus and clay flocculate at concentrations of electrolytes intermediate between the concentrations required to flocculate each one separately. On the whole, however, the flocculation of organic colloids parallels the flocculation of inorganic colloids.

Deflocculation of Soil Colloids. — Apparently the ion of opposite sign to that of the colloidal particle is responsible for flocculation, and the ion having the same sign as the colloid particle has a dispersing effect.

In the continued use of nitrate of soda on soils, a deflocculation of the soil colloids results, as a rule. This effect is due to an increased alkalinity of the soil which is the residual effect of such an addition. The residual effects of additions of carbonate of potash and certain other fertilizing materials are likewise similar to that of nitrate of soda.

In a general way, hydrogen ions are efficient coagulants of electronegative colloids. Under soil conditions, however, this effect is not so apparent. Increased hydrogen-ion concentrations of the soil tend to remove the soil colloids from their iso-electric point, and bring about their dispersion or deflocculation, for reasons given before. Under these conditions, there is a tendency for drainage to aid in the deflocculation of soil colloids.

Volume Changes of Soil Colloids. — The removal of water from soil colloids may result in a concentration of *sols* and eventually bring about a *gel* formation. As a matter of fact, soil colloids occur normally as *gels* rather than as *sols*. This is due to loss of water and to the resulting increased concentration of dissolved salts.

The loss of water by soil colloids is of importance in soil fertility, because it is accompanied by a pronounced shrinkage of the soil mass. This shrinkage is not only due to the loss of water to a point where the capillaries begin to fill with air, but also to a residual shrinkage, which is much less than the volume of water lost. It is interesting to note that clay soils may shrink 18 to 50 per cent in volume after drying.

That emulsoid colloids increase in volume on wetting is a well-known fact. A soil high in emulsoid colloids, as in the case of organic soils, may swell to such a marked extent, on wetting, as to be harmful to plant growth. An increase in the hydroxyl-ion concentration is sometimes

known to favor the swelling power of clay colloids and emulsoid colloidal gels such as humus and silicic acid.

TABLE 38

SWELLING (VOLUME INCREASE) OF COLLOIDS IN WATER

(*Data of Anderson and Mattson, U S. D A Department Bulletin* 1452)

Kind of colloid	Volume per gram in benzene, cc	Volume per gram in water, cc	Swelling, per cent
Gelatin	1 8	18 0	900
Silica gel	1 1	1 14	4
Bentonite	1 1	9 0	718
Fallon	1 0	2 5	150
Sharkey	1 0	1 9	90
Susquehanna.. .	1 1	2 0	82
Marshall .	1 1	1 8	64
Miami	1 1	1 8	64
Sassafras .	1 1	1 8	64
Huntington	1 3	1 9	46
Cecil	1 3	1 9	46
Norfolk	1 3	1 8	38

Adsorption by Soil Colloids — In recent years considerable attention has been directed to the surface factor of soil colloids. It has been shown that when a certain soluble foreign substance is introduced into a two-phase system, such as water and silicic acid, although there is a tendency for the added material to become distributed throughout the system, it is often found localized on the surface of the solid phase. This phenomenon has already been defined as *adsorption*. Soil colloids, being negatively charged, readily adsorb positively charged ions. Of the adsorbed cations, Ca, Mg, Na and K usually predominate. As a rule, however, and certainly in the case of very fertile soils, calcium is the principal adsorbed base, or what may be termed *replaceable* base. In *alkali* soils, sodium predominates.

Extraction with a neutral salt solution removes adsorbed material from samples of soil, substituting for the adsorbed base the base of the salt solution. Mutual replacement of ions may take place, depending upon the salt solution used. If, however, a calcium salt is used, the soil particles tend to aggregate, rendering the soil more porous and granular, as should be the case. On the other hand, if a sodium salt is used, the

conditions are reversed — the soil colloids taking the form of hydrosols and becoming highly dispersed.

TABLE 39

BASE ABSORPTION POWER OF THREE SOILS BEFORE AND AFTER OXIDIZING THE ORGANIC MATTER PRESENT WITH HYDROGEN PEROXIDE

(*Data of Kerr, Journal of the American Society of Agronomy*, 1928)

Soil type	Absorption capacity per 100 gm. soil		Difference, milli-equivs	Absorption capacity due to organic matter, per cent
	Before, milli-equivs	After, milli-equivs		
Carrington silt loam	23 9	18 8	5 1	21
Colby silt loam	14 8	13 8	1 0	7
Waukesha silt loam	25 1	18 2	6 9	28

If a soil sample is suspended in water and subjected to electrodialysis, all adsorbed ions are removed, with the exception of hydrogen. The soil is then said to be *unsaturated*. This term is used, also, to designate a soil in which hydrogen ions make up part of the adsorbed materials. On the other hand, when the adsorptive capacity of a soil is satisfied by metallic ions, the soil is said to be *saturated*. If such a soil is saturated with potassium ions only, it is designated as a *potassium soil* and is an alkaline system quite similar to that of a salt made up of any strong base and weak acid, such as K_2HPO_4.

The system of a *potassium soil* and water becomes neutral on the addition of a certain quantity of hydrogen ions, just as H_3PO_4 will be neutralized by much less than its chemical equivalent of KOH. Therefore, it is apparent that an unsaturated soil is not necessarily an acid soil.

When ordinary fertilizer salts react with unsaturated soils, hydrogen ions naturally participate in the *exchange* phenomenon. These ions will not necessarily exceed the hydroxyl ions in the solution, unless the soil or the salt itself is acid in reaction. If neutral salts, such as those mentioned above, are brought in contact with acid soils, there is an increase in hydrogen-ion concentration on account of ionic exchange. Under these conditions, it is entirely possible for the acidity so developed to aid in dissolving certain of the soil constituents. As a matter of fact, a dissolution of aluminum, iron and manganese has been known to take place

under these conditions, but at a very slow rate. This increased solubility is not directly due to replacement, since replacement is a relatively rapid process.

TABLE 40

EXCHANGEABLE CATIONS IN MILLIGRAM EQUIVALENTS PER 100 GRAMS OF AIR-DRY SOIL, HYDROGEN-ION CONCENTRATION OF SOILS BEFORE AND AFTER ELECTRODIALYSIS

(*Data of Wilson, Soil Science*, 1928)

Soil	Cations, Ca+Mg +K	Total cations in terms of NaOH	*p*H	
			Before dialysis	After dialysis
Dutchess silt loam, Dutchess Co.	6 53	7 48	5 2	4 2
Dutchess silt loam, Orange Co	3 10	6 73	4 3	4 0
Ontario loam, Monroe Co	12 12	12 13	7 2	4 3
Ontario loam, Oneida Co	12 26	12 63	7 4	4 6
Vergennes clay, Jefferson Co	14 25	14 24	5 7	3 9
Vergennes clay, Washington Co.	12 15	12 10	5 4	4 1
Volusia silt loam, Allegany Co	8 18	8 88	4 8	4 2
Volusia silt loam, Cortland Co	7 34	8 60	5 0	4 1
Volusia silt loam, Tompkins Co (Cayuta)	10 24	11 39	4 9	3 8
Volusia silt loam, Tompkins Co (Turkey Hill)	5 85	6 36	5 2	4 6
Dunkirk silty clay loam, Tompkins Co	6 20	7 00	5 7	4 3
Wooster gravelly silt loam, Cayuga Co	4 57	5 70	4 8	4 4

The fact that a soil, when leached with a solution of potassium chloride, tends to hold to the potassium and not necessarily to the chloride ion, was formerly referred to as a case of *selective* or *hydrolytic* adsorption. This was explained by assuming that incipient hydrolysis of the salt occurred, followed by the absorption of the base, leaving the acid free, and that this acid, on dissolving certain of the soil bases, formed soluble chlorides. When such a phenomenon occurs in colloidal soils, it is now thought to be due to the replacement of adsorbed hydrogen, the base taking its place on the surface of the colloid.

If a sample of humus is placed in a neutral salt solution, an apparent decomposition of the salt takes place. If leached with a salt solution, however, until no more acidity is developed, the humus is found to behave as a neutral mineral soil and to exchange bases in equivalent amounts. What is true for humus is also true to a certain extent for colloidal manganese dioxide and silicic acid. There follows, however,

an adsorption of negative ions which is illustrated by the increase in negative charges of these substances. The increase in negative charge for the different ions follows in the order $SO_4 > Cl > Br > NO_3$, the order for the decrease in charge being $H > Ba > Rb > K > Na > Li$. It would appear, therefore, that these results are due to a preferential adsorption of hydroxyl ions.

The extent of adsorption of cations by soil colloids differs according to the reaction of the medium. Those colloids having a high silica content adsorb much greater quantities from acid or neutral solutions than do those high in the oxides of iron and aluminum. In the presence of a large excess of base, however, the adsorbing power of the latter may equal or exceed that of the former.

Anions are not adsorbed by soil colloids to any appreciable extent. When the $SiO_2/Al_2O_3 \cdot Fe_2O_3$ ratio is narrow, considerably more anions are adsorbed than when the ratio is wide. As a matter of fact, some soil scientists are inclined to believe that no adsorption takes place with the latter complex. In some cases, negative adsorption has been noted.

A superficial examination of an inorganic soil shows what class of colloidal material predominates. If the ratio of $SiO_2/Al_2O_3 \cdot Fe_2O_3$ is narrow, the color is red or yellow. If the ratio is wide, the color is gray.

Some investigators believe that ferric hydrate has the power of absorbing phosphoric acid, yielding a solid solution. For this reason, therefore, the *fixing* of phosphorus by soils rich in iron is sometimes referred to as *absorption* rather than *adsorption*.

The effect of temperature on the adsorption by soil colloids is quite noticeable. As a rule, a relatively high temperature tends to decrease the adsorptive power. This may be attributed to the partial destruction of the colloidal system.

Fundamental studies on simple colloidal systems, such as *activated* charcoal in water suspension, have shown that the hydrogen and hydroxyl ions are readily adsorbed. This also holds true for soils. Not only are both ions adsorbed, but the hydrogen ion is adsorbed much more readily than any other ion, and is more difficult to replace.

Adsorption by soils is of far-reaching importance from the standpoint of plant nutrition, because the adsorbed materials constitute practically the main source of mineral nutrients. Moreover, it appears that adsorbed ions tend to be absorbed by plants in more or less the same ratio as they exist in adsorbed form. In order to obtain the proper *physiological balance* of mineral nutrients for any particular plant, we must treat the soil in such a way as to bring about the proper ratio of adsorbed materials in the soil.

The fertility of the soil, therefore, is more or less directly related to its

exchange capacity. A good soil always has a high content of mutually replaceable bases.

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CHAPTER X

SOIL ACIDITY

All soils of humid regions tend to become acid in reaction, and in some cases the acidity becomes so pronounced, in the course of time, that it interferes seriously with the growth of plants. As a rule, however, agricultural soils rarely exceed an acidity of *p*H 4.

Causes of Soil Acidity

The principal cause of soil acidity is to be sought in the action of carbonic acid on the soil-forming rocks and minerals Such a reaction is modified to some extent by soluble organic materials which usually tend to increase acidity.

The carbonic acid formed under humid conditions dissolves the basic elements slowly, but more rapidly than the acidic elements. For example, calcium and magnesium are more readily leached away than iron and aluminum, leaving acid silicates.

Organic additions, such as starch, always result in an increased acidity. The addition of green manure or stable manure may cause an increase during the first stages of decomposition, but as decomposition becomes more complete this effect is lessened somewhat, owing to the release of basic materials. Plants may remove basic ions more rapidly than acidic ions and in this way contribute to acidity. Because of the replacement of adsorbed hydrogen ions in somewhat acid soils, the action of a number of our common inorganic fertilizing materials serves to increase the acidity. The absorption of potassium from muriate of potash, KCl, for example, may lead to an increase in acidity for this reason.

The Correction of Soil Acidity

Although not all scientific and practical men are as yet agreed as to the causes of soil acidity, all are convinced that such a condition may be rectified through the additions of specific compounds of calcium or magnesium. The lack of replaceable calcium is always apparent in acid soils. When calcium is the only component of the adsorptive complex, the latter is chemically non-hydrolyzable. This does not hold true in the case of potassium, for such a complex hydrolyzes readily and an alkaline condition is thus brought about.

The addition of lime may produce various effects on acid soils; acids are neutralized; calcium is introduced into the exchange system and

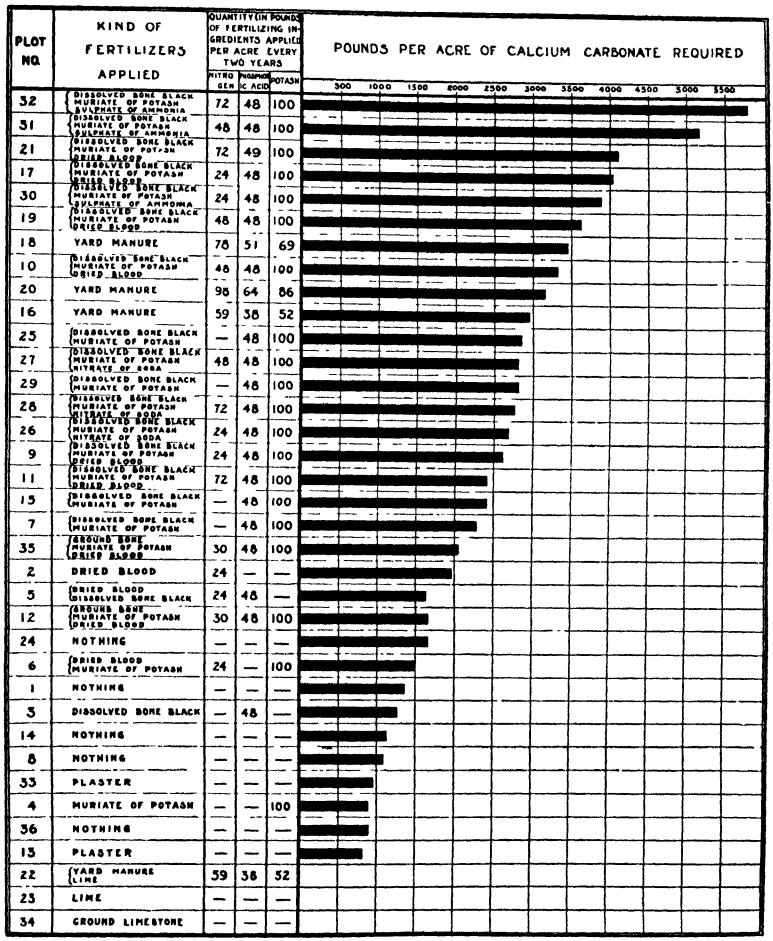

FIG. 29. These studies were conducted at the Pennsylvania State College in 1915 and represent thirty-four years of treatment Results expressed in terms of calcium carbonate per acre (seven inches)

there appears to be a reduction of soluble aluminum. Moreover, it has been found that, when either CaO or $CaCO_3$ was added to acid soils, considerable quantities combine with the organic matter present.

The Relation of Acidity to the Buffering Power of Soils

All properties of an acid solution depend upon the hydrogen-ion concentration at the moment. Many factors, however, tend to affect this concentration at any one time. Thus colloids, acid phosphates, lime salts and many other soil materials may act on the soil solution in such a way as to preserve a quite uniform reaction. For this reason, it is more difficult to modify the acidity of a soil than the acidity of an aqueous solution. This effect is known as *buffer action*, the substances which cause the condition being known as *buffers* or *reaction regulators* and the solutions themselves as *buffered solutions*. By lowering the state of saturation of the exchange complex of the soil, the soil becomes more acid. On the other hand, if the soil is well supplied with respect to bases, the normal production of acids does not markedly increase the hydrogen-ion concentration of the soil solution.

The buffering effects of soils are primarily concerned with the adsorption complex and are expressive of the replacing activities of the hydrogen ion. For example, the addition of an acid solution to a soil generally results in hydrogen ions being adsorbed and the formation of a simple salt of the substituted base In such a case, the hydrogen-ion concentration of the solution is necessarily greater than that of the adsorption complex. The buffering power of a soil against acids is, therefore, conditioned by the presence of bases in replaceable forms, and its extent is governed by the quantity present. For this reason, a heavy soil should be more highly buffered than a sandy soil.

Soils of high fertility invariably show a high buffering power; soils of low fertility generally show a poor buffering power Through disintegration and decomposition, as modified by intense cultivation, the buffering power may be decreased considerably. By proper fertilizer practice, however, the buffering power may be increased.

The Relation of Acidity to Soil Fertility. — It is generally recognized that a relatively high acidity has a number of unfavorable influences on soil fertility from the standpoint of physical, chemical and biological conditions

Soil acidity and lack of calcium acid carbonate appear to go hand in hand; the particles of acid soils are not in a flocculated condition and therefore do not have the necessary *crumb* structure so desirable for maximum biological activities. Soil acidity may thus lead to the preservation of organic matter, but, under these conditions, toxic organic substances may tend to accumulate.

Soil acidity tends to lower the availability of many of the mineral nutrient soil materials. Under these conditions, phosphorus unites with iron

TABLE 41

THE HYDROGEN-ION CONCENTRATION OF SOILS AS AFFECTED BY THE USE OF DIFFERENT AMOUNTS OF $(NH_4)_2SO_4$ FERTILIZER

(*Data of Pierre, Journal of the American Society of Agronomy*, 1927)

Soil type	Untreated	Reaction of soil	
		500 pounds $(NH_4)_2SO_4$ per acre	1000 pounds $(NH_4)_2SO_4$ per acre
	*p*H	*p*H	*p*H
Norfolk sand	5 85	5 00	4 80
Ruston sandy loam	5 10	4 65	4 35
Orangeburg sandy loam	6 00	5 07	4 75
Greenville sandy loam	5 80	5 00	4 77
Norfolk sandy loam	5 35	4 70	4 45
Decatur silt loam	5 75	5 20	5 05
Cecil clay loam	6 15	5 88	5 60
Decatur silt loam	5 85	5 15	5 20
DeKalb fine sandy loam	5 90	5 15	4 85
Mississippi delta soil	6 00	5 65	5 50
Tifton sandy loam	5 80	5 10	5 00
Portsmouth fine sandy loam	5 15	4 90	4 70
Cecil sandy loam	5 75	5 18	5 18
Lintonia silt loam	6 10	5 85	5 65

and aluminum, forming insoluble phosphates, or with the organic matter present, forming complexes in which the phosphorus is less available for plant growth There is likewise a tendency for the hydrogen ion to displace adsorbed mineral bases, and thus aid their loss from the soil or perhaps lead to the increased solubility of iron, which, as a rule, is not desirable from the standpoint of plant growth.

Soil Acidity in Relation to Plant Diseases and Plant Competition. — Many harmful disease organisms are adapted to acid soil conditions; others thrive under alkaline conditions For these reasons it is highly desirable to control the reaction of the soil in such a manner as to check the development of deleterious organisms Moreover, different plants respond in different ways to soil acidity. In fact, such descriptive terms as *acid-loving, neutral* and *alkali* plants are sometimes used to designate the response of plants to soil reactions Barley, legumes, sugar beets and mangolds are quite susceptible to acid conditions. Wheat is more resistant than those mentioned, whereas potatoes and rye are extremely

resistant to an acid soil condition. In uncultivated soils one can obtain a superficial measurement of the degree of acidity from the character of the vegetation grown thereon. On strongly acid soils, for example, sorrel, mosses and other plants are found to predominate, whereas clover, alfalfa or other legumes may be found in soils of low acidity.

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CHAPTER XI

FARM MANURE

The term *farm manure* as more commonly used, signifies the solid and liquid excrement of farm animals, alone or mixed with those parts of crop plants which are more or less indigestible and therefore unsuitable for use as animal foods. There is, however, a tendency even at the present time to allude to the mixture of horse excrement and litter as *stable manure* and to the mixture of cattle excrement and litter as *barnyard manure.* The term *manure* is sometimes applied to any fertilizing material, but is more often used in the same general way as the term *farm manure.*

The value of farm manure as a soil addition is well recognized, and it is regarded as one of the most valuable by-products of general farm practice. Probably no other fertilizer material possesses to so great a degree the power of restoring lost soil fertility as barnyard manure, and it is for this reason that much investigation has been and is now being conducted on the relation of this material to soils and plant growth.

The Chemical Composition of Farm Manure

The composition of farm manure varies greatly, and we will now consider some of the factors which have a tendency to modify its composition and fertilizing value.

Kind of Animal. — The composition of farm manure varies according to the animal from which it is derived. Table 42 shows the most important differences in the composition of types of manure.

Fresh animal manure from any source usually undergoes a considerable loss of many of its fertilizing ingredients before it is changed into the ordinary farm manure. It is difficult, therefore, to give the true chemical composition of farm manure. Any figures are at best only a close approximation.

Manure from cows or steers contains considerably more water than that from any other domestic animal. It is slow to ferment and is therefore classed as a " cold manure." Horse manure, on the other hand, contains much less water than solid excreta from cattle and is classed as a " hot manure " because it ferments readily with the production of considerable heat. Sheep and poultry manures are quite dry, and fer-

ment readily. Poultry manure is the richest of farm manures, owing to the fact that the urinary secretions are semi-solid and are voided with the solid excreta.

TABLE 42

THE COMPOSITION OF MANURE FROM DIFFERENT FARM ANIMALS AS BASED ON ONE TON QUANTITIES

(Data of Duley, Missouri Station Bulletin 166)

Animal	Wt manure, pounds	Nitrogen, pounds	Phosphorus, pounds	Potassium, pounds
Horse — Solid	1632 2	8 06	2 12	3 26
Liquid	367 8	4 41	Trace	4 56
Total	2000 0	12 47	2 12	7 82
Cow — Solid	1456 5	4 71	1 31	1 80
Liquid	543 5	5 16	06	4 29
Total	2000 0	9 87	1 37	6 09
Sheep — Solid	1200 0	7 80	2 40	22 8
Liquid	800 0	13 44	10	14 08
Total	2000 0	21 24	2 50	16 36
Hog — Solid	1290 3	7 74	2 58	4 77
Liquid	709 7	2 12	39	5 89
Total	2000 0	9 86	2 97	10 66
Hen	2000 0	23 00	8 10	7 46

Farm animals, on maintenance rations, may excrete the same quantity of mineral nutrients originally present in the feed. Milch cattle, on the other hand, may excrete 65 to 85 per cent of the constituents present in the feed. Therefore, the utility of the animal, as well as the kind of animal, is a factor of considerable importance in modifying the chemical composition of the excretory materials.

Age of the Animal. — Rapidly growing animals excrete less of the mineral elements of their food than do full-grown animals, since the latter neither gain nor lose any appreciable weight. According to Thorne, a growing calf, gaining 50 pounds per month in live weight, will store as much as 4 pounds of phosphorus annually in its bones and other tissues. Contrary to the general belief, the pasturing of land with immature animals will not necessarily lead to an increased fertility of the soil. It must be remembered also that hair, hoof, wool and other animal tissues require considerable quantities of nitrogen for their formation, and these are taken indirectly from the soil.

Ration of the Animal. — The composition of animal manures varies directly with that of the food consumed, at least in the majority of cases. An animal fed on corn and timothy hay will produce manure much poorer in nitrogen than those which are fed clover and wheat bran.

TABLE 43

VARIATION IN QUANTITY AND COMPOSITION OF MANURE CAUSED BY FEED

(*Data of Jensen, Michigan Station Circular* 25)

	Cow fed mangels			Cow fed alfalfa hay		
	Solid excrement	Urine	Total excrement	Solid excrement	Urine	Total excrement
	pounds	pounds	pounds	pounds	pounds	pounds
Voidings per day	42	88	130	48	14	62
Content of voidings	per cent	per cent	per cent	per cent	per cent	per cent
Water	83 00	95 94	91 51	79 70	88 23	81 80
Nitrogen	0 33	0 12	0 19	0 34	1 54	0 61
Phosphoric acid	0 24	0 01	0 08	0 16	0 01	0 12
Potash	0 11	0 60	0 45	0 23	1 69	0 56

Feeding stuffs vary greatly in regard to their fertilizing constituents as shown by the following table:

TABLE 44

FERTILIZING CONSTITUENTS IN ONE TON OF FEEDING STUFFS

(*Data of Jensen, Michigan Station Circular* 25)

Material	N, pounds	P_2O_5, pounds	K_2O, pounds
Alfalfa hay	46 8	12 2	35 8
Clover hay	39 4	11 0	37 4
Timothy hay	18 8	6 6	28 4
Corn	33 0	14 2	11 4
Wheat	38 0	11 0	17 4
Wheat bran	49 2	53 8	30 4
Oil meal	120 0	34 8	26 8
Cottonseed meal	145 0	60 8	31 6
Corn silage	8 6	2 2	7 4
Wheat straw	10 0	4 4	12 6

The dry material of animal excrement rarely, if ever, exceeds 50 per cent of the food consumed. The kind of food eaten, though modifying its chemical composition, would not necessarily affect the quantity of manure produced.

Nature of the Litter. — In addition to variations caused by the kind of feeding stuff eaten by the animal, the composition of manure is also materially altered by the nature of the litter used. Straw produces a very bulky product which is somewhat difficult to handle and has a low fertilizing value per unit weight, although it is an excellent litter material for the absorption of urine. On the other hand, sawdust or wood shavings may produce a very compact product when used as litter, although these materials are low in manurial value and are poor absorbents. Moreover, they tend to injure the quality of manure in that they decompose slowly and prevent rapid decomposition of the manure and the liberation of its mineral constituents. Peat is an excellent absorbent and increases the percentage of the chief fertilizing constituents in the prod-

TABLE 45

WATER-HOLDING CAPACITY OF, AND FERTILIZING ELEMENTS CONTAINED IN, BEDDING MATERIALS*

(*Data of Ames and Gaither, Ohio Station Bulletin* 246)

Material	Water retained by 100 pounds of material after 24 hours, pounds	Fertilizing elements in 100 pounds of material		
		Nitrogen, pounds	Phosphorus, pounds	Potassium, pounds
1 Wheat straw	220	0 53	0 092	0 830
2 Oat straw	220	0 58	0 087	1 096
3 Dead leaves . .	162	0 80	0 131	0 249
4 Peat moss . .	1300	0 80	..	
5 Sawdust .	435	0 20–0 70	0 131	0 581
6 Spent tan .	450	0 50–1 00		
7 Peat	600	1 00–2 00		

* Analyses 1 and 2 by Ohio Experiment Station Other data from Farmers' Bulletin 192, U S. D A

uct, but it decomposes very slowly under soil conditions. The material to be used as litter must possess good absorbent powers in order to prevent the loss of nitrogen, phosphoric acid and potash. Finely chopped straw is ideal in this respect. The litter should decompose rapidly when

added to soil and likewise should not temporarily deplete the soil of nitrates. The addition of nitrate nitrogen to the manure tends to overcome the undesirable effects of straw and similar litters when used on the soil.

The Loss of Manurial Constituents

The composition of manure is altered considerably by the methods of handling, and some of the factors involved will now be considered.

Leaching. — It should be understood that equal quantities of the main fertilizing constituents of the manure are found in both the liquid and solid portions; therefore, manure should not be exposed to the weather for any length of time before it is utilized, or in other words, the liquid portion should not be allowed to drain away. It is important not only to protect the manure pile but also to have water-tight floors and to use the proper absorbent material. According to Weaver, experiments have shown that, in ordinary agricultural practice, at least one-third to one-half of the manurial plant food is lost through leaching of the liquid manure.

Undoubtedly, the greatest loss incurred in the handling of manure is due to leaching. This source of loss, however, may be overcome, as shown by Ames and Gaither. An average of three tests conducted by these investigators showed that, where fattening steers were fed in stables having good floors, 61 per cent of the nitrogen, 82.8 per cent of the phosphoric acid and 82.4 per cent of the potash contained in the feed and bedding were recovered in the manure produced. In these investigations, the manure was allowed to accumulate in the stalls.

The loss by leaching represents the loss of the more available fertilizing constituents as the latter are water-soluble. As decomposition proceeds, however, considerable quantities of the more unavailable fertilizing materials are made soluble and may be lost in the same manner.

Fermentation. — Manure contains numerous bacteria which have the power of bringing about decomposition as soon as it is voided by the animal. The first evidence of decomposition or fermentation is the odor of ammonia which soon becomes apparent. This comes from urea the decomposition of which should be controlled since it is the most important fertilizing constituent of the manure.

In fresh manure, bacteria of the *B. coli* strain predominate, but these disappear in a relatively short time. In the course of time there is usually a gram-positive spore-forming strepto-bacillus predominating. Ammonifying bacteria, however, are always present, and there are more spore-formers than non-spore-formers at all times. In general, aerobic bacteria are more numerous than anaerobic bacteria, although a number

of facultative organisms, i.e., those which function either in the presence or absence of oxygen, are present to a certain extent. The nature of the fermentation at first is aerobic and is furthered by the presence of air and of soluble carbohydrates. A considerable rise of temperature is thus produced, and the carbohydrates present are consumed in the process. As time goes on, there is a normal settling of the manure which tends to interfere with the entrance of air, and the temperature is reduced

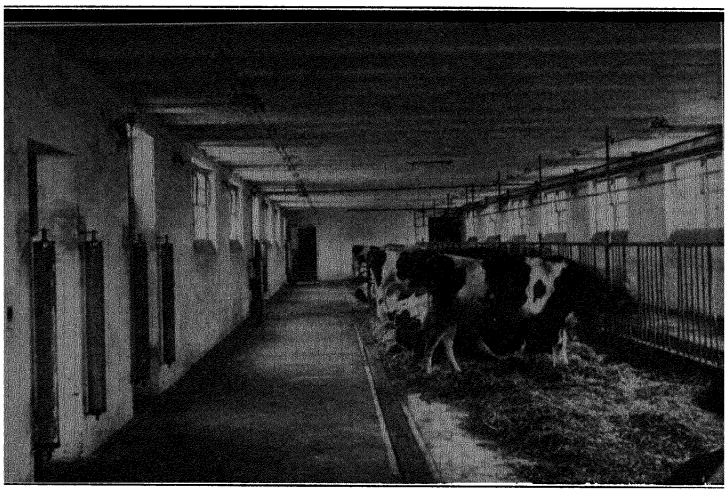

FIG. 30. Modern Type of Dairy Barn.

as a result. The biological changes now produced are anaerobic or putrefactive in nature and take place much more slowly than under aerobic conditions.

Under aerobic conditions, carbohydrates are changed to carbon dioxide and water, lactic, acetic and other organic acids, methane, hydrogen and sometimes nitrogen. The product remaining is practically the same as soil humus. In this series of reactions, however, unions are formed between the free humic acids and the alkaline carbonates of ammonia and potassium, forming soluble compounds which impart a dark color to the liquid present in the manure.

The fermentation of the nitrogenous matter is even more important. In this process the decomposition of urea may be represented by the following equation:

$$\underset{\text{urea}}{(NH_2)_2CO} + HOH = \underset{\text{ammonium carbamate}}{NH_4 \cdot O \cdot CO \cdot NH_2}$$

$$\underset{\text{ammonium carbamate}}{NH_4 \cdot O \cdot CO \cdot NH_2} + HOH = \underset{\text{ammonium carbonate}}{(NH_4)_2CO_3}$$

Part of the nitrogen-bearing compounds of the manure are quite insoluble, for example, the proteins. These pass through a series of complex changes, being converted into amino acids and finally into ammonia.

Some of the nitrogen is lost in elemental form. Inasmuch as nitrite-forming bacteria are present, such a loss may be increased by the action of nitrous acid or ammonia, on compounds containing amino nitrogen:

$$R \cdot NH_2 + HONO = R \cdot OH + N_2 + HOH$$

Some of the readily available nitrogen compounds of manure may be utilized for protein manufacture by the organisms themselves. This change is exactly the reverse of those previously described. There is a tendency, therefore, for some of the nitrogen to be conserved. These two processes take place simultaneously, and the quantity of simple or complex compounds present at any one time depends, in a measure, upon the relative rate at which these reactions take place

In the fermentation of manure, therefore, nutrients may be lost in several ways, including:

1. The formation and liberation of carbon dioxide.
2. The formation and liberation of ammonia
3. The liberation of free nitrogen.
4. The loss of certain constituents through leaching.

The Preservation of Manure

Many methods are employed in the handling of manure in order that losses of the more valuable fertilizing constituents may be kept at a minimum.

Direct Utilization. — In order that unsanitary conditions may be avoided in dairy and other barns, manure cannot be left in the stalls for any considerable period of time. If possible, the manure should be removed and hauled to the fields at regular intervals Any leaching that may occur later will carry the plant food directly into the soil. At the same time, losses due to fermentation are limited.

On steep hillsides the rains may wash considerable quantities away. It is not desirable, therefore, to spread manure on fields thus located until just before the time for plowing. Under these conditions, the manure should be stored until it is ready for use.

Storage. — When manure is to be stored for any length of time it is important to know just how losses of valuable constituents may be kept at a minimum, some loss being inevitable. Since the two chief sources of loss are due to leaching and bacterial decomposition, it is obvious that those processes must be controlled as much as possible. Protection of

the manure against leaching is accomplished by keeping it under cover. Air-tight floors in the stalls are likewise of advantage from this standpoint. In order that bacterial decomposition may be controlled, the manure should be kept in a moist compact condition. In this way, the action of aerobes is inhibited and nitrogen losses are prevented.

Chemical Preservatives. — Powdered gypsum is sometimes mixed with manures in order that it may absorb free ammonia. It is thought by some that the absorption of ammonia by this material is chemical in nature and takes place in the following way:

$$CaSO_4 + 2\,NH_3 + H_2CO_3 = CaCO_3 + (NH_4)_2SO_4$$

Sulfates of magnesium and iron, sodium bisulfate, superphosphate and sulfuric acid have been used for similar purposes. It is not desirable, however, to use acid materials because of the danger of injury to animals that may tramp in the manure. The use of sulfates may give rise to sulfides, which is not desirable from the standpoint of soil fertility. In some instances, antiseptics are used to counteract bacterial activity. For obvious reasons, this is not a desirable practice. It would be much better to add superphosphate, because this material tends to limit the losses of nitrogen as ammonia. Some investigators are of the opinion that nothing exceeds the effectiveness of soil additions in preventing losses of manurial constituents, especially if the soil contains considerable humus material.

The Reinforcement of Manure

Manure is not a well-balanced fertilizer, since it is quite low in phosphoric acid. This unbalanced condition is rendered more noticeable by the fact that ordinary agricultural soils are quite low in available phosphorus. The rather wide P/N ratio in manure may be counteracted by the use of superphosphates or similar materials as supplements. The use of rock phosphate as a supplement has led to varying opinions as to its efficiency. On the whole, however, the value of manure has been increased by its use, although it is less effective than superphosphate.

The Availability of Manurial Constituents

Fresh manure, used on soils containing large quantities of clay, tends to warm the soil and make it more porous, thus aiding decomposition processes and setting free its fertilizing constituents and, in addition, part of the insoluble plant food from certain of the soil minerals as well. Such a manurial application tends to make conditions more favorable for root development and results in a better utilization of the fertilizing constituents.

Rotted manure has a less marked effect in this connection. On light or sandy soils, however, rotted manure is more beneficial than fresh manure, as the latter would have the effect of heating and drying out such soils, and of increasing their porosity to an undesirable extent. These conditions would lead to a loss of manurial constituents by heavy rains.

Fresh manure induces rank growth, and for this reason it is undesirable for use on many crops. Especially is this true for the cereals, with the possible exception of corn. Even in the case of a leaf crop such as tobacco, a rank growth is undesirable. It is desirable to add fresh manure to the corn crop and to follow with those plants which are less susceptible to the unbalanced condition of the fresh manure, but this practice has its limitations. In this way, a more efficient utilization of the manure constituents may be effected and no rank growth will be induced.

Although manure may be rotted by keeping it in a moist compact heap, it is probably better, where possible, to compost the material. Especially is this true of horse manure, which heats readily. In composting this material, clay, peat, or muck soil may be used. This is done by making a foundation of about six inches of dirt and on top of this placing alternate layers of manure and soil, moistening the mass as the heap grows At the end of two months the heap should be torn down and thoroughly mixed, if necessary, and remoistened with water Sometimes it is desirable to add lime to hasten decomposition of the manure, although this practice may lead to the volatilization of ammonia.

Composting manure tends to prevent undue heating and also retains practically all of the most desirable constituents in a form in which they are best utilized by growing plants.

The Effects of Manure on the Soil

The effects of manure on the soil are physical, chemical and biological in character.

Physical Effects. — The beneficial physical effects of manure on soils are due to the action of its organic constituents. These help to flocculate clay soils and reduce them to a state of tilth which it is rather difficult to produce by any other means. On sandy soils it seems to bind the particles together and aid in their power of retaining water. However, it is considered best to make light applications at frequent intervals.

Biological Effects. — Organic soils, formed as they are under extremely moist conditions, usually do not contain appreciable quantities of available mineral nutrients. Moreover, such soils as marshes and peats are sometimes decidedly lacking in bacterial life and unless treated with

manure may require a considerable period of time before crops can be grown on them advantageously. Since manure contains considerable bacterial life, an application of it results in a more rapid breaking down of these organic soils with the liberation of their inorganic constituents which may be absorbed and utilized by plants.

The biological changes produced by applying manure to soils may be more far-reaching than the physical or chemical changes. Many of the manure bacteria will not thrive under soil conditions, but a number will continue to multiply and in so doing will prove of material benefit in a number of ways. It is possible, therefore, for the bacterial content of the soil to be changed both quantitatively and qualitatively, as there are added in the manure many new species. The physical and chemical effects of the manure on the soil will greatly modify these already present, for the microflora and microfauna of the soil were due, originally, to the specific properties of that soil.

Chemical Effects. — The chemical effects brought about by manure additions to the soil are rather striking when considered from the point of view of composition. If used in quantities comparable to ordinary commercial fertilizers, the mineral nutrients added would appear to be rather insignificant. As ordinarily used, however, the quantity of mineral nutrients is sufficient to disturb the chemical equilibrium of the soil.

When fresh manure is added, a decrease of soluble nitrogenous plant food material always results, so that crops grown on a soil so treated may have their growth retarded. For this reason, fresh manure should be allowed to decompose for a time before a crop is planted, or the manure should be so treated that this effect may be counteracted to a certain degree.

The Residual Effect of Manures. — No other fertilizing material is said to be more beneficial from the standpoint of soil fertility than manure. It is a complete fertilizer, although unbalanced for most plants, and the effect of a single application may be apparent for years. At the Rothamsted Station, where one application of 14 tons of manure had been made to a field, the results on the fertility of the soil were still apparent at the end of 40 years.

The Production of Artificial Manure

Experiments conducted at the Rothamsted Station relative to the conversion of straw and similar material into manure, without the intervention of live stock, have resulted in the production of a material quite similar to ordinary farm manure. It was found that, if straw was inoculated with a culture of aerobic cellulose-decomposing organisms (*Spiro-*

chaeta cytophaga), with the proper quantity and kind of mineral nutrient material, satisfactory results could be obtained under conditions favorable in other respects. It was found later that the bacteria normally present were likewise efficient in this regard. This process has been patented.

In the production of well-rotted artificial manure from straw, it was found that a liberal air supply, a fairly high temperature and a suitable supply of soluble nitrogen compounds were essential for the optimum activity of the organisms involved. Moreover, it was found that a neutral or slightly alkaline reaction of the medium was desirable.

According to Albrecht, a reagent consisting of 45 pounds of ammonium sulfate, 15 pounds of superphosphate and 40 pounds of limestone, when added at the rate of 150 pounds per ton of straw, yielded a satisfactory mineral balance of the organisms under proper conditions of temperature and aeration, if the decomposing mass was kept liberally supplied with moisture.

Many investigations have been and are now being conducted on the production of artificial manure. It is hoped that these experiments may lead to a better utilization of refuse plant materials on the farm.

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CHAPTER XII

FERTILIZERS

All cultivated soils lose, in the course of time, their ability to produce good crops. This failure may be due to poor drainage, abnormal physical conditions, the presence of parasitic organisms, the absence of organic matter, the absence of beneficial soil organisms, the presence of toxic organic and inorganic compounds or a lack of mineral plant nutrients. Materials which supply the lacking mineral plant food are called *fertilizers*. The elements usually lacking in soils are phosphorus, potassium, nitrogen, calcium, magnesium and sulfur.

PHOSPHATE FERTILIZERS

Agricultural soils, shortly after being placed under cultivation, usually respond to applications of phosphatic materials. This should indicate that phosphorus is lacking in these soils, or that it is not made available as rapidly as the crop can absorb and use it.

Phosphorus, in the form of bones, has been added to soils for many years and is still being used. About the middle of the last century, the use of mineral phosphates became rather popular, especially after the discovery was made that sulfuric acid rendered the phosphorus in these materials more available for plant nutrition. These are now being used in increasing quantities, and the phosphorus problem of today constitutes one of the most important of our agricultural problems, owing to the fact that the visible supplies are not inexhaustible.

Available Supplies of Phosphatic Materials. — The phosphates used for fertilizers are derived from residual, replacement and sedimentary deposits. On the whole, the most important are the sedimentary deposits. There are three very large phosphate fields, the Western American, the Northern African and the Russian. However, many smaller deposits are found in practically all parts of the world. At the present time practically one-half of the world's supply of phosphate comes from the North African fields. A great deal of the remainder comes from the eastern fields in the United States and from oceanic islands. The Florida fields supply the highest grade of hard rock phosphate in the United States. It is of the residual type and contains 76 to 80 per cent or more of tri-calcium phosphate. The Tennessee fields produce a rock of a lower

grade as compared with the deposits in Florida, containing from 68 to 77 per cent, whereas that of the western fields contains approximately 72 per cent or more of tri-calcium phosphate.

In 1924, according to Mansfield, about 414,157,000 tons of raw phosphate rock were available in Arkansas, Kentucky, South Carolina, Tennessee and Florida, as compared to 6,430,565,000 tons available in Idaho, Montana, Utah and Wyoming, the states comprising the western deposits.

It is estimated that these supplies should meet the needs of agriculture for centuries to come, at the present rate of consumption, but it should be borne in mind, however, that the ordinary systems of mining phosphate rock are decidedly wasteful, and much of the low-grade rock cannot be used economically. Moreover, the figures given above are but rough approximations at best. For these reasons, some authorities are inclined to believe that not many centuries will elapse before a world-wide phosphorus famine may be in evidence.

Phosphorus is continually being lost from the soil and must be replaced from an outside source Whitson estimates that 6 pounds of phosphorus per acre are lost annually. A great deal of this is removed by crop plants, a part of which eventually finds its way into the sewage systems of cities from which it is never recovered. It has been estimated that the loss in the United States by this means alone totals considerably more than the equivalent of 1,000,000 tons of phosphate rock annually.

Various phosphorus-bearing fertilizers are on the market, and the merits of some of these will now be considered.

Rock Phosphate. — Rock phosphate, $Ca_3(PO_4)_2$, appears on the market as a very finely ground powder of light gray color, of approximately colloidal dimensions. Owing to the fact that these particles remain suspended for a time when blown into the air, the term *floats* is sometimes used to designate this product. It generally carries about 27 per cent of P_2O_5.

Rock phosphate, or raw phosphate rock as it is sometimes called, is a very slow-acting fertilizer and is generally used in quantities of from 1000 to 2000 pounds per acre, larger quantities than would ordinarily be necessary if the material were more available. It is generally agreed that, under soil conditions, carbonic acid increases its availability to a limited extent, especially if the products of the reaction are removed from the system. For this reason, it is thought that the presence of decomposing organic matter tends to aid in the availability of the phosphate, according to the following equation:

$$Ca_3(PO_4)_2 + 4\ H_2CO_3 \rightleftharpoons CaH_4(PO_4)_2 + 2\ Ca(HCO_3)_2$$

In the course of this process, the carbonate of lime is changed to gypsum, and carbon dioxide with some chlorine and fluorine is evolved, and the raw rock is converted partly into gypsum and partly into phosphoric acid and mono- and di-calcium phosphates.

The mixture enters the den at a temperature of about 120° C. Steam and other gases pass through the slowly drying mass, leaving a porous material which is rendered stable through the presence of gypsum. The changes in the den, therefore, involve among other things the formation of a dry and friable product from a semi-liquid material.

At the end of 24 hours, the contents of the den are removed and dumped on a pile, where they "air," that is, they tend to dry out further, and

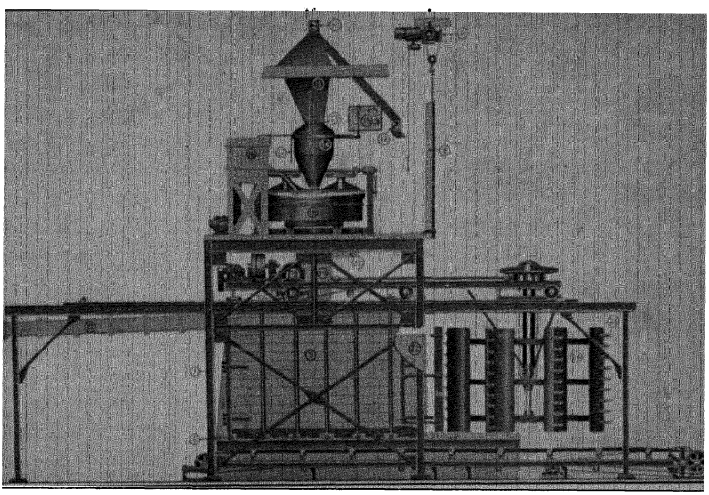

FIG. 31. Phosphate Acidulating Unit complete with mechanical den and excavator. (Courtesy of the Sturtevant Mill Company.)

the free phosphoric acid reacts with the unchanged tri-calcium phosphate. After a few weeks it is reground and prepared for shipment.

Figures 31, 32 and 33 show apparatus used in the manufacture of superphosphate.

The reactions involved in the manufacture of mono-calcium phosphate are as follows:

(1) $3\ Ca_3(PO_4)_2 + 6\ H_2SO_4 \rightarrow 4\ H_3PO_4 + Ca_3(PO_4)_2 + 6\ CaSO_4$

(2) $4\ H_3PO_4 + Ca_3(PO_4)_2 \rightarrow 3\ CaH_4(PO_4)_2$

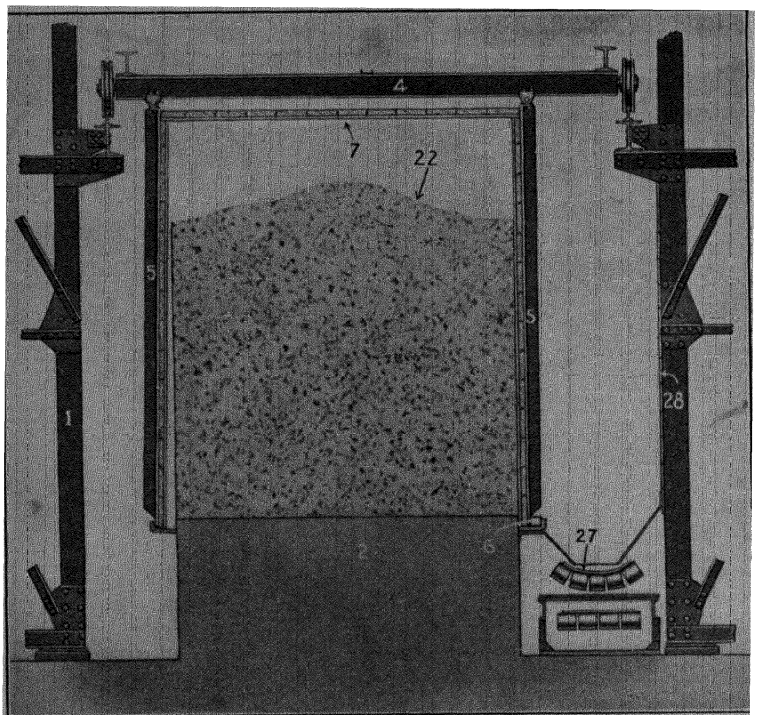

FIG. 32. Vertical cross section through den, back of revolving cutters. (Courtes of the Sturtevant Mill Company.)

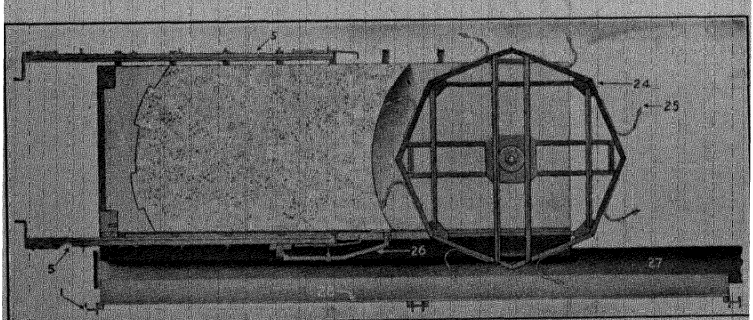

FIG. 33. Den and excavator. (Courtesy of the Sturtevant Mill Company.)

It is also possible for the mono-calcium phosphate to react with tri-calcium phosphate, forming di-calcium phosphate:

$$CaH_4(PO_4)_2 + Ca_3(PO_4)_2 \rightarrow 2\ Ca_2H_2(PO_4)_2$$

Since fluorine as calcium fluoride is generally present in phosphate rock, the sulfuric acid reacts with it to form hydrofluoric acid:

$$CaF_2 + H_2SO_4 \leftrightharpoons CaSO_4 + 2\ HF$$

Since free silica is generally present, hydrofluoric acid reacts with it forming silicon tetrafluoride:

$$4\ HF + SiO_2 \rightarrow 2\ H_2O + SiF_4$$

If sufficient water is present, the silicon tetrafluoride reacts with it to form hydrofluosilicic acid:

$$3\ SiF_4 + 2\ H_2O \rightarrow SiO_2 + 2\ H_2SiF_6$$

The presence of fluorides is quite objectionable on account of the toxicity of the compounds formed in the course of the manufacturing process and the excessive quantities of sulfuric acid required by their presence.

The calcium carbonate that is invariably present reacts with sulfuric acid as follows:

$$CaCO_3 + H_2SO_4 = CaSO_4 + H_2O + CO_2$$

Unless calcium carbonate is present in relatively large quantities, it is not considered entirely objectionable because the heat generated in the reaction with H_2SO_4 serves to increase the rate of the main reaction and the carbon dioxide serves to assist the reaction by leaving the mass porous. If the calcium carbonate is rather high, care should be taken to insure the presence of sufficient water to aid in the formation of gypsum, which is hydrated calcium sulfate.

Another of the common impurities of phosphate rock is iron in the form of ferrous or ferric phosphate, often as pyrites and sometimes as oxides. Aluminum is likewise present in the form of the phosphate or silicate. A phosphate rock high in iron and aluminum is undesirable, since there is a tendency for part of the phosphoric acid to be rendered unavailable for plant growth when these are present, owing to their reaction with sulfuric acid and subsequent reaction with phosphoric acid to form the highly insoluble iron and aluminum phosphates.

Mono-calcium phosphate is quite available to plants. Its solubility and availability are decreased, however, when it is brought into contact with calcium acid carbonate, forming the relatively insoluble tri-calcium phosphate:

$$CaH_4(PO_4)_2 + 2\ Ca(HCO_3)_2 \leftrightharpoons Ca_3(PO_4)_2 + 4\ H_2O + 4\ CO_2$$

When added to soils, mono-calcium phosphate dissolves and is fairly well distributed over a small area. Although the action of calcium bicarbonate, as shown above, is to render it unavailable, the particles of tri-calcium phosphate formed are quite small and possess a maximum of surface for solution activities. In the presence of relatively large quantities of carbon dioxide, the direction of the above reactions may be reversed.

An application of mono-calcium phosphate does not necessarily result in permanently increasing the acidity of soils, although such effect may be apparent immediately after the application. Owing to the tendency of mono-calcium phosphate to react with the soluble iron and aluminum compounds present in acid soils, it is desirable to add relatively large quantities to such soils. Moreover, it also supplies calcium in a form available for plant growth which is extremely desirable under such conditions. The soil should be neutral or very slightly acid for maximum beneficial effects of phosphate additions.

It is considered inadvisable to mix superphosphate with any form of lime, basic slag, wood ashes, or any other substance which would react with it and change its chemical nature. Above all, according to Murray, it should not be mixed with sodium nitrate because the following reaction takes place, resulting in a loss of nitrogen:

$$3\ CaH_4(PO_4)_2 + 8\ NaNO_3 \rightarrow Ca_3(PO_4)_2 + 4\ Na_2HPO_4 + 8\ NO_2 + 2\ O_2 + 4\ H_2O$$

Ammonium sulfate, on the other hand, does not react with mono-calcium phosphate.

A very high grade of phosphate fertilizer called double superphosphate, containing 35 per cent P_2O_5, has been manufactured in Germany and in the United States. In this process the purest phosphate is treated with an excess of sulfuric acid, the phosphoric acid is removed from the gypsum and more phosphate rock is added:

$$Ca_3(PO_4)_2 + 4\ H_3PO_4 \rightarrow 3\ CaH_4(PO_4)_2$$

Basic Slag. — This is a by-product of the steel industry. In the manufacture of the best quality of steel, air is blown through molten *pig-iron* in order to oxidize the carbon. Care is taken to prevent the phosphorus in the ore from uniting with the steel by the introduction of a basic mixture consisting of lime and magnesium. When the phosphorus is present to the extent of 2 to 4 per cent of the weight of the ore, the basic slag so obtained contains from 17 to 19 per cent of P_2O_5. More than 75 per cent of the lime in basic slag is in combination with phosphorus and

silicon. The iron present adds considerably to the difficulty of grinding but is not objectionable otherwise.

Basic slag, $P_2O_5 \cdot 4\,CaO$, is comparatively insoluble. Therefore, it acts best when relatively large quantities of water are present, as in clay soils. Owing to its lime content, it may be used to advantage on acid soils. Although basic slag is not as readily available as mono-calcium phosphate, it is far more available than raw phosphate rock. It should not be mixed, however, with ammonium sulfate, as the free lime reacts with this material, resulting in a loss of nitrogen as ammonia.

$$(NH_4)_2SO_4 + CaO \rightarrow CaSO_4 + 2\,NH_3 + H_2O$$

It does not react with nitrate of soda. Under soil conditions it becomes available according to the following equation:

$$P_2O_5 \cdot 4\,CaO + 6\,CO_2 + 5\,H_2O \rightarrow CaH_4(PO_4)_2 + 3\,Ca(HCO_3)_2$$

Bone Phosphate. — The inorganic constituents of bones vary to a considerable extent. Those from mature animals are richer in phosphorus and lime than are those of immature animals. Differences occur also in the composition of bones taken from different parts of the same animal. Raw bones have been used as a phosphate fertilizer; they contain from 19 to 25 per cent of P_2O_5 and from 2 to 4 per cent of nitrogen. In order to prepare bones for the manufacture of phosphate fertilizers they are first steamed or cooked to remove the fat and to convert the protein materials to glue and gelatin. The bones are then ground and sold as steamed bone meal. This should contain approximately 25 per cent of P_2O_5 and about $2\frac{1}{2}$ per cent of nitrogen. Steamed bone meal is much more available than raw bone meal on account of the difference in fat content. The fat tends to protect the particles against the processes of decomposition

If bones are subjected to the process of destructive distillation practically all the organic matter is removed. The resulting product, bone black, may be used for the clarification of solutions in the manufacture of cane sugar. After it loses its absorptive powers it may be utilized as a phosphate fertilizer, although it is extremely slow-acting in this respect. Its availability, however, may be considerably increased by treatment with sulfuric acid, as in the case of raw rock.

Ammonium Phosphates. — In the manufacture of ammonium phosphates, phosphoric acid is obtained by treating tri-calcium phosphate with an excess of sulfuric acid and neutralizing, or partially neutralizing, this acid with ammonia. Some attention is now being devoted to the treatment of superphosphate with strong ammonia.

POTASH FERTILIZERS

Agricultural soils, as a rule, contain considerable quantities of potassium minerals. The availability of these minerals is quite low, however, and in certain cases the addition of readily available potash fertilizers is to be recommended.

Available Supplies of Potash Materials

Foreign Supplies. — For many centuries, the town of Stassfurt, in Germany, has been noted for its salt works. At considerable depths, in that locality, are immense deposits of rock salt, and these have been

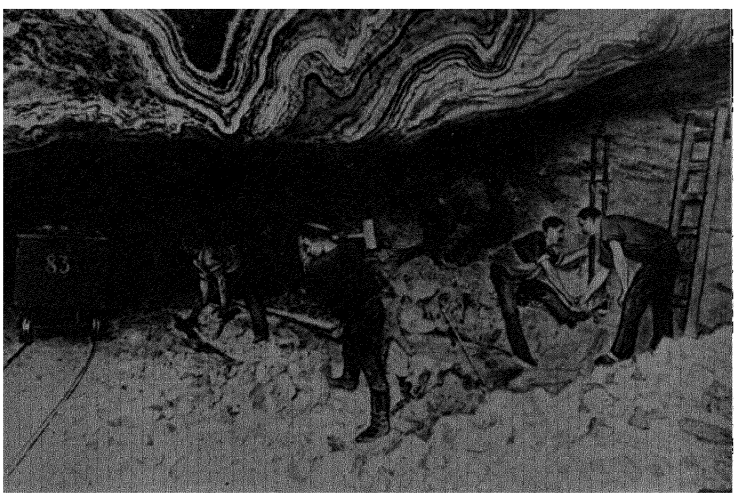

FIG. 34. Mining carnallite. (Courtesy of the Agricultural and Scientific Bureau, N. V. Potash Export My.)

mined for the production of sodium chloride. More recently, however, it was found that these beds were overlaid with potash-bearing minerals, and these deposits at the present time constitute the chief source of potash fertilizers. These minerals were laid down by the evaporation of sea-water confined in lakes which had no outlets, but which were connected with the ocean by channels through which sea-water was forced by storms. The constant evaporation of the sea-water under these conditions eventually produced a supersaturated solution from which the various salts were precipitated, forming more or less definite strata according to their solubilities.

Although there is no sharp line of demarcation between these layers, it is considered that there are four principal regions containing, approximately, 16 kinds of salts. The upper portion, consisting of about one-twentieth of the entire depth of the deposits, yields, according to Turrentine, 55 to 60 per cent carnallite, $KCl \cdot MgCl_2 \cdot H_2O$, and about 18 per cent kieserite, $MgSO_4 \cdot H_2O$. The next lower region, considerably more extensive, yields about 17 per cent kieserite, 13 per cent carnallite and 65 per cent rock salt. This is known as the *kieserite region.* Below this is the *polyhalite region,* which forms a layer equal to the kieserite, and contains about $6\frac{1}{2}$ per cent of polyhalite, $K_2SO_4 \cdot MgSO_4 \cdot 2\,CaSO_4 \cdot$

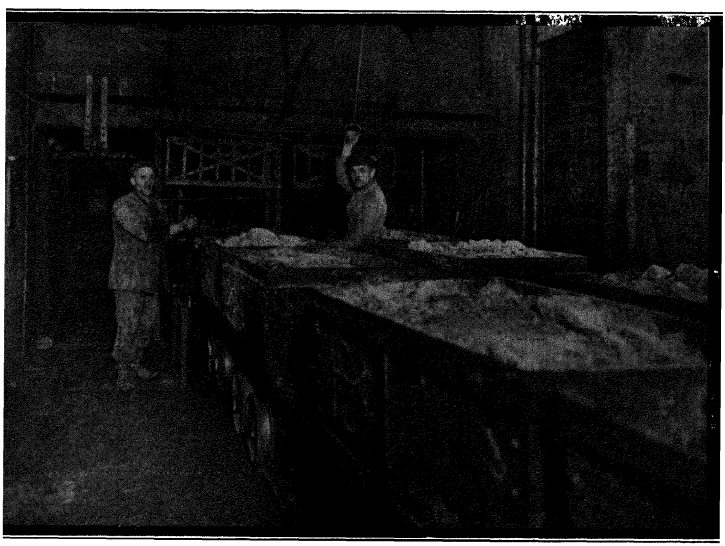

FIG. 35. Cars loaded with potash salts being transported to the surface. (Courtesy of the Agricultural and Scientific Bureau, N. Y. Potash Export My.)

$2\,H_2O$, and 91 per cent of rock salt. The region below the polyhalite contains rock salt and anhydrite, $CaSO_4$, and constitutes about 70 per cent of the depth of the whole deposit.

At the present rate of consumption, it is said that the supplies of potash from the Stassfurt deposits alone will supply the world for the next thousand years.

The crude salts must undergo a process of refinement before they can be used as fertilizers, and it is possible to manufacture different products of more or less constant chemical composition from the crude material.

In the manufacture of muriate of potash, KCl, for example, the coarsely ground carnallite is transferred to a large vat of boiling water saturated with the chlorides of sodium and magnesium. Under these conditions, muriate of potash dissolves readily where rock salt and certain other

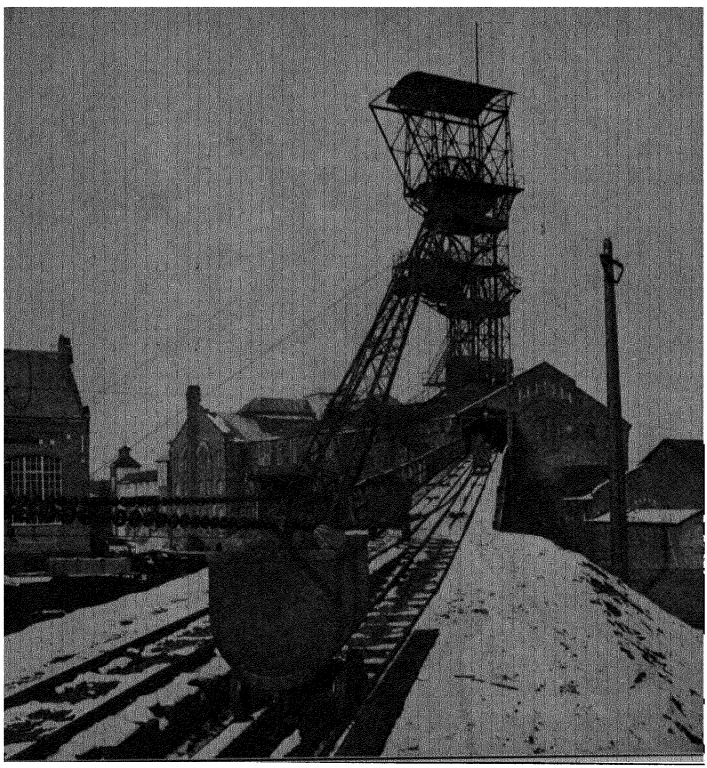

FIG. 36. Headgear and overhead cable-railway for transporting potash salts from the pit-head to the factory. (Courtesy of the Agricultural and Scientific Bureau, N. V. Potash Export My.)

impurities remain undissolved. The hot solution is then drawn off and allowed to remain in settling tanks for a period of 3 or 4 days, in order that crystallization may take place. Later, the crystals are washed with cold water in order to remove more of the impurities. This method is modified according to the kind of crude salt used, but, in any case, the product obtained is never pure. Muriate of potash as it comes on the market may contain 80 per cent or more of KCl.

In the manufacture of sulfate of potash, K_2SO_4, both muriate of potash and kieserite are mixed in the same solution, and a product containing sulfate of potash, with considerable magnesium, is subsequently obtained. When this product is mixed with an equal proportion of muriate of potash and dissolved in boiling water, the less soluble sulfate of potash is precipitated on cooling. This product has a purity of from 90 to 98 per cent as it comes on the market.

In addition to the muriate and sulfate, a number of other more or less refined fertilizer products are obtained from the Stassfurt salts. These contain magnesium and sodium in addition to potassium as chlorides and sulfates and are especially valuable for use on soils deficient in magnesium.

Potash salts also occur in Alsace-Lorraine. These deposits consist of sylvinite, $KCl \cdot XNaCl$, to the extent of approximately 300,000,000 tons,

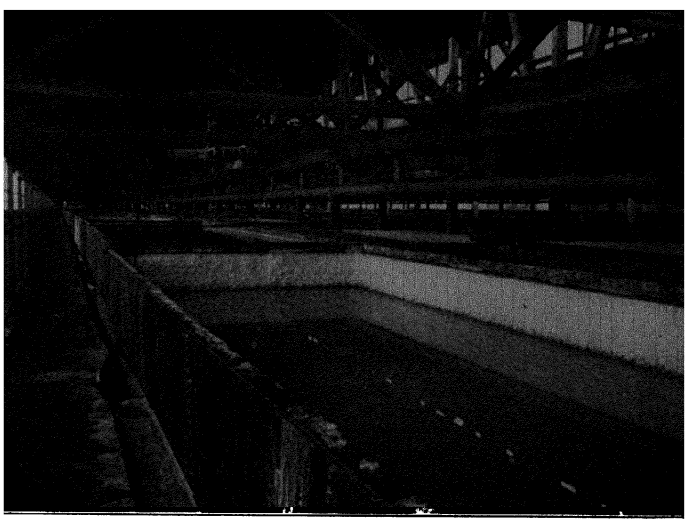

FIG. 37. Crystallizing plant; in the foreground a half emptied vat after the potash salts have crystallized out. (Courtesy of the Agricultural and Scientific Bureau, N. V. Potash Export My.)

which are enough to supply the potash fertilizer needs of the world for more than 300 years. At the present time, the French and German governments are working in coöperation in the disposal of their potash supplies. Deposits of potash also occur in Spain and Poland, but these are not of international importance.

American Potash. — In 1910, because of some difficulty over contracts and the formation of a government monopoly by the Germans, the dependence of the United States on the Stassfurt supplies was so forcibly brought to the attention of our government that appropriations were made for the investigation of possible sources of a domestic supply. This work was placed under the direction of the Bureau of Soils and the United States Geological Survey. As a result, it was found that potash from various sources could be obtained for fertilizer purposes in different parts of this country.

Natural Brines. — Various brines, rich in potash salts, occur in many of the inland lakes of Nebraska. These lakes vary from one acre to two square miles in area and have originated from the leachings of the surrounding areas, which are concentrated through evaporation of the water. The dry salts carry from 27 to 28 per cent of potash salts, largely as the sulfate and carbonate.

Of the federal supply of potash brines, that of Searles Lake, San Bernardino, California, is the most important. This is not a lake in the true sense of the term, but a saturated brine which maintains a practically constant level very close to the top of a salt plain about 12 square miles in area, situated in a basin-like valley The average thickness of this salt deposit is about 70 feet; it consists of sodium chloride, sodium carbonate and other salts. The brine consists of a solution of these salts and potassium chloride, in addition. As a matter of fact, about one-eighth of the total solids of this brine is made up of potassium chloride. It has been estimated that the brine contains quantities exceeding 30,000,000 tons of potash calculated as KCl.

At first, muriate of potash was obtained from solution by means of steam evaporations, but difficulties were encountered from the presence of saponin-like organic substances which produced frothing. This particular trouble was overcome, however, by means of the addition of soap.

The method of manufacture employed at the present time consists essentially of the evaporation of the brine in multiple-effect evaporators, with the elimination of the surplus sodium salts and to the point where the remaining solution is practically saturated with potassium chloride and sodium borate. On cooling this solution, the potassium chloride crystallizes; it is about 98.5 per cent pure. Considerable difficulty was encountered with the utilization of the first potash fertilizer obtained from Searles Lake, owing to the fact that the product contained sufficient sodium borate to be toxic to vegetation. This difficulty was overcome and the trace of boron it now contains is more likely to be beneficial than harmful to plant growth, as boron is now looked upon by some authorities as being essential to the growth of plants.

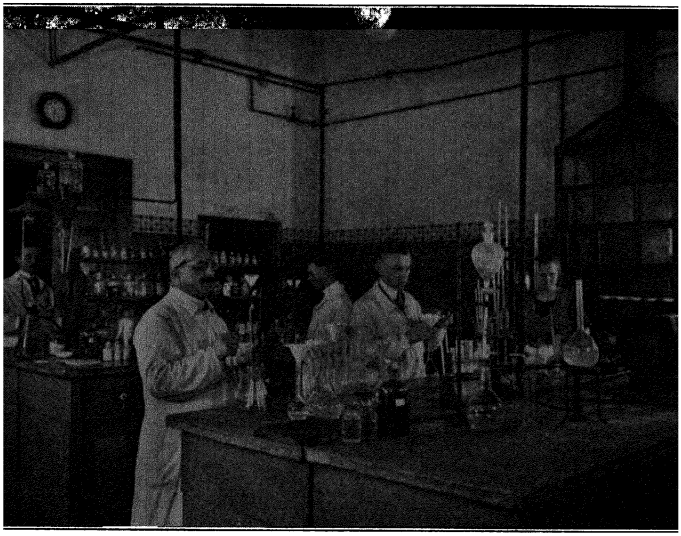

FIG. 38. Chemical control laboratory. (Courtesy of the Agricultural and Scientific Bureau, N. V. Potash Export My.)

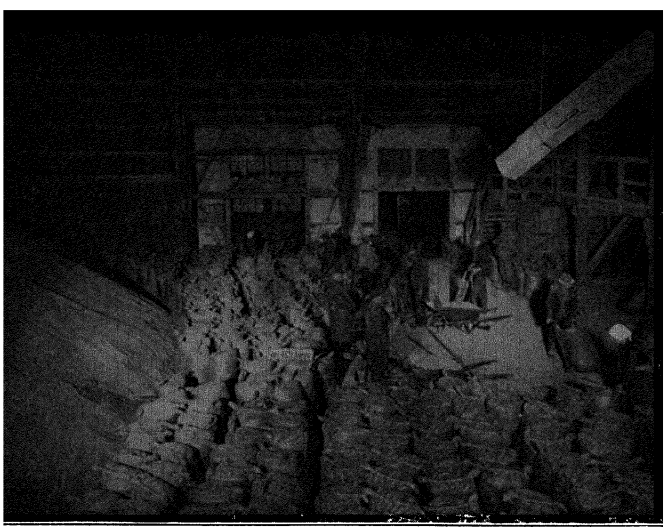

FIG. 39. Bagging, weighing, sampling and loading salts for transport. (Courtesy of the Agricultural and Scientific Bureau, N. V. Potash Export My.)

Minerals. — Alunite, or hydrous sulfate of aluminum and potassium, $K_2O \cdot 3\ Al_2O_3 \cdot 4\ SO_3 \cdot 6\ H_2O$, containing 11.4 per cent of potash, is found in southern Utah. By calcination the potash is rendered soluble and can be dissolved, concentrated and crystallized as the sulfate. By suitable modifications of this process high-grade alumina and other compounds can be obtained as by-products. The supply of alunite is limited in quantity.

Leucite, $KAlSi_2O_6$, a potassium-bearing mineral, is found in Wyoming, in comparatively large quantities. Upon treatment with sodium

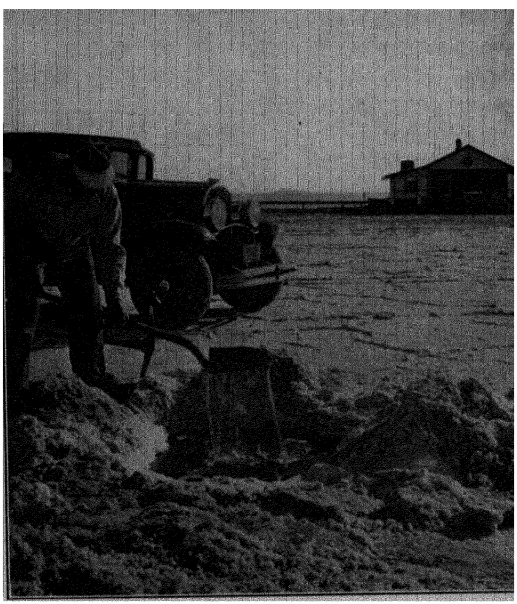

FIG. 40. Showing nature of Searles Lake formation, or salt body in which the brine rises to within an inch or two of the surface. This brine is pumped to the plant, and becomes the source of KCl. (Courtesy of the American Potash and Chemical Corporation.)

chloride and subjecting the mixture to a high temperature, potassium is volatilized as the chloride. It is estimated that this source may yield approximately 200,000,000 tons of potash and an equal quantity of alumina.

Polyhalite, $K_2SO_4 \cdot MgSO_4 \cdot 2\ CaSO_4 \cdot 2\ H_2O$, another potassium-bearing mineral, is found in large quantities in Texas and New Mexico

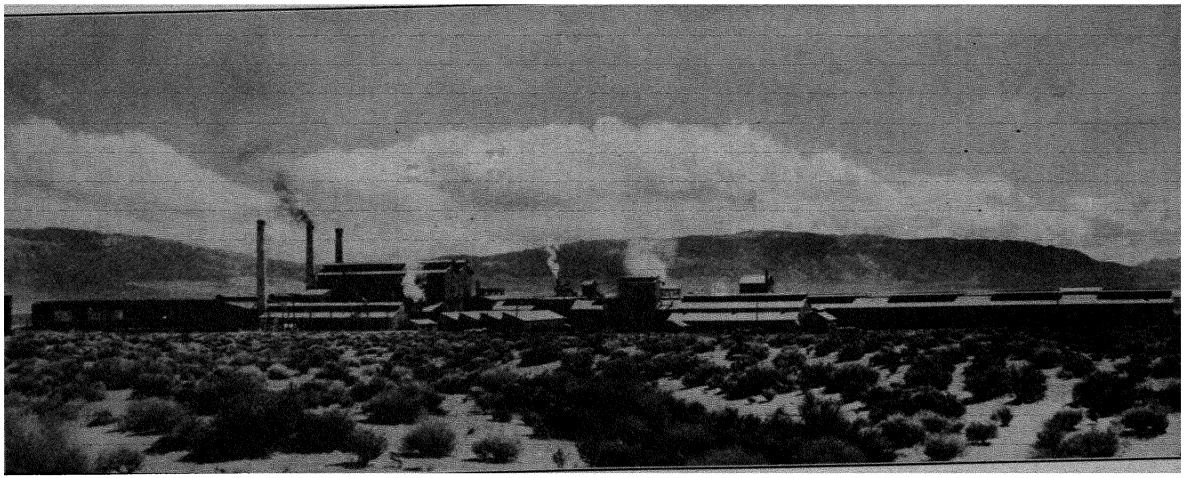

FIG. 41. General view of potash plant of Searles Lake. (Courtesy of the American Potash Corporation.)

and will probably constitute an important source of potassium in this country. Many other less valuable domestic supplies of potassium-bearing minerals are known but at present are not utilized to any considerable extent for the production of potash fertilizers.

Cement and Flue Dusts. — In the manufacture of cement and in the smelting of iron, potash is volatilized and may be recovered in a form suitable for use as a fertilizer. Potash is precipitated from the dusts and gases by the Cottrell Electrical Precipitation Process. It has been estimated that, in the manufacture of cement alone, 90,000 tons of potash could be obtained annually by this method.

Potash from Organic Sources. *Kelp.* — Kelp is a seaweed which grows along the Pacific coast. Certain of these species contain a high percentage of potassium chloride. The air-dried material contains 13.65 per cent of potassium chloride, and the ash contains approximately 30 per cent. Both the plant material and ash have been used as potash fertilizers. Ordinarily, however, the ash is extracted and a product obtained which contains about 90 per cent of KCl. The production of potash from kelp depends, primarily, on the development of by-products, because the cost of producing potash from this source is rather expensive.

Other Organic Sources. — In the crystallization of sugar from both sugar cane and sugar beets, the potash present in the juices becomes concentrated in the molasses. If the sugar is removed by calcium salts the potash may be recovered from the filtrates. By recrystallization, a product can be obtained containing 50 per cent or more potash, principally in the form of the carbonate. Moreover, the tops of sugar beets may be burned and an ash obtained which is also high in carbonate of potash.

Fertilizing Value of Potash Materials

The fertilizing value of a potash fertilizer depends both on the quantity of potassium present and its form of combination.

Muriate of Potash. — This form of potash is not desirable as a fertilizer for such plants as mangolds and cigar-leaf tobacco. Muriate of potash has a deleterious action on the burning quality of the latter. The ash of such a plant shows the presence of a relatively small amount of potash in the carbonate form. This would indicate that certain quantities of the potash within the plant tissue were not in organic combination. This is undesirable from the standpoint of burning quality, as a good-burning tobacco has, as a rule, a considerable quantity of potash combined with organic acids. For many crops, however, the muriate is a desirable form of potash.

Sulfate of Potash. — When the sulfate of potash is used as a fertilizer for cigar-leaf tobacco, the ash of the plants contains more carbonate of potash than when equivalent quantities of muriate of potash are used. One of the probable reasons for this condition is that more sulfur is assimilated by the plant. Potassium is thus rendered available for its normal functions, one of which is to neutralize organic plant acids. Both the sulfate and chloride, however, may be used advantageously in complete fertilizers for the growth of most crops.

Carbonate of Potash. — The supplies of this material are rather limited at the present time. It should be more desirable than either the muriate or sulfate, in that it carries no undesirable residual material and is already in proper form for assimilation. It has, however, a puddling effect on the soil which the other forms mentioned do not have. It should never be mixed with ammonium salts because it decomposes the latter, with a resulting loss of ammonia

Nitrate of Potash. — This is a desirable form of both potassium and nitrogen. At the present time, however, its supply is rather limited. Murray states that, for the proper utilization of potash fertilizers, the soil should contain plenty of available lime compounds. When replacement of adsorbed hydrogen takes place by the addition of these fertilizers, it is desirable to have the acids neutralized which are formed in the course of such a reaction It must be remembered, however, that, in the case of certain soils, the presence of excess quantities of calcium is not desirable because the potash is rendered less available for plant growth than when smaller quantities of calcium are present.

NITROGENOUS FERTILIZERS

Nitrogen is usually the most expensive of the various elements commonly used as fertilizers. It is usually deficient in soils, notwithstanding the fact that considerable quantities are added to soils by the aid of specific organisms which have been discussed in a previous chapter. For this reason, therefore, nitrogen must be added to the soil in a form which is available for utilization by plants. Fortunately, such materials are now obtainable from a number of organic and inorganic sources.

Sources of Nitrogenous Materials

Nitrate of Soda. — Natural nitrate of soda is found in extensive deposits on the arid plateau of northern Chile. It occurs in a mixture of various salts (principally of sodium, potassium, and magnesium) which cement together clay, sand, pebbles, and stones of various sizes to form a conglomerate known as " caliche." The caliche occurs as an

undulating layer which varies from about one foot to ten feet in thickness. In the workable deposits it lies at different depths, in places as deep as six or eight feet below the surface of the ground.

For approximately the last fifty years, the methods used in the mining of the caliche and the extraction of the nitrate have experienced but few changes. According to these methods, the mining was done by hand and the extraction of the nitrate was effected by what is known as the "Shanks process." A brief description of this process follows:

The Shanks Process. — The miners first remove with hand shovels any soft material that may overlie the caliche. Special drillers then sink pits of large diameter through the caliche layer, the work being carried out wholly by means of an iron bar which is wielded by hand. The bottoms of these holes are enlarged and filled with a low-grade blasting powder which is made locally. These charges are then exploded, and the caliche is broken up. The miners hand-sort the caliche that is of sufficiently high grade for treatment in the plant, which is then loaded into railway cars, either directly or after a short haul by mule cart to a gathering point. It is then shipped to the plant by rail for treatment.

At the plant the caliche is crushed to about 3-inch size and placed in small steel tanks which hold from 60 to 100 tons of material and which are equipped with steam coils. A solution, saturated with the various caliche salts, is then run onto the caliche and the charge is boiled. As a result, the nitrate is dissolved from the caliche, making a hot solution with a high nitrate content. This solution is then run to settling tanks to be clarified. The caliche, from which most of the nitrate has been removed, is washed with weaker solutions and the remaining material is finally discharged as waste. The strong solution, after it is clarified in the settling tanks, is run into shallow pans and allowed to stand in the open air where it is cooled. In the cooling, the nitrate crystallizes out. This nitrate is removed from the pans, allowed to drain, and is then stored in large piles where in time it becomes sufficiently dry to be bagged and shipped to the consuming markets. The solution from the cooling pans is returned to the boiling tanks and is used to treat new caliche as it is received from the crushers.

The above methods proved fairly economical so long as a supply of high-grade caliche was procurable; but as the higher-grade deposits (which at one time yielded caliche that contained about 60 per cent sodium nitrate) gradually became exhausted, the plants had to treat lower and lower grades until they are now treating caliches of about 15 to 20 per cent sodium nitrate, with resulting increased costs.

The New Process. — During the last ten years a new process has been developed by the firm of Guggenheim Brothers, latterly through the

Anglo-Chilean Consolidated Nitrate Corporation. This process has allowed mechanical methods to be applied to the nitrate industry, and in this way the cost of production has been lowered and the utilization of much lower-grade caliches has become possible through mining by mechanical methods. This new process, together with its mechanical mining, is now in use in only one plant, but, as a similar plant is being constructed for the Lautaro Nitrate Company, it would seem that, gradually, the old-time methods must be replaced by the new.

Under the new process, the caliche is drilled with compressed-air-driven jackhammers, blasted by high-grade blasting powders, loaded into cars by large electric shovels in pieces which may be as large as 6 feet in length, and hauled to the plant where it is crushed to a size of less than $\frac{5}{8}$-inch in diameter. It is then loaded into large concrete tanks, each holding approximately 7500 tons, and treated with warm solutions which have received their warmth from waste heat that becomes available in the plant. These solutions are then cooled down by mechanical refrigeration in large cooling tanks, to about freezing point, during which time the nitrate crystallizes out. The crystallized nitrate resulting therefrom is separated from the liquid by centrifugal driers which discharge the nitrate with a moisture content of about 2.5 per cent. The solutions are then re-warmed and returned to the dissolving tanks where the same cycle of operations is repeated.

The nitrate from the centrifugal driers is sent to a graining plant where it is melted in large iron pots and then sprayed in the molten form in an enclosed chamber. The drops, on cooling, solidify into small shot-like pellets, a form most suitable for agricultural purposes. The product thus obtained, having lost all its moisture in the fusion operation, is packed in water-proof, paper-lined burlap bags which protect it to such an extent that it is delivered to the consumer with a moisture content of less than 0.5 per cent, and (according to standard methods of analyzing nitrate of soda) with a sodium nitrate content of about 99 per cent.

The outstanding features of the improved process are: first, the ability to extract sodium nitrate efficiently from rock of lower grade than is considered economical for use in the Shanks process; second, the conservation of all heat wastes in the operation; and third, the employment of about one-fourth of the men necessary for similar production under the old process.

Thus it is possible to introduce mechanical methods for handling the material on a larger scale than heretofore, the available reserves of nitrate are greatly expanded, the output per unit of fuel consumed is materially increased, and, incidentally, a purer product is delivered.

The Composition of Nitrate of Soda. — Pure nitrate of soda is a colorless crystalline substance containing 16.47 per cent of nitrogen. As it ordinarily comes on the market, however, it contains about 95 per cent nitrate of soda, or about 15.65 per cent of nitrogen.

The Fertilizing Value of Nitrate of Soda. — The form of nitrogen in this fertilizer is most suitable for plant growth. An application of sodium nitrate brings about a growth response in a relatively short period of time. It is not so desirable as a nitrogenous fertilizer on light soils because of its extreme solubility and the ease with which it leaches out of such soils. The same is true to a greater or less extent for all agricultural soils, and it is mainly for this reason that authorities advise not to make a single application unless it is necessary, but to divide the entire quantity into two or three portions and add each portion at different intervals, preferably as a top dressing. In order to insure proper distribution, however, it should previously be mixed with soil or some other inert material. Such applications are highly desirable for grasses and cereals.

The value of nitrate of soda as a source of nitrogen is at a maximum when the environmental conditions are not the best for nitrification, as in the early spring. It is extremely important, also, in the effect that it has on the decomposition of organic matter, for reasons given elsewhere. Excessive quantities of nitrate of soda are undesirable from the standpoint of permanent soil fertility, as they tend to inhibit somewhat the processes of nitrification and nitrogen fixation. Moreover, such additions would not only leave an undesirable residue in the soil, but also cause an alkaline reaction, which may be undesirable The continued use of even small additions tend to bring about a *puddling* of the soil.

Sulfate of Ammonia. — When bituminous coal is subjected to destructive distillation, as in the manufacture of coke, part of the nitrogen contained in the coal may be recovered by the employment of suitable methods. The gases given off are hydrogen, methane, and other hydrocarbons, carbon monoxide and dioxide, sulfur compounds, ammonia, water vapor, and tarry materials. To recover the ammonia, the gases are cooled and brought into contact with water which readily dissolves the ammonia and many other compounds. Owing to the fact that the ammonia tends to combine with carbonic acid and other compounds present, the solution must be treated with lime. The ammonia is liberated by contact with boiling limewater, and the mixture is distilled by steam into sulfuric acid with which the ammonia unites, forming ammonium sulfate.

The Composition of Sulfate of Ammonia. — When pure, ammonium sulfate is a white crystalline salt, but the commercial product is usually

gray in color. Unlike nitrate of soda, it is not hygroscopic. It contains 20.75 per cent nitrogen.

The Importance of Sulfate of Ammonia as a Fertilizer. — Sulfate of ammonia may be mixed with any inert fertilizing material but should not be used with caustic lime or any similar material which may liberate the ammonia. When added to moist soils, sulfate of ammonia dissolves as readily as nitrate of soda and is quickly distributed throughout a given area. The ammonium ion may displace the adsorbed bases present in the soil, which eventually unite with the sulfate radical, forming sulfuric acid or sulfates. For this reason, the nitrogen of ammonium sulfate is more readily retained by soils than the nitrogen of sodium nitrate.

Some agricultural crop plants appear to use ammonia nitrogen directly, and to grow as well, when this form of nitrogen is present, as when nitrates predominate. Most plants, however, respond to additions of nitrate nitrogen. In order, therefore, for sulfate of ammonia to be of maximum value in the nutrition of the common agricultural crop plants, it is necessary for the ammonia to be changed to nitrate by the process of nitrification. Ordinarily, if the environmental conditions are of the best, this change takes place rather quickly. Sulfate of ammonia, on the whole, is not as readily available for plant growth as nitrate of soda. It should be applied rather early, therefore, in order to insure its nitrification

On soils deficient in lime, sulfate of ammonia may be injurious. This is probably due to the fact that under these conditions sulfuric and nitric acids are formed, which, if not completely neutralized, may cause the soil solution to become sufficiently acid to interfere seriously with normal plant growth. On the other hand, an excess of lime should not be present because it may lead to the loss of nitrogen through volatilization of ammonia.

On the whole, the effects of sulfate of ammonia on soil acidity have been over-emphasized. The continued use of this fertilizer will deplete the soil of lime, but if proper attention is given to satisfying the lime requirement, the use of sulfate of ammonia as a carrier of nitrogen is to be recommended for most crop plants.

Nitrogen-fixation Methods. — A marked effort has been made in recent years to fix atmospheric nitrogen in such a form that it may be used for plant nutrition. Several methods have proved successful, and other methods, now in the experimental stage, show considerable promise. The three general methods now in use are the electric arc, the cyanamide and the synthetic ammonia process.

The fundamental principle underlying the so-called arc process is based on the fact that, under conditions involving the dissociation of

both oxygen and nitrogen by means of the electric arc, nitric oxide is formed as a result of recombination of the ions involved. In this method, electric furnaces of various types have been employed for the production of a broad or long electric arc through which air is allowed to pass. These methods involve large quantities of electrical energy and can be used advantageously only in those countries having large amounts of water power. In this process, nitric oxide may be formed very readily, but, unfortunately, it dissociates under approximately the same conditions. It is necessary, therefore, to cool the gases from a temperature of about 3000° C., the temperature of the arc, to about 1200° C. in order to prevent complete dissociation. This is rather difficult to do even under the best of operating conditions. Usually no more than $2\frac{1}{2}$ per cent of the air is converted to nitric acid.

In the Birkeland-Eyde and the Schoenherr processes, the nitric oxide is subsequently drawn from the electric furnace into large chambers where it is allowed to react with more oxygen to form nitrogen dioxide:

$$2\ NO + O_2 \rightleftarrows 2\ NO_2$$

A mixture of NO and NO_2 behaves as N_2O_3, nitrous anhydride, and when brought into contact with water reacts as follows:

$$N_2O_3 + HOH \rightleftarrows 2\ HNO_2$$

Subsequent oxidation of the nitrous acid forms nitric acid:

$$2\ HNO_2 + O_2 \rightleftarrows 2\ HNO_3$$

This acid may then be neutralized with calcium hydroxide or other bases, forming nitrates, which may be utilized as fertilizers.

The Haber process for the fixation of atmospheric nitrogen involves the production of ammonia. In this process, nitrogen and hydrogen are subjected to a pressure of 100 to 200 atmospheres and brought to a temperature of about 600° C. Under these conditions of temperature and pressure, and in the presence of spongy platinum containing traces of Al_2O_3 and K_2O, ammonia is formed. The mechanism of the process is rather involved but the power required is quite small, consequently it offers great possibilities for the economical fixation of atmospheric nitrogen. The ammonia so derived may be led into sulfuric acid, forming sulfate of ammonia, or may be oxidized to nitric acid. The nitric acid may then be neutralized by basic materials.

The Bucher process involves the fixation of nitrogen as a cyanide by heating a mixture of carbonate of soda, coke and iron in an atmosphere of nitrogen:

$$Na_2CO_3 + N_2 + 4\ C \rightleftarrows 2\ NaCN + 3\ CO$$

The iron acts as a catalyst in this reaction. The sodium cyanide obtained may be used for the production of ammonia.

Nitrogen-fixation Products. *Calcium Cyanamide.* — "Calcium cyanamide is prepared by heating calcium carbide and nitrogen together at a very high temperature:

$$CaC_2 + N_2 \rightleftharpoons CaCN_2 + C$$

"The calcium cyanamide obtained in this reaction is a dark crystalline powder, containing as impurities, graphitic carbon and the original impurities found in the limestone and coke which comprise the raw materials.

"The product manufactured on the American Continent contains 21 to 22 per cent of nitrogen. Calcium cyanamide was the first nitrogen-fixation product to be used as a nitrogenous fertilizer. In this country it finds its largest use as a constituent in the manufacture of mixed fertilizers. It contains a large amount of lime, a fact which limits the quantity that may be used in mixed fertilizers containing superphosphates. In European countries, cyanamide is usually applied broadcast to the soil as a single fertilizer material. This use is spreading in the United States, particularly in those regions and for those crops where nitrogen alone is indicated. When added to the soil as a single material cyanamide is first hydrolyzed to urea. The urea thus formed then undergoes the same transformation that urea from animal sources undergoes in the soil. Some investigators are inclined to believe that calcium cyanamide alone does not always prove beneficial as a fertilizer. The manufacturers, however, point out that unsatisfactory results with cyanamide in the past have been due largely to the fact that the material, being comparatively new, has not been used with intelligence. There is no doubt that, as American farmers become familiar with cyanamide, they will secure the same reliable and effective field results which are achieved by European users."

Urea. — The production of urea may be effected by treating calcium cyanamide with sulfuric acid, or by subjecting a solution of ammonium carbonate to the action of a suitable catalyst. Although both sulfate of ammonia and nitrate of soda leave undesirable residues in the soil, after the utilization of the nitrogen which they contain, this is not true of urea. When added to soils, urea is rapidly converted into ammonia, two-thirds of the total amount being converted in 24 hours. Eventually it is changed to nitric acid by the nitrifying bacteria present.

Nitrate of Calcium. — As stated before, the nitric acid obtained by the process of nitrogen fixation can be neutralized by lime. By evaporation of the solution, nitrate of calcium is obtained. Ammonia is obtained by

treating calcium cyanamide with steam:

$$CaCN_2 + HOH \rightleftarrows CaCO_3 + 2\,NH_3$$

and by oxidation may be changed to nitric acid which may be neutralized by lime.

In the oxidation of ammonia to nitric acid, ammonia gas and air are passed through a red-hot fine-meshed platinum gauze, a reaction taking place according to the following equation:

$$4\,NH_3 + 5\,O_2 \rightleftarrows 4\,NO + 6\,H_2O$$

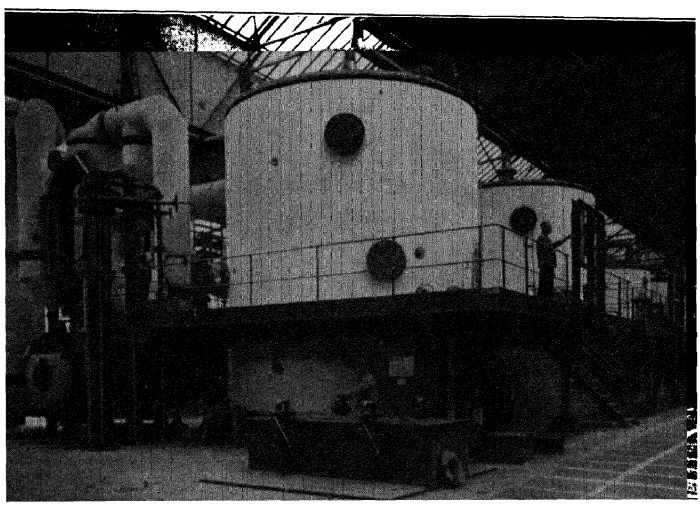

FIG. 42. Oxidation ovens for the manufacture of fixed-nitrogen products. Oppau Works. (Courtesy of the Kuttroff, Pickhardt & Co., Inc.)

The gaseous mixture is then cooled, and the nitric oxide combines with the excess oxygen according to the following equation:

$$2\,NO + O_2 \rightleftarrows 2\,NO_2$$

If nitrogen peroxide is allowed to pass through water the following reaction takes place:

$$3\,NO_2 + H_2O \rightleftarrows 2\,HNO_3 + NO$$

Nitrate of calcium contains about 13 per cent of nitrogen and is quite popular as a nitrogenous fertilizer, especially in Europe. If ammonium nitrate and calcium nitrate are fused and sprayed into the air, a product less hygroscopic than either ammonium nitrate or calcium nitrate is obtained.

Leuna Saltpeter. — At the Leuna works in Germany and at Billingham, England, synthetic ammonia is converted into ammonium sulfate by what is known as the gypsum process. Sometimes ammonia is used to neutralize the nitric acid to form ammonium nitrate, which in itself has much to recommend it as a fertilizer. Large quantities of ammonium nitrate are mixed with ammonium sulfate to form what is known as *Leuna saltpeter.*

The air-nitrogen industry has now reached a point where it is producing more fixed nitrogen than is available from any other source. As a matter of fact, two large synthetic-ammonia plants in Germany produce annually more nitrogenous fertilizer than is obtained from the Chilean nitrate fields in the same period of time. As time goes on, more nitrogen-fixation products will probably be used as fertilizers

Miscellaneous Organic Nitrogenous Fertilizers. *Dried Blood and Tankage.* — These substances are only slightly inferior to sulfate of ammonia as carriers of nitrogen. Where a slowly available fertilizer is desired, these have much to recommend them. Blood is collected in packing-houses, dried at a low temperature, and ground. If not kept dry it is liable to undergo decomposition Dried blood contains from 11 to 16 per cent of nitrogen Since it is slowly available, it should be applied early in the season. It is quite adaptable to light soils because the nitrogen it contains is quite insoluble and therefore not likely to be lost by leaching

Tankage is a mixture of refuse from the packing-houses, such as meat scraps, bone, and other by-products. These materials are steam-cooked and the gelatin and fat removed. Tankage contains from 5 to 10 per cent of ammonia and from 3 to 8 per cent of phosphoric acid, the latter being present as tri-calcium phosphate Like dried blood, this product undergoes ammonification and nitrification when added to soils, and its continued use increases the lime requirement of a soil

Cottonseed Meal. — This is a very valuable fertilizer, not only from the standpoint of the nitrogen it contains but also from the standpoint of other desirable plant nutrients, particularly phosphorus. It is the residual material left after the cotton and oil have been removed from the seed. It is especially desirable as a fertilizer for cigar-leaf tobacco. It contains more nitrogen than castor pomace or linseed meal.

Fish Scrap. — The utilization of menhaden fish for oil and soap has long been a prosperous industry on the Atlantic Coast. The residues are treated in much the same way as the by-products of the packing-house industry. Fish scrap contains about 8 per cent each of nitrogen and phosphoric acid. It is similar to tankage in its effect on plant growth.

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CHAPTER XIII

THE ATMOSPHERE

The atmosphere, like the soil, is made up of gaseous, liquid, and solid materials. Some of the atmospheric constituents are beneficial, whereas others may be detrimental to plant growth. As an environmental factor in the growth of plants, the importance of the atmosphere can hardly be over-estimated.

The Chemical Composition of the Atmosphere

Under ordinary conditions, the chemical composition of the atmosphere is remarkably constant, although in certain localities there may be at times a wide variation from the normal. Some consideration will now be given to the individual atmospheric constituents.

Oxygen. — Approximately 21 per cent of oxygen, by volume is found in the atmosphere at sea-level. Like the other gaseous elements present, it occurs in the molecular form. Under the influence of sunlight and electrical disturbances, oxygen becomes ionized. It may later revert back to its original form, O_2, or three atoms may combine to form ozone, O_3. It may also combine with nitrogen, forming NO, or with hydrogen forming H_2O_2. All the compounds mentioned have been identified as constituents of the atmosphere.

In the atomic state or in the form of ozone, oxygen is more chemically active than it is in the molecular state. However, it is utilized in molecular form by plants and caused to unite with carbonaceous material to form carbon dioxide and water and various intermediate compounds. It may be taken up through the leaves, or it may enter the root systems or other parts of the plant. The oxygen absorbed by plants may be returned to the atmosphere as a result of photosynthetic and other plant processes. The mechanism involved in the activities of oxygen within the plant will be considered elsewhere.

Nitrogen. — Nitrogen makes up more than 78 per cent by volume of the atmosphere. Practically all of it is present in molecular form. On ionization it may unite with hydrogen as well as with oxygen. Certain oxides of nitrogen may unite with moisture to form nitric acid, which may then combine with some of the ammonia present to form ammonium nitrate. According to Crowther and Ruston, the total

nitrogen as ammonia and nitric acid or nitrates brought from the atmosphere annually by rains, amounts to as much as 8.37 pounds per acre of soil. Moreover, these investigators found that about 1.5 pounds of organically combined nitrogen were added annually to an acre of soil from the atmosphere. These figures, however, are based on abnormal, rather than normal, atmospheres.

Nitrogen serves to dilute the oxygen present and in this way is very important from the standpoint of plant growth, because a high concentration of oxygen in the atmosphere is toxic in its effect. Nitrogen in the gaseous state is utilized by some of the lower plant organisms, but this is an exception to the general rule.

Carbon Dioxide. — The atmosphere contains about 0 03 per cent by volume of carbon dioxide, although the percentage probably fluctuates to a greater extent than that of the other gaseous constituents of the atmosphere. The percentage of carbon dioxide is far greater in the neighborhood of cities than in rural districts, particularly during warm weather Whereas nitrogen occurs 35 miles, and oxygen 30 miles, above the earth's surface, carbon dioxide, because of its greater density, is rarely found at a greater height than 10 miles. Plants utilize enormous quantities of carbon dioxide, but equal quantities are returned to the atmosphere. The chief processes by which it is returned are: the respiration of plants and animals, the processes of decay and fermentation, the combustion of carbonaceous materials other than those within living organisms, and the decomposition of carbonates.

Carbon dioxide, in addition to being a nutrient for plants, may, through its powers as a solvent in the soil, aid plants in the utilization of other materials. Increasing the amount of carbon dioxide in the atmosphere to approximately 25 per cent has been found to increase plant growth. Above this concentration, however, it appears to exert a toxic effect.

Water Vapor. — The quantity of water vapor present in the atmosphere is influenced by a number of different factors The total amount present in winter is about one-third the amount in summer. However, the average amount of water is approximately 1.3 per cent by volume or 0.84 per cent by weight. One-half of the total moisture is found at less than 6000 feet elevation, and one-tenth above an elevation of 20,000 feet.

When the air is cooled to a temperature below that corresponding to the pressure of saturated vapor, condensation takes place, if dust particles or other *condensation nuclei* are present. The condensate may take the form of fog, rain, snow or hail. The water vapor of the atmosphere, in a measure, governs the quantity of solar energy reaching the

surface of the earth. This is due, mainly, to its high specific heat, although increases in volume likewise play a part. The presence of water vapor also affects the rate at which heat is radiated from the earth back into space during the night time.

The activity of water vapor in regulating the temperature of the atmosphere, and in preventing excessive transpiration, affects the character of plant growth in a number of different ways. A very dry air will accelerate transpiration of the growing plant to a greater extent than humid air. This is of importance, because plants respond more quickly to changes in moisture content than to any other single environmental factor. It is for this reason that artificial means are sometimes employed in order that the moisture content of the air surrounding the plants may be increased.

Free Acids. — The presence of oxides of nitrogen and sulfur, and chlorine, leads to the development of an acidity in the atmosphere. This is counteracted to a certain extent by the presence of ammonia.

Crowther and Ruston found that free acids to the extent of 20 pounds per acre, calculated as sulfuric acid, were annually washed into the soil of a certain locality. They found that in foggy weather the acidity of the atmosphere increased over that on a clear dry day These figures, however, are rather abnormal. As a rule, the acid content of the atmosphere is quite low, and in some cases it has been shown that basic exceeds acidic materials.

Sulfur. — Experiments at the Rothamsted Station have shown that 17.41 pounds of sulfur trioxide from the atmosphere are added annually per acre of soil by means of rains. Crowther and Ruston have found that this figure is exceeded in manufacturing districts, where the amount may be 95 7 pounds per acre. The sulfur present in the atmosphere comes from the combustion of sulfur-containing material where the sulfur present is oxidized to sulfur dioxide. This may unite with moisture present, forming sulfurous acid, which on oxidation forms sulfuric acid. Both acids, as well as some of the free sulfur dioxide gas, have been known to be washed into the soil and to react with the basic soil constituents to form sulfites and sulfates.

An atmosphere containing appreciable amounts of combined sulfur is not desirable from the standpoint of plant growth, since these gases and acids are directly harmful to living plant tissues and eventually may lower the fertility of a soil through the formation of sulfites and an increased acidity.

Chlorine. — The chlorine content of the atmosphere is rarely very high. It has been estimated, however, that in some instances quantities of chlorine have been brought down from the atmosphere by means of

rain to the extent of 15 pounds per acre of soil, annually. This figure is exceeded in the neighborhood of industrial towns and near the sea-shore. A high chlorine content of the soil is very undesirable for the growth of some plants. Near the sea-coast the atmosphere contains considerable quantities of chlorine as sodium chloride. This is due to the salt spray of the sea being broken up into particles of colloidal dimensions which are carried great distances inland by air currents. These distances may be measured, in a way, by determining the quantity of chlorine in the soil, and sometimes from the character of the vegetation.

Dust Particles in the Atmosphere. — Suspended solids are always present in the atmosphere Very little is found in suburban districts, but in manufacturing districts large quantities generally occur.

TABLE 46

THE COMPOSITION OF THE ATMOSPHERE AS INFLUENCED BY LOCATION

(Data of Crowther and Ruston, Journal of Agricultural Science, Vol 6, 1914)

Station			Annual precipitation, pounds per acre			
No	Locality	Position	Total suspended matter	Total sulfur expressed as SO$_3$	Free acid expressed as H_2SO_4	Chlorine
1	Hunslet	Industrial area	1565	215	90	198
2	Park Square	Heart of city	849	197	15	75
3	University	1 mile north	399	134	26	51
4	Headingley Hill*	2 miles north	273	103	19	43
5	Weetwood Lane	3 miles north	147	73	11	34
6	Garforth	6 miles east		91	28	22

* This station lies roughly half-way between stations 3 and 5, and the data given are the means of the results obtained at these stations

The suspended materials found in the atmosphere may influence plant growth because of the effect of inducing rainfall. Traces may prove beneficial from the standpoint of precipitation. Larger quantities will seriously interfere with normal growth by reducing the intensity and quality of the sunlight reaching the growing plant, or by blocking the stomatal openings of the leaf. The mechanical effects of dust particles and tarry materials from the atmosphere may be more deleterious to conifers than to deciduous trees, because the stomata of the former are on the upper, rather than on the lower, surfaces of the leaves.

The Soil Atmosphere

The so-called pore space of the soil is occupied by water and atmospheric gases This is known as the " soil atmosphere " and its chemical composition is radically different from that of the free atmosphere above the soil. The reason for this difference may be attributed to the fact that under soil conditions the atmospheric constituents are enclosed by the soil complexes and are not as free to move about as they are in the air overlying the surface.

The Chemical Composition of the Soil Atmosphere. — In general, the soil atmosphere contains a little less oxygen and more carbon dioxide than the true atmosphere. According to Russell and Appleyard, there are two quite distinct atmospheres in the soil, one being present in the pore spaces and the other dissolved in films of water on the surface of soil particles and other substances. The latter is almost free of oxygen and consists mainly of carbon dioxide and nitrogen. The former, on the other hand, usually contains considerable quantities of oxygen. These two atmospheres, however, are not separated by a distinct line of demarcation.

Oxygen. — As stated above, very little oxygen is found in the surface films surrounding the soil particles It would appear, therefore, that the utilization of oxygen from the soil solution exceeds the rate at which fresh supplies come in from the main portion of the soil air, viz., that portion high in gaseous oxygen.

A well-drained cultivated soil is generally regarded, and rightly so, as being a suitable environment for the activities of aerobes Under the same conditions, however, owing to the presence of this second atmosphere, an organism may work under anaerobic conditions. In other words, there is a possibility for aerobic and anaerobic activity to take place in the same soil, if it is at all suitable for the growth of crop plants.

The most important constituent of the soil atmosphere is oxygen. The oxidation of inorganic constituents leads to their availability so far as the plant is concerned The organic matter must also undergo oxidation, before it can be utilized advantageously by plants

Carbon Dioxide. — Microorganisms are largely responsible for the presence of large quantities of carbon dioxide in the soil atmosphere. Additional quantities may be produced by the adsorption of bases from carbonates and bicarbonates The carbon dioxide given off from the roots of growing plants augments the quantity present in the soil.

Since most of the carbon dioxide comes from the activity of soil bacteria, then all factors such as temperature, moisture, oxygen and others, would indirectly affect the quantity of this gas present at any one time.

Nitrogen. — The percentages of nitrogen of the true and of the soil atmosphere are quite comparable. According to Comber, the nitrogen of the former is 78.95 per cent, and that of the latter amounts to 79.2 per cent.

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CHAPTER XIV

INSECTICIDES, FUNGICIDES AND RELATED MATERIALS

The control of those organisms which are deleterious to plant life is, at present, a problem of major importance. Even though the soil and other environmental conditions may be satisfactory, crop production may be considerably impaired by the attack of many harmful insects and disease organisms. In some cases, complete destruction of crops has been noted as a result of insect depredations alone, notwithstanding the fact that the agriculturist is doing all that appears humanly possible in matters of control. The losses to vegetation of all kinds, from these causes, appear to be increasing from year to year.

There are many reasons to account for the increased activity of insects in relation to plant destruction. The introduction of plants which apparently are more desirable as food material than the naturally occurring vegetation appears to be a factor. Moreover, the introduction of foreign insects is an important factor, since the lack of a *natural enemy* enables them to multiply at an enormous rate if other conditions are optimum for their development. The destruction of insectivorous birds, a practice so prevalent in this country, is to be deplored, for an excellent natural means of control is lost as a result. The growing of large acreages of crop plants which afford unlimited food materials for many insects is probably one of the most important factors in their propagation, and very naturally this will continue to be so for years to come.

At present, various artificial methods of insect control are practiced by agriculturists. The proper fertilization of the soil is, to a certain extent, an effective method, because a healthy plant is said to be less susceptible to insect injury than one suffering from malnutrition. Crop rotation is quite effective as a means of control, although this practice has its limitations. The times of plowing and of planting are likewise important, for obvious reasons.

Few of the methods now used in insect control are entirely satisfactory. Some cases have been reported where the materials employed appeared to be beneficial to plant growth, whereas others have proved to be deleterious. Moreover, as many of the materials used are likewise quite toxic to higher animals, care must be taken at all times to avoid leaving a noticeable residue on those plant parts which are used as foods.

INSECTICIDES

Insecticides may be classified as follows:

1. Stomachic Poisons. — These, as their name indicates, kill by being taken internally.

2. Contact Poisons. — These either kill by closing the openings of the respiratory systems, either wholly or in part, thereby causing death by suffocation, or they may cause death by their corrosive action on the tissues of the insect.

3. Fumigants. — These are gaseous poisons that kill when inhaled.

4. Repellents. — These do not necessarily kill, but serve to keep insects away from vegetation.

5. Attractants. — These do not kill but serve to attract large numbers of insects to a given point where they may be killed by the use of the proper material.

Stomachic Poisons

In general, insects feed either on the solid portions of the plant or on its sap. Stomach poisons function for the first group but not necessarily for the second. Practically all the poisons employed in the control of "chewing" insects contain arsenic in one form or another. Several of the most outstanding commercial preparations will now be considered.

Arsenicals

Arsenic Trioxide. — Arsenic trioxide, As_2O_3, is a by-product obtained in the production of many elements from their respective ores, as for example, copper and nickel. It is also called "white arsenic" or "ratsbane," and is the basis of many poisonous preparations. It is slightly soluble in water but more so in carbonated water. It dissolves readily in alkaline solutions. Cultural solutions containing as little as 0.0002 per cent are capable of destroying plant life.

It is quite safe to use arsenic trioxide in combination with Bordeaux mixture (which will be considered later) and with other preparations containing a slight excess of lime, which, although a base, reduces solubility of the arsenic. Its solubility and high toxicity, however, prevent its direct application to living plants. No arsenical should be used with a solubility exceeding 0.75 per cent. If an arsenical such as arsenic trioxide is used, the leaves of the plant turn black. Some plants possess foliage which is more susceptible to the destructive action of arsenic oxide than that of others; thus the peach tree has foliage which is easily destroyed by arsenicals of a concentration which has very little effect on the foliage of the more resistant apple tree.

TABLE 47

EFFECT OF HYDRATED LIME ON THE STABILITY OF LEAD ARSENATE
(2 4 GM TO 1000 CC. H_2O)

(*Data of Mogendorff, New Jersey Station Bulletin* 419)

Flask number	Calcium hydroxide		Water-soluble arsenic in As_2O_5-equivalent present after digesting for 9 days
	millimols	gm	gm
1	1	0 074	0 0409
2	5	0 370	0 0019
3	50	3 700	0 0005
4	100	7 400	0 0010

Arsenic, to be toxic, does not necessarily have to be water-soluble when ingested. The digestive juices of insects which are available for reaction with arsenicals have been found to have an alkaline reaction. Hydrolysis takes place quite rapidly under these conditions and even where the acidity approximates *p*H 6

Paris Green. — Paris green, $3\,Cu(AsO_2)_2 \cdot Cu(C_2H_3O_2)_2$, or acetometarsenite of copper, was the first arsenical to be used as a spray, having been used prior to 1868 for the control of the Colorado potato beetle. It is usually prepared by boiling an aqueous solution of copper acetate in the presence of arsenic oxide, and is freely soluble in ammonium hydroxide, which may serve as a test for its purity

Paris green is a rather heavy substance and does not remain long in suspension. This feature is objectionable as the result is unequal distribution of the insecticide over the plant. At best it does not adhere readily to foliage, making frequent sprayings necessary, involving additional time, labor, and materials. Because of its high toxicity it makes an excellent material for poison baits. Its bright color also makes its presence easily visible on substances to which it is applied, and this is a fact of considerable importance.

By federal law, not more than 3 per cent of the arsenic of Paris green should be water-soluble. Mainly, on account of its solubility, it is not recommended for general use as a spray.

London Purple. — London purple, a product of indefinite composition, was first used in the United States in 1878 as a substitute for Paris green in the control of the Colorado beetle. It is obtained as a waste product in the aniline dye industry and is made up, primarily, of calcium and

arsenic. Large quantities of this material are utilized in the United States for the control of the cotton-leaf worm, the tobacco worm, and the potato beetle. It is relatively soluble and tends to " burn " foliage; for this reason it is not generally recommended as a spray.

Zinc Arsenite. — Zinc arsenite (meta), $Zn(AsO_2)_2$, is a light powder, which was introduced in 1911 but has never come into general use as an insecticide. In solubility it resembles Paris green, London purple, and arsenious oxide, and therefore tends to burn foliage unless a sufficient quantity of lime is present.

Calcium Arsenite. — Calcium arsenite (meta), $Ca(AsO_2)_2$, first introduced in 1912, is prepared by treating a mixture of arsenious oxide, sodium carbonate and lime with water, and boiling the solution. The reaction involved may be illustrated by the following equation:

$$As_2O_3 + Na_2CO_3 + Ca(OH)_2 \rightleftarrows Ca(AsO_2)_2 + CO_2 + 2\,NaOH$$

It is a satisfactory spray for potatoes when used with Bordeaux mixture, but on account of its solubility it should not be used alone.

Calcium Arsenates. — Calcium arsenate, $Ca_3(AsO_4)_2$, is prepared by adding calcium hydroxide to an aqueous solution of sodium arsenate:

$$2\,Na_2AsO_4 + 3\,Ca(OH)_2 \rightleftarrows Ca_3(AsO_4)_2 + 6\,NaOH$$

In this reaction, however, other compounds are formed, such as *calcium acid arsenate*, $CaHAsO_4 \cdot H_2O$, *neutral calcium arsenate* (hydrated), $Ca_3(AsO_4)_2 \cdot 2\,H_2O$, and *basic calcium arsenate*, a product of rather variable composition.

Calcium arsenate, as the commercial preparation is called, injures foliage when used alone. It is generally used to advantage with Bordeaux mixture or lime sulfur. It is a white powder, it has good suspension and adhesive qualities, and it can be prepared easily. The acid salt is quite insoluble in water but the neutral salt is more soluble. Carbonic acid may increase the solubility of these arsenates, causing a tendency to burn foliage owing to the liberation of free arsenic acid:

$$Ca_3(AsO_{12}) + 3\,H_2CO_3 \leftrightharpoons 3\,CaCO_3 + 2\,H_3AsO_4$$

An excess of lime, therefore, should be present. Calcium arsenates are now used on a relatively large scale in arsenical dusts.

Lead Arsenates. — Three forms of these materials are known: *neutral lead arsenate*, $Pb_3(AsO_4)_2$; *acid lead arsenate*, $PbHAsO_4$; and *basic lead arsenate*, $Pb_5OH(AsO_4)_3$. If a solution of lead nitrate is treated with sodium acid arsenate the reaction is said to take place according to the following equation:

$$Pb(NO_3)_2 + Na_2HAsO_4 \rightleftarrows PbHAsO_4 + 2\,NaNO_3$$

On the other hand, if lead acetate is used, the following equation obtains:

$$3\,Pb(C_2H_3O_2)_2 + Na_2HAsO_4 \rightleftharpoons Pb_3(AsO_4)_2 + 4\,NaC_2H_3O_2 + 2\,CH_3COOH$$

The acid, the neutral, and basic lead arsenates are insoluble in water. The acid salt is the richest in arsenic and therefore appears to be the best, but, on the other hand, it seems to be the general opinion that the neutral and basic forms are the safer to use, especially in combination with lime sulfur, a combination in which this arsenical is often necessarily applied. The acid salt is, however, a more quickly acting poison as it hydrolyzes as follows:

$$5\,PbHAsO_4 + HOH \rightleftarrows Pb_5OH(AsO_4)_3 + 2\,H_3AsO_4$$

Colloidal lead arsenate may sometimes be used to advantage. This is obtained by dissolving gelatin in an aqueous solution of disodium acid arsenate, and adding an aqueous solution of lead nitrate. Gelatin, in this case, functions as a protective colloid. In this connection, sodium silicate should never be used, for it increases the solubility of arsenic:

$$5\,PbHAsO_4 + 2\,Na_2SiO_3 + H_2O \rightleftharpoons Pb_5OH(AsO_4)_3 + 2\,Na_2HAsO_4 + 2\,H_2SiO_3$$

At the present time the use of arsenicals is quite common in the control of soil insects.

Arsenical Injury. — As stated elsewhere, the application of an arsenical to actively growing plants may act in an injurious manner. This is due to the increased solubility of arsenic under humid conditions. Ammonia and carbonic acid of the atmosphere will materially increase the amount of soluble arsenic which tends to injure the plant. This action is intensified by high temperatures.

Arsenical poisoning may be both *acute* and *chronic*. Where large areas of the leaf turn black within 24 hours after the application, it is referred to as *acute* arsenical poisoning. In case of *chronic* poisoning, the leaves turn yellow prematurely and drop off. In this type of poisoning the absorption of arsenic probably takes place at a very slow rate. Arsenicals should be applied to foliage only under conditions of relatively high temperature, low humidity and good air circulation. Damp weather not only aids in the solubility of arsenic but facilitates its entrance into the tissues.

Considerable difficulties are sometimes experienced in having the arsenical adhere to the tissues. Many surfaces to which such poisons are applied possess active electrical charges. A living leaf, for example, holds on its surface an active negative charge. In such cases, therefore, the application of a suspensoid colloid, carrying an opposite charge,

would result in an attractive force that would permit of better contact. Unfortunately, however, most spray suspensions bear negative rather than positive charges.

For any substance employed which does not inherently possess marked "sticking" qualities, a number of materials are available which, when used in this connection, serve to produce or to improve these adhesive qualities. Such substances are colloidal in nature and include calcium caseinates, resin soaps, glue, molasses, and like materials.

Arsenical Residues. — Sometimes arsenicals adhere to foliage to a maximum degree. For this reason, a considerable amount of attention is now being given to the question of *arsenical residues*. The federal government now recognizes an "international tolerance" of 0.01 grain of As_2O_3 per pound of fruit. If a fruit contains arsenic in excess of this figure, it cannot be sold legally. In order to guard against the possibility of excess, care is now being taken in the use of arsenicals; amounts just sufficient to control insects are employed, and the residues are removed with 1 per cent hydrochloric acid solution. Alkali solutions remove arsenic, but they likewise remove wax and related materials, thereby lowering the quality of the fruit.

The long-continued use of arsenicals has been known to result in increasing the arsenic content of the soil to such an extent that it interferes with economic crop production.

Arsenic is a cumulative corrosive poison and seriously interferes with the normal respiration of plants and animals, if present in sufficient concentrations.

TABLE 47(*a*)

RESULTS OF ANALYSIS FOR ARSENIC IN SPRAY RESIDUES ON PENNSYLVANIA APPLES IN 1926

(*Data of MacLeod, Haley and Sudds, Journal of Economic Entomology, Vol* 20, 1927)

Sample No.	County	Date of last spray application	As_2O_3 in grains per pound of fruit
1	Montgomery	July 16	0 0019
2	Montgomery	July 16	0 0026
3	Bucks	June 1	0 0003
4	Bucks	July 18	0 0010
15	Adams	June 30	Trace
16	Adams	July 1	0 0002
17	Adams	July 23	0 0009

Non-Arsenicals

These comprise a number of chemically unrelated materials whose toxic action is due to some other principle than arsenic.

Hellebore. — This is a powder prepared from the roots of plants bearing the names of *Veratrum album* and *Veratrum viride.* "White" hellebore is obtained from the first, and "green" hellebore from the second. The active principles of these plants are powerful alkaloids, such as *protoveratrine*, $C_{32}H_{51}O_{11}N$, *cevadine*, *jervine*, *pseudojervine* and *rubijervine.* The total content of these poisons is about 1 per cent. Hellebore is not only an efficient stomachic poison but is also a good contact insecticide and repellent. It does not have the wide range of usefulness possessed by the various arsenicals, but it is quite useful under many conditions

A number of other materials are sometimes stated to be useful as stomach poisons under special conditions. These include copper cyanide, cresylic acid and other materials. However, these are not of sufficient importance to receive more than passing mention.

A new insecticide, called *Rotenone*, has been obtained recently from the "fish-poison plant" *Deguelia* (*Derris*) *elliptica*, and from the roots of such plants as cube, haiari and timbo. It is said to be more potent than nicotine as a contact insecticide and more effective than lead arsenate in poisoning leaf-eating insects. Rotenone is not poisonous to higher animals. A spray residue of rotenone, therefore, is not a menace to human health.

Contact Poisons

For the "sucking" insects the use of arsenicals is not to be recommended. Effective control of these insects requires that they must be brought in close contact with the poisons used. This is accomplished by means of sprays which tend to bathe the insect in the aqueous solution employed. This method, however, is not entirely satisfactory, since it is extremely difficult, in some cases, to bring the poison in immediate contact with the insect. The escape of a single insect may result in a complete infestation of the plant shortly after the spray is applied, for many insects have remarkable powers of reproduction. For this reason, therefore, every kind of an insect which can be controlled by a stomach poison should be so controlled. For the others, the following materials are used:

Pyrethrum. — Pyrethrum, or Persian insect powder, is prepared by grinding the petals of the pyrethrum blossom. Its value as an insecticide is due to the presence of substances exceedingly poisonous to in-

sects but harmless to man and other animals. The powder is found to contain a complex monohydric alcohol, an oily aromatic substance, and a liquid and a solid acid, all of which may contribute to the suffocating lethal properties of this material. Standinger and Ruzika have isolated two poisonous esters from the powder, both being derived from the same alcohol but with different acids. These esters are designated as *Pyrethrin I* and *Pyrethrin II*.

Large quantities of insecticides are now being prepared commercially by extracting this powder with light mineral oils, in which the active principles dissolve quite readily.

Oils. — The use of oils, as contact insecticides, was first introduced in 1870, and this practice has become quite popular in recent years. The active principles of these preparations are hydrocarbons, substances which, when properly prepared, will spread and adhere well and which are very penetrating when brought into contact with the bodies of insects. The principal oils used are crude petroleum, lubricating oils and kerosene. They are applied in a highly emulsified condition. The emulsifiers used are soaps, calcium caseinate, saponin, or other like materials. At the present time, the lubricating oils appear to be the most popular of the oil sprays, especially for dormant spraying. The highly refined white oils are rather expensive but are otherwise quite satisfactory.

Miscible oil preparations, which are mineral oils emulsified with vegetable oils in the presence of some alkali, are sometimes used. A stable stock preparation can be prepared and mixed with water when needed. When applied to plants, these oils tend to spread over and cover the surface more rapidly and thoroughly than do the ordinary oil emulsions.

Oil sprays may be used in many cases instead of lime-sulfur preparations, which will be considered later. They are much cleaner and easier to handle and not as unpleasant to apply as lime-sulfur. Likewise they are cheaper and more effective against San José scale, on account of their better spreading and penetrating powers.

Experience has shown that saturated petroleum oils, although not generally toxic to plants, are nevertheless capable of interfering with normal metabolism. Oil applied as a film to the foliage persists, as such, for approximately three weeks, depending upon the morphology of the leaf and the viscosity of the oil. The disappearance of the oil is due, mainly, to its absorption by the leaf. The resistance offered to this penetration is mostly epidermal, and may become extremely rapid once ingress to the interior is obtained. During the period of oil penetration, transpiration is sharply decreased and respiration is increased enormously, while photosynthesis becomes temporarily inoperative. This may be due, in part, to the solvent action of the oil on the chloroplasts,

since intercellular oil rapidly turns green because of the presence of dissolved chlorophyll. If plants are dormant when treated with oil sprays, the liability of injury is reduced to a minimum.

Oil sprays bring about the death of insects by closing their breathing pores. They have, in addition, a corrosive action on their tissues. According to De Ong, any oil containing unsaturated hydrocarbons appears to pass through the entire body of the insect and exert a solvent action on many of the compounds present. This is not necessarily true of oils free of unsaturated hydrocarbons, which may penetrate but a short distance into the main tracheal tubes, where they may remain and interfere with normal gas exchange.

Soaps. — Soap solutions are very effective against soft-bodied insects and scales. Their action as insecticides may be attributed to the caustic effect of the alkali used in their manufacture, and to their effect on respiration, since they tend to cover the insect, thereby excluding the air.

The most effective soap is a sodium or potassium soap made from whale or any other fish oil. The advantages of fish oil over ordinary soaps lie in their greater penetrating power, and the fact that they form more liquid suspensions, and the fish oil itself, probably through its fatty acids, seems to be more fatal to insects than the ordinary animal or vegetable oils and fats.

Resin soaps are especially employed as *scalecides*. These act by penetrating the trachea and clogging the spiracles by the settling out of the resin when brought into contact with carbon dioxide.

Fumigants

Maximum efficiency of a spray can be obtained only if all the vegetation is touched by it. This is impossible in many cases. For this reason, fumigants are thought to be more efficient than sprays, as they penetrate the air surrounding the insect. Of these *nicotine*, *hydrocyanic acid*, *carbon bisulfide*, and *paradichlorbenzene* are among the most important.

Nicotine. — When either tobacco extracts or tobacco dusts are used as insecticides, their efficiency is considerably increased by the addition of basic materials which replace nicotine from its forms of combination in the tobacco. When nicotine is liberated in this manner it volatilizes, and if breathed by insects, exerts a paralyzing action on their nervous system. Nicotine readily penetrates the tissues of soft-bodied insects as well.

A number of nicotine preparations are on the market. Some of these have nicotine in the form of the sulfate, $C_{10}H_{14}N_2 \cdot H_2SO_4$. Fairly pure

TABLE 48

A STUDY OF THE EFFECT OF VARIOUS MIXTURES OF *Nicotiana rustica* DUST (4 57 PER CENT NICOTINE), WATER, AND HYDRATED LIME UPON THE VOLATILIZATION OF NICOTINE AS MEASURED BY THE DEATH RATE OF CABBAGE APHIDS (*Aphis brassicae Linnaeus*). TEMPERATURE 25.5° C.

(*Data of Haley, Olson and Follweiler Journal of Economic Entomology, Vol.* 18, 1925)

Composition of mixture			Death rate after various periods			
Quantity of tobacco, gm	Quantity of hydrated lime, gm	Quantity of water, cc	20 minutes mln	24 hours mln	48 hours mln	72 hours min.
5	5	1	4 5	9 2	13 5	16 0
5	5	2	3 5	8 5	13 2	15 8
5	5	3	3 0	8 4	13 4	15 7
5	5	4	3 25	8 2	13 3	15 9
5	5	5	5 2	8 4	13 1	16 2
5	5	6	5 8	8 6	13 2	16 5

nicotine is likewise obtainable and may be used as a fumigant in solutions of a 0.05 to 0.10 per cent concentration.

If finely ground tobacco is mixed with calcium hydroxide or a similar material, and water in the proper proportions, a dust is produced which gives up its nicotine rather readily. Dusts may be made of nicotine sulfate and hydrated or caustic lime which are efficient also. Nicotine has practically no effect upon higher plants. Care should be taken in applying nicotine sulfate preparations to vegetable and like materials shortly before they are prepared for human consumption, as the nicotine residue may prove toxic. The addition of lime, however, will prevent this occurrence.

Hydrocyanic Acid. — Hydrocyanic acid, HCN, is prepared by treating sodium cyanide with sulfuric acid:

$$2\,NaCN + H_2SO_4 \rightleftarrows Na_2SO_4 + 2\,HCN$$

It is sometimes used in liquid form as a fumigant under carefully controlled conditions, as it volatilizes readily.

Hydrocyanic acid is used extensively in the fumigation of greenhouses and nursery stock, and likewise in the control of scales on orchard trees.

It is also employed for insect control in grain elevators and granaries, and its lightness brings about a rapid spreading, particularly upward, in contrast to heavy vapors of carbon bisulfide and similar fumigants. For this reason, it can be used with success in tents and similar enclosures only, since otherwise a sufficient concentration would be difficult to maintain for any given period of time.

The applicability of this chemical as an insecticide was first demonstrated in 1886, when it was used in the control of the cushionary scale attacking citrus trees in California. It acts as a respiratory poison by stopping oxidation, thereby causing suffocation of the insects. If used in too great a concentration it injures vegetation, especially young plants.

A recent development in cyanide fumigation has been the introduction of calcium cyanide, $Ca(CN)_2$, which may be applied as a dust. Hydrocyanic acid is slowly given off under the influence of moisture.

Carbon Bisulfide. — Carbon bisulfide, CS_2, is a liquid which volatilizes readily at ordinary temperatures. Its vapors are poisonous and, since they are 2.63 times heavier than air, they tend to work downward very rapidly when placed at the top of grain in elevators or in the soil

Carbon bisulfide functions both as an anaesthetic and an asphyxiant and acts very readily after entering the body of the insect. It may destroy fungi if used in relatively large quantities. Like hydrocyanic acid, it must be used in enclosures. It is somewhat poisonous to plants. According to Sanderson, it inhibits the movement of plant protoplasm and for this reason must be employed with care

Paradichlorbenzene. — Paradichlorbenzene, $C_6H_4Cl_2$, is a crystalline substance which volatilizes slowly under ordinary conditions, yielding a gas which is five times as heavy as air. Its vapor is quite toxic to insects. Paradichlorbenzene is a fumigant which has been introduced for controlling the peach tree borer, but it may be used for similar fumigation activities. Its vapor will injure tender roots and young growth of plants but has no effect on mature trees.

Repellents

Materials which have the power of repelling both biting and sucking insects are sometimes used for plant protection. Some contact insecticides and fumigants may act in this way, but the materials usually employed as *repellents* cannot properly be called insecticides. Inert materials, such as soil, are sometimes used to cover plants with the view of warding off insects. Phenol, naphthalene, oil of citronella, and similar materials may be used in this manner.

Attractants

Sometimes it is deemed advisable to attract insects to a certain point where they may be killed by the use of the proper material. In the control of the Japanese beetle, it was found that oil of geraniol served to attract these insects This is rather a new field of investigation, and very little material is available on this subject.

FUNGICIDES

Fungicides are preparations used to destroy or prevent the growth of fungi. Ordinarily, however, they are used as a means of prevention and may be applied as dusts or as sprays. The most common fungicidal materials are sulfur and copper.

Sulfur. — Sulfur was early recognized as an efficient fungicide and was introduced in 1821. The more finely it is divided, the more effective it becomes and the better it adheres to vegetation. Sulfur is somewhat volatile under ordinary conditions and thus surrounds the organism with sulfur vapor, according to Fulmer. Definite information is now available which proves that sulfur may oxidize under ordinary conditions, forming oxides of sulfur, such as pentathionic acid, which is a hydrated oxide. The more surface exposed to the oxygen and moisture, the more efficient the application becomes.

TABLE 49

SHOWING THE EFFECT OF THE PRESENCE OF OXYGEN ON THE TOXICITY OF POWDERED SULFUR TO SPORES, *Botrytis cinerea*

(*Data of Doran, New Hampshire Technical Bulletin* 19)

Conditions	Spores germinating
	Relative numbers
Spores sulfured, oxygen present	0
Spores sulfured, oxygen absent	100
Check, spores not sulfured	100

When lime and sulfur are boiled together, various compounds are formed, depending upon the ratio of lime to sulfur. According to Thompson and Whittier, when the ratio of lime to sulfur is 1 to 2.25, the following reaction occurs:

$$3\ Ca(OH)_2 + 12\ S \rightleftharpoons 2\ CaS_5 + CaS_2O_3 + 3\ H_2O$$

On the other hand, if the ratio is more narrow, oxysulfites and sulfides are formed, including hydrosulfides and polysulfides. The active prin-

ciples of lime-sulfur are polysulfides which may undergo oxidation when applied to vegetation.

$$2\,CaS_5 + 3\,O_2 \rightleftharpoons 2\,CaS_2O_3 + 6\,S$$

The advantages of using polysulfides rather than powdered sulfur, as fungicides, are that they are soluble in water and precipitate free sulfur in its most active fungicidal form. Polysulfides are corrosive in their action on organisms such as scale insects and, on oxidation, may lower the oxygen pressure in their environment, causing suffocation. Sulfur preparations are used also as contact insecticides

Copper Compounds. — Various copper compounds are now used as fungicides, the best known of which is *Bordeaux mixture*, which may be prepared by mixing equal quantities of copper sulfate and lime (CaO) in the presence of water. Under these conditions a voluminous gelatinous precipitate is formed which remains as a colloidal suspension for several hours. It was first introduced in 1807.

Bordeaux mixture is regarded as a mixture of hydrated oxide of copper and calcium sulfate, with an excess of lime It surpasses all other fungicides in regard to its power of adhering to plant tissues, and may become active through the solvent action of compounds secreted by plants or by the action of carbon dioxide Some of the copper appears to be made soluble by the fungi themselves

Bordeaux mixture is sometimes harmful to plant tissues. As the copper becomes soluble, it may enter the tissues through the epidermal walls or through the stomata, bringing about the destruction of the living cells through precipitation of some of the vital compounds. This injury may be recognized by the presence of brown spots on the tissues or by defoliation. On fruits, the death of some of the cells incites the formation of cork cells, thus causing the russet areas so frequently observed on apples after spraying.

Soda Bordeaux, copper carbonate, ammoniacal copper carbonate and copper sulfate are other important copper fungicides

Formaldehyde. — Formaldehyde, HCHO, is used as a fungicide. Its ability to kill organisms appears to be due to its reducing powers, although it has the power of altering the colloidal structure of living cells. For this reason care should be taken in its use. It is quite soluble in water and, in aqueous solution (40 per cent by volume) it is known as *formalin*. It may be used either in this form or as a gas.

HERBICIDES

Herbicides are chemical substances used in destroying noxious plants. Among these may be mentioned sodium arsenite, ferrous sulfate, copper sulfate, sodium chloride, sodium chlorate, calcium chlorate and many other like materials.

About a pound of any of the above-mentioned materials may be dissolved in a gallon of water and used as a spray. The plants to be destroyed are sprayed until thoroughly wetted, which usually requires about 100 gallons per acre.

The application of this method for destroying vegetation is usually possible only where all plant life is to be destroyed along railroad tracks, tennis courts, and the like. However, some success has been attained through the use of copper sulfate and ferrous sulfate in destroying mustard, dandelion, and other rough-leafed plants growing in grass areas.

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CHAPTER XV

THE ABSORPTION OF MINERAL NUTRIENTS BY PLANTS

Under the subject of seed germination, Chapter VIII, the statement was made that, after the mineral matter of the seed was utilized, the seedling was dependent on its environmental conditions for additional supplies necessary for its continued existence. It was for this reason that considerable attention was given to the soil because it represents a storehouse of plant nutrients. Certain factors modifying the solubility of soil minerals and those modifying the chemical composition and concentration of the soil solution were stressed. We are now concerned with the mechanism involved in the withdrawal of mineral nutrients from the soil solution.

The Absorbing System

Numerous microscopic absorption organs occur on the roots of plants; these are designated by the term *root-hairs*. These are not separate cells but rather thread-like projections of the epidermal cells of the roots. Their walls are thin and unmodified, and they are lined with a protoplasmic layer. The cells appear to be covered externally with a calcium pectate layer. Root-hairs form an intimate contact with colloidal soil constituents. Most soil particles, as stated before, if not wholly colloidal in nature, are at least covered with organic or inorganic colloidal material of a hydrophilic character. When a root-hair becomes imbedded in this material, separation cannot be effected readily. Where such an intimate contact obtains, it is apparent that a possibility exists for the absorption of adsorbed substances from the soil particle, if not the absorption by the plant of the colloidal material itself, as well as the substances existing in true solution.

If a growing plant is placed in a solution of mineral nutrients, the passage of the dissolved materials into the root-hairs and body proper may be observed. This line of investigation has resulted in the recognition of many of the factors which are involved in the mechanism of absorption by living plants.

Factors Influencing the Process of Absorption by Plants

A number of factors are normally involved in the absorptive process of plants, a few of which will now be considered.

The Osmotic Concentration of the Nutrient Medium. — The osmotic concentration of the soil solution is normally less than that of the root-hairs. This appears to be due, among other things, to the salts of organic acids present in the protoplasm. Dissolved materials in the soil solution usually range from a concentration of 0.01 to 0.1 per cent, although this is exceeded in alkali soils. A concentration equal to 0.2 per cent is not necessarily injurious, but beyond this concentration considerable injury to the plant is apparent, since the plant loses more water than it absorbs and the normal passage of dissolved materials through the protoplasmic film is seriously hindered.

Investigations dealing with the growing of plants in nutrient solutions have brought out the fact that osmotic pressures equivalent to 0.1 to 0.6 atmosphere were the lowest that can obtain for normal growth, and osmotic pressures equal to 1.50 to 1.70 atmospheres were the most beneficial. According to Hoagland, the osmotic concentration of a very fertile soil is approximately 0.78 atmosphere. It is evident that the osmotic concentration of plant cells must be given due consideration in crop production. The use of fertilizers should never result in an osmotic concentration greater than that of the cells.

The Hydrogen-ion Concentration of the Soil Solution. — The hydrogen-ion concentration of the nutrient solution may affect the absorptive process by modifying the absorbing efficiency of the root-hairs. If the reaction of the solution is alkaline, some of the necessary mineral nutrients may be rendered less soluble. Of these, calcium, iron, and phosphorus may be mentioned. On the other hand, although a high acidity might render mineral nutrients more soluble, it may cause an increased permeability of the protoplasm, owing, in part, to its effect on the electrical charge of certain cell colloids. Increased permeability is not conducive to good plant growth.

It has been noted that *anions* are absorbed more readily from an acid than an alkaline solution and *cations* more readily from an alkaline solution. The injurious effects of a high or low acidity, therefore, may be due, in part, to a deficiency of mineral nutrients.

Attention has been called to the fact that the colloids of plant cells may be either positively or negatively charged. Under normal conditions, it is quite probable that both kinds are present, and this may affect the absorption of both *anions* and *cations*. It is equally possible that the hydrogen-ion concentration of the soil solution may affect the hydrogen-ion concentration of the plant cell and so modify the affinity of the colloids for the various nutrient ions of the opposite charge. Cases are known where the hydrogen-ion concentration of the nutrient medium has affected the hydrogen-ion concentration of the living cells.

According to Hoagland, however, this does not hold true for *Nitella*, a unicellular water plant.

Normal plant growth is obtained only where *anions* and *cations* are absorbed simultaneously. Care should be taken, therefore, to insure a reaction of the nutrient medium which would not affect the absorption of one kind of ion at the expense of another.

Through the difference in the rate of absorption of different ions, the reaction of the nutrient solution may undergo a change. The ammonium ion from ammonium sulfate may be absorbed more readily than the sulfate ion, causing the residual solution to become more acid. Conversely, the nitrate ion is absorbed more readily than the sodium ion where both are present as sodium nitrate, leaving the residual solution more alkaline than before. The soil solution must, therefore, be well buffered to prevent the possibility of pronounced changes in reaction taking place, and this condition invariably exists in a fertile soil.

Transpiration. — Ordinarily one would believe that, the greater the rate of transpiration, the more water and dissolved materials would be carried within the plant. Apparently, this is not wholly true, for many investigators have showed that the absorption of water and mineral nutrients may take place independently of one another.

The Influence of One Ion on the Rate of Absorption of Another Ion. — The results of many investigators have shown that it is possible for one ion to exert a beneficial or detrimental influence on the rate of absorption of another ion, which may bear a like or unlike electrical charge.

It is quite possible for an inorganic compound to be absorbed in molecular form. In the majority of cases, however, it appears that ions are differentially absorbed by the growing plant. For example, Hoagland found that potassium was absorbed more readily than chlorine from a solution of potassium chloride. Where sodium was present, there was a lowering of the rate of absorption of the potassium ion. In like manner, the sulfate ion lowers the rate of absorption of potassium, as noted where potassium was supplied as the sulfate rather than the chloride. The nitrate ion is absorbed more readily than any other ion from a nutrient solution, the maximum rate being obtained where it is present as calcium nitrate or free nitric acid.

Where a more rapid absorption of the anion than the cation takes place, the carbonate or bicarbonate ion takes its place. On the other hand, where the converse is true, hydrogen takes its place. This may provide an added possibility for molecular absorption.

The Composition of the Cell in Relation to the Absorption of Ions. — Numerous investigators have shown that *different species* of plants growing in media of identical composition possess selective powers with re-

TABLE 50

ABSORPTION OF IONS FROM SOLUTIONS OF DIFFERENT MAGNESIUM SALTS*

(*Data of Hoagland. Soil Science, Vol.* 16, 1923)

Name of salt	Initial concentration	Ions absorbed by plant		Ions entering solution			Reaction		H_2O absorbed
		Mg	Anion	Ca	K	HCO_3	Initial	Final	
	atmos.	milli-equivalents					*p*H	*p*H	cc
$Mg(NO_3)_2$	21 1	4 75	5 40	0 70	0 03	2 48	5 0	6 7	130
$MgSO_4$	20 7	1 82	1 17	0 60	0 06	0 08	5 0	5 3	135

* Age of plants was 28 days Length of absorption period was 2 days.

spect to specific ions. For example, one plant may absorb relatively large quantities of calcium whereas another may absorb very little. The reason for these differences may be due, partly, to their relative root development, but the main factor involved appears to be the composition of the cell itself.

The concentration and composition of the cell sap determines the osmotic concentration of the cell, and this has a great deal to do with the absorption and translocation of nutrient ions. It does not appear possible for ions to be absorbed and translocated unless a protoplasmic layer is held firmly against the cell walls as the result of osmotic pressure.

The permeability of protoplasm is markedly affected by its chemical composition. Plants have been known to absorb a nutrient ion, such as potassium, from a very dilute solution and to concentrate it within the cytoplasm. It appears, therefore, that potassium can penetrate the protoplasm in one direction but not in another. This can be explained only by assuming chemical compounds within the cytoplasm which act upon the protoplasm in such a way as to render it less permeable to potassium. If, on the other hand, the chemical composition of the plant itself is of such a nature as to intensify the permeability of the protoplasm, the cellular compounds may be lost, as the result of an equilibrium being established between the cell and nutrient solution.

The rate of assimilation of absorbed materials within the plant is a factor of importance in the absorptive process. It is readily conceivable that, if ions or molecules are utilized rather rapidly, the diffusion of

similar ions and molecules may be hastened materially. For example, if the plant in question is a high protein producer, the chances are that the absorbed nitrogen will be quickly assimilated and thus removed from solution. Therefore, further diffusion of nitrate ions should take place normally in order that the conditions of equilibrium may become re-established.

A high organic acid content of the cells is generally conducive to a rapid absorption of basic nutrients such as calcium and potassium, if the latter are present in sufficient quantity and in the proper form. It is in this way, mainly, that the proper reaction of the cell from the standpoint of metabolic activity can thus be maintained or preserved.

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CHAPTER XVI

THE ASSIMILATION OF MINERAL NUTRIENTS BY PLANTS

The intelligent feeding of plants necessitates, among other things, a knowledge of the mineral nutrients necessary for growth, and the rôles they play in metabolism. In order to ascertain whether an element is essential as a plant constituent, it is necessary to grow the plant under conditions as nearly normal as possible and to exclude the element in question from the nutrient medium. This practice has its limitations, since the seed itself may carry a quantity of the nutrient sufficient for normal development. It is mainly for this reason that it is not possible to enumerate, at the present time, all the elements that are necessary for plant growth. It is no less a difficult matter to ascertain the functions of each mineral nutrient in plant metabolism. Notwithstanding these difficulties, however, advancement has been made, and is still being made, along these lines of research

The Rôles of Mineral Nutrients in Plant Metabolism

Calcium. — Calcium is indispensable for the growth of higher plants but is not necessary for many of the lower forms. If an attempt be made to grow a crop plant in the absence of calcium, it is found that normal growth may be obtained until all the calcium reserves in the seed have been utilized and for a short period thereafter. In the course of time, however, defoliation occurs and growth ceases. This illustrates the fact that calcium, though not a necessary protoplasmic constituent, is essential for the normal development of the plant. In his work with the tobacco plant, Garner found that, where calcium was present in minimum quantities, only the lower leaves grew normally. The growing point was affected to such an extent that flowers were unable to form.

One of the main functions of calcium is to neutralize the acids formed in the course of metabolism. Uniting with pectic acid it forms calcium pectate, a necessary cell-wall material, and in neutralizing oxalic acid (forming the very insoluble calcium oxalate), it removes from the reactive system one of the most poisonous products of metabolism. This likewise holds true for other organic acids, the presence of which, in sufficiently high concentrations, may result in a cessation of cellular

activities. In old tissues considerable quantities of insoluble calcium salts are found.

Calcium is said to make other elements *physiologically available* for plant growth. If the root system of a plant is placed in a solution of potassium chloride, the potassium cannot be utilized to advantage. On the other hand, considerable quantities of cellular material may leach out of the plant into the nutrient solution. There are several reasons why this is true, but the most important is probably the effect of potassium on the state of emulsion of the oils in the protoplasmic films Potassium is favorable to the condition of an " oil-in-water " type of emulsion, thereby increasing cell permeability The addition of calcium overcomes the deleterious effect of potassium, both elements being utilized for growth and practically no leaching of cellular materials taking place. Calcium is antagonistic to the effect of potassium on the state of the oils in the protoplasmic film, favoring a condition of " water-in-oil " type of emulsion, which is not freely permeable to dissolved constituents. Normal growth can be obtained only where the protoplasm of the cells is slightly, but not freely, permeable to dissolved materials

The presence of calcium is closely connected with root-hair development. This is probably due to its stabilizing effect on the pectic acid layer surrounding these cells. As a result of its effect on the development of root-hairs, calcium materially increases the feeding capacity of plants.

The presence or absence of calcium appears to play a relatively unimportant rôle in starch formation. Its absence, however, seriously interferes with the hydrolysis and subsequent translocation of carbohydrates. Plants suffering from calcium hunger usually respond to direct applications of soluble carbohydrates.

Some plants require so much calcium for their metabolism that they are usually referred to as " lime-loving " plants. The legumes are an example of this group. Others appear to thrive best on soils low in calcium, hence they are referred to as " lime-avoiding " plants. The rhododendron is a good example of this type of plant. It is interesting to note that plants grown on soils low in calcium are usually low in mineral constituents which makes them of less value for animal nutrition. This is due, not only to a deficiency of calcium, but also to a deficiency of other desirable mineral nutrients which results from a lack of calcium or to the fact that they are not as available from a physiological standpoint.

The absence of calcium results in a decrease in the amount of cytoplasm and in the appearance of large vacuoles. There is no substantial proof that cells disintegrate owing to the absence of a calcium pectate

TABLE 51

THE CHEMICAL COMPOSITION OF BLUE-GRASS (*Poa pratensis*) AS MODIFIED BY THE CALCIUM CONTENT OF SOILS

(*Data of Forbes, Whittier and Collison. Ohio Station Bulletin* 222)

Sample No.	Ash %	Nitrogen %	Calcium %	Potassium %	Phosphorus %	Source of sample
1	8 32	1 76	0 424	2 18	0 286	High-calcium soil
2	5 94	1 78	0 185	1 80	0 177	Low-calcium soil

layer in the cell walls, as is held by a number of investigators, but the cell contents do not have as rigid a structure as where calcium is present in normal quantities. Calcium is important because of its function in the nucleus and its effect on cell division.

Magnesium. — Magnesium is a necessary plant nutrient and is an essential constituent of chlorophyll. The lack of magnesium produces a peculiar type of *chlorosis* in which the yellow as well as the green pigments are affected. Thus, in the affected tissues, the color is a very pale yellow, and in extreme cases almost pure white. This color is readily distinguished from the orange color of *etiolated plants*.

A plant may be grown in the presence of limited quantities of magnesium and normal growth may proceed for a time. Later, however, the lack of magnesium becomes apparent, owing to a breaking down of the chlorophyll complex as evidenced by a striped effect in the leaves or to other somewhat analogous changes. Since this is true, it is to be expected that assimilation processes should be affected first, and this has been found to hold true.

Magnesium may be toxic to plant growth in high concentrations. Water-soluble magnesium is readily absorbed by the plant, and it has been found by Garner that only 12 pounds per acre, applied as the sulfate, resulted in abnormal plant growth. This occurs, however, only where calcium is markedly deficient and may be overcome by calcium additions. *Magnesium poisoning* and *calcium deficiency* are thus quite closely related. According to True, magnesium poisoning can take place only after it has replaced calcium in the pectate layer of the cell walls. Magnesium appears to be localized in regions of newly formed tissues. It is thought to aid in the manufacture of nucleoproteins and in the assimilation of phosphorus. A more or less direct relationship exists between the phosphorus and magnesium content of tissues; especially is this true of oil-bearing seeds.

Magnesium acts as a carrier of phosphorus, in the form of phosphates, as $MgHPO_4$, which on hydrolysis yields free phosphoric acid which may be assimilated quite readily:

$$4\ MgHPO_4 + 2\ HOH \rightleftarrows Mg_3(PO_4)_2 + Mg(OH)_2 + 2\ H_3PO_4$$

The insoluble tertiary salt formed in this reaction may dissolve in a sufficient concentration of organic acids, forming the primary and secondary salt, both of which function in phosphorus assimilation.

Potassium. — Potassium is necessary for plant growth but does not necessarily enter into the actual composition of the structural material of the cell. It occurs, mainly, in solution and is found within the vacuole, although it is likewise present in the protoplasm This solubility of potassium compounds, some of which are highly ionized, materially increases the moisture-retaining powers of the plant, although possibly not to the same extent as is true with chlorine compounds.

In the normal metabolic activities of living plants a number of organic acids are constantly being produced, a few of which, such as oxalic acid, are toxic and therefore interfere with normal development. There should be at all times sufficient quantities of potassium to neutralize these compounds so that such a condition will not arise, and to prevent undue changes in actual acidity. If a plant tissue does not contain sufficient quantities of potash to meet these requirements, an abnormal metabolism takes place, which if it does not result in death, at least renders the plant less resistant to the invasion of parasitic disease organisms, among which may be mentioned the various " rusts." Especially is this true when plants are liberally supplied with nitrogenous materials, since such compounds markedly increase the production of organic acids.

Potassium may neutralize an acid such as oxalic, either wholly or in part, forming a soluble compound. If it is brought into contact with calcium, a reaction takes place which results in the formation and precipitation of calcium oxalate, thereby permitting the potassium ion, once more, to function normally in its original capacity. This may likewise hold true for a number of other acids as well. It is thought that sodium may partially replace potassium in this particular rôle.

Certain salts of potassium, such as potassium malate, may aid in the translocation of iron and some other elements through the formation of soluble complex compounds. Iron is not translocated throughout the plant unless potassium is present in sufficient quantities. This is a factor of considerable importance from the standpoint of carbon assimilation, since the presence of iron is necessary for the formation of one or more compounds which go to make up the chlorophyll complex.

Potassium appears to play an important rôle in carbohydrate manufac-

ture and translocation. There are a number of reasons why this is true, among which may be mentioned its property as a condensing agent and its effect on the water-holding powers of the plant, both properties of which are of major importance in this connection. Indirectly, therefore, potassium plays a rôle in the formation of other plant compounds. In addition, it plays a direct rôle in protein formation.

The proper functioning of potassium within the cell appears to depend to a great extent on the form in which it is supplied to the plant. If it is present as the nitrate, the nitrate nitrogen is utilized in the formation of organic nitrogen compounds, thereby liberating potassium in basic form — the form in which it best serves the organism. If it were in combination with an element or radical that was not capable of being utilized in metabolism, this condition would not necessarily obtain, unless it were replaced from this form by a base such as calcium.

Iron. — A variety of heavy metal compounds are present in living cells, and some of these, such as iron, are essential to plant growth. These appear to be involved in the catalysis of biological oxidations.

Substances which are stable when exposed to oxygen outside the organism are readily oxidized within the cell. It is now known that the heavy metal present in the highest concentrations is iron, but it does not give a test for either the ferrous or ferric ion. Therefore, it must be in the form of a complex ion. A number of these compounds are known, the most important of which are probably the *heme* compounds, one of which, at least, is known to play an important rôle in cellular oxidations. *Hemes* are pigments formed by the combination of iron with the various porphyrins; they appear to be universally distributed throughout the vegetable and animal kingdoms, but never in any great quantity. According to von Euler and Fink, only about 1/160 of the total iron of yeast is in the form of *heme*.

If iron is absent from the nutrient medium, chlorophyll formation will not take place. Although iron is not an essential constituent of the chlorophyll complex, it is believed to function as a catalyst in the formation of compounds such as pyrrole dicarboxylic acid, which is utilized in the synthesis of chlorophyll

Where the growth of the plant is quite rapid there is usually a deficiency of chlorophyll, and this is due, in a measure at least, to the slow absorption and translocation of iron compounds. It sometimes happens that iron is precipitated in the cells owing to an excess of calcium being present or to other causes. Under these conditions, chlorophyll formation can be brought about by spraying the plants with a solution of an iron salt.

An important rôle of iron compounds in the living plant is that of

nitrite reduction. According to Baudisch, nitrogen has a great affinity for iron, and the reduction of nitrites is induced by a primary linkage of the nitrite nitrogen to the iron nucleus. On the other hand, the affinity of iron for nitrate oxygen is not sufficiently great to bring about a primary addition and subsequent splitting off of an oxygen atom. This reduction of nitrite compounds aids in the utilization of inorganic nitrogen in the formation of organic nitrogen compounds. This reaction is aided by sunlight.

Phosphorus. — Phosphorus is necessary for the formation of many plant compounds, among which may be mentioned *phytin* (the calcium-magnesium salt of *inositol phosphoric acid* which probably functions as a reserve phosphorus supply); *phospholipids* (such as *lecithin*); *hexose phosphates* (intermediate products in the alcoholic fermentation of sugar by yeasts); and *nucleic acids* (combinations of phosphoric acid with carbohydrates and *purine* and *pyrimidine bases*).

Inorganic phosphates found dissolved in plant juices may function to a certain extent as buffer materials according to the following equation:

$$K_2HPO_4 + CH_3 \cdot COOH \rightleftarrows KH_2PO_4 + CH_3 \cdot COOK$$

There appears to be a correlation between the quantity of phosphorus absorbed by plants and the quantity of proteins synthesized. Experiments performed on lower plant organisms grown in the absence of phosphorus show that simple proteins may be formed, but that no cell division takes place. If a phosphate is added, however, cell division occurs with the simultaneous production of nucleo-proteins, which is followed by a lowering of the simple protein content.

Seeds rich in carbohydrates are correspondingly low in lecithin. On the other hand, seeds rich in glycerides contain relatively large quantities of this compound. This is to be expected since glycerides constitute part of its molecule. On germination, as the oil decreases, the lecithin content increases. As one of its main functions is that of aiding in the oxidation of glycerides, it is looked upon, to a certain extent, as an *autocatalyst*, a substance which is formed in the course of a reaction.

Phosphorus, through the formation of an hexose-phosphate, plays a part in the decomposition of glucose:

$$2\,C_6H_{12}O_6 + 2\,K_2HPO_4 \rightleftarrows 2\,CO_2 + 2\,C_2H_5OH + C_6H_{10}O_4(K_2PO_4)_2 + 2\,H_2O$$

The hexose-phosphate on hydrolysis yields the original phosphorus compound:

$$C_6H_{10}O_4(K_2PO_4)_2 + 2\,HOH \rightleftarrows C_6H_{12}O_6 + 2\,K_2HPO_4$$

This reaction is very important in plant respiration, especially where

oxygen is lacking. Phosphorus, however, causes an acceleration of both the aerobic and anaerobic forms of respiration, thereby increasing the intensity of metabolism with the final result of hastening maturity, being antagonistic to nitrogen in the latter respect.

The effect of phosphate additions to plants is to increase root development, which increases the feeding capacity of the plant and in this way materially increases crop yields.

Sulfur. — Sulfur is an essential plant food but is usually required in smaller amounts than nitrogen, phosphoric acid or potash. In some cases it has been observed that seeds do carry sufficient quantities of this element to suffice for the needs of the plant. On the other hand, such plants as alfalfa require considerable quantities of sulfur for normal growth. Sulfur in the sulfate form stimulates root development and is used also for the synthesis of many plant proteins.

FIG. 43. View of experiment station at "Limburgerhof," Germany, where considerable investigational work in relation to nitrogen fertilization is conducted. (Courtesy of the Kuttroff, Pickhardt and Co., Inc.)

Manganese. — Manganese is necessary for plant growth, but in minute quantities. Its place in the nutrition of the green plant has been investigated in detail for some years, and numerous claims have been advanced in regard to its function, but there does not seem to be general agreement relative to its specific rôle in the activities of the plant. In all probability, however, it functions as a catalyst in biological oxidations. On account of its activity as an oxidizing catalyst, plants are not able to tolerate high concentrations of this element.

Nitrogen. — Plants respond to nitrogen additions more readily than to any other nutrient material. It is an essential constituent of all proteins. A plant liberally supplied with nitrogen in the proper form

shows a well-developed leaf system, which is desirable from the standpoint of carbon assimilation as more surface is exposed for gaseous interchange. The leaf proteins which invariably parallel the assimilation of nitrogen likewise aid in carbon assimilation since they have an affinity for the carbon dioxide of the atmosphere.

Nitrogen stimulates cell reproduction to a remarkable extent, and thereby favors the vegetative phase of plant growth. It likewise contributes to the formation of chlorophyll and is an essential constituent of this complex. It aids in the hydrolysis of starch and in the conversion of sugars to organic acids, thereby increasing the acidity of the plant. Where a high starch content and a low acidity are desired, as in the case of tobacco, considerable attention must be given to the quantity of nitrogen present in the nutrient medium and to the physiological balance of all nutrients.

The Ash Content of Plants

The ash content does not serve as an index of the number of different elements present in plants during their growing period. For example, a plant may contain nitrogen in the form of nitrates which were not utilized in metabolism, yet no nitrogen is found in the ash. This is due to the fact that nitrates decompose when subjected to high temperatures. Other elements may be lost in much the same manner.

Neither does the relative abundance of mineral constituents in the ash necessarily serve as an index of the plant's requirements. A plant may absorb more of one element than it is able to utilize advantageously in metabolism. For example, a buckwheat plant may tolerate considerably more calcium than is necessary for its development. However, the total quantity of ash constituents, as well as the ratio of one element to another, may vary according to the type of plant, and a possibility exists that where a plant absorbs more of one element than does another, this element is required in larger quantities by the first than by the second.

The ash content of different plant parts shows marked differences. More potassium is found, for example, in the leaves than in the stems. There is also a pronounced difference in the ash content of the same tissue at different periods of the growing season. As a rule, there appears to be a close correlation between the quantity of ash present and the chemical activity of the tissue.

The composition of the nutrient medium does not have a direct bearing, necessarily, on the character of the plant ash. Plants grown under the same nutritive conditions may exhibit great differences between the quantity and character of their ash content. In a few cases, however, a more or less definite relationship is found between the materials avail-

able and those absorbed. Especially is this true in respect to the bases held in adsorbed form by the soil colloids. If this is proved to hold true in general, for plants grown under soil conditions, it will be of considerable importance in determining the proper physiological balance of mineral elements in the soil for the nutrition of plants.

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CHAPTER XVII

THE PLANT CARBOHYDRATES

The greater portion of the food reserve and structural network in plants is composed of carbohydrate materials. These are utilized by the plant for the synthesis of all other organic plant compounds and constitute either directly or indirectly the main source of energy for the various metabolic activities of the plant.

The most fundamental study of the biochemistry of plants is that which involves the mechanism of carbohydrate synthesis. Although a considerable amount of investigational work has been conducted on this particular subject, our knowledge of this important process leaves much to be desired.

The Formation of Carbohydrates in the Vegetable Kingdom

The Synthesis of Carbohydrates from Carbon Dioxide. — Laboratory experiments conducted by Butlerow in 1861 have shown that if trioxymethylene $(CH_2O)_3$, a condensation product of formaldehyde, was subjected to the action of hydrated lime in aqueous solution, a soluble carbohydrate or hexose mono-saccharide was obtained, which was neither sweet nor fermentable. This experiment probably led Von Baeyer in 1864 to offer his hypothesis that carbohydrates were formed in green leaves through the breaking down of CO_2 and H_2O under the influence of sunlight into CO and H_2, the hydrogen liberated uniting with CO to form $H \cdot CHO$ which would later undergo a process of condensation to form a sugar, as represented by the following equation:

$$6\,H \cdot CHO = C_6H_{12}O_6$$

The oxygen liberated from the CO_2 and H_2O was supposed to take no part in the reaction. That sunlight was necessary under normal conditions for the synthesis of carbohydrates has never been doubted, and the process is called *photosynthesis* in order to show this relationship.

Closely related to the hypothesis of Von Baeyer was that advanced by Erlenmeyer some years later. Erlenmeyer considered that carbonic acid was hydroxy-formic acid, and that it was capable of reacting with water according to the following equation:

$$HO \cdot COOH + H \cdot OH = H \cdot COOH + H_2O_2$$

Erlenmeyer believed that free oxygen was liberated from the hydrogen peroxide and that the formic acid was reduced to formaldehyde, which on condensation would form a sugar.

In recent years the principal investigations conducted on the formation of carbohydrates by green plants have been concerned with establishing the validity of Von Baeyer's hypothesis. These studies have dealt with the existence of free formaldehyde in plants, the production of formaldehyde from carbonic acid, the production of carbohydrates from formaldehyde, and the assimilation of formaldehyde or its derivatives by plants.

The Existence of Formaldehyde in Living Plants. — Many difficulties are involved in a study of this nature. For example, free formaldehyde is extremely toxic to plants. It would be natural to expect, therefore, that, if formaldehyde was produced by plants, these organisms would possess a mechanism which would convert it into a less toxic compound at a rapid rate. The absence of a test for free formaldehyde, therefore, would not necessarily be an indication that it was not a product of normal metabolism. Many experiments have shown that, although it was very difficult to obtain a trace of formaldehyde under ordinary conditions, it was possible to obtain a test in the leaves after they had been subjected to intense illumination. As the plant contains many substances which yield formaldehyde on decomposition, among which may be mentioned chlorophyll, these results have been questioned. More recently, however, Klein and Werner discovered that formaldehyde was found as a normal product in leaves undergoing active photosynthesis, but, if the leaves were exposed to sunlight in a carbon dioxide-free atmosphere, no formaldehyde was produced. In their work they made use of dimethylhydroresorcinol, which condenses with formaldehyde to form an extremely insoluble and characteristic crystallization product.

The Production of Formaldehyde from Carbonic Acid. — It is a very easy matter to oxidize formaldehyde to formic acid and to carbonic acid. It was thought, therefore, that it was fundamentally possible for the reactions to proceed in the opposite direction. In 1907 Fenton found that carbonic acid was reduced by metallic magnesium with the formation of formaldehyde. Lob also obtained the latter compound on subjecting a solution of carbonic acid to the silent electrical discharge. Others have claimed that they obtained formaldehyde by exposing carbonic acid to ultraviolet light of shorter wave-lengths. Baly, Heilbron, and Barker claim that this is possible if the liquid is kept agitated, the explanation being that under these conditions some of the formaldehyde formed is removed from the zone of reaction and kept from undergoing polymerization by ultraviolet light of longer wave-lengths.

The synthesis of formaldehyde from carbonic acid by means of light energy is a laboratory, if not an industrial, possibility. The conditions existing in the plant are so different by comparison, however, that the question arises as to whether the plant possesses the mechanism necessary for the production of formaldehyde in like manner.

The Production of Carbohydrates from Formaldehyde. — The experiments of Butlerow, Loew, and many other investigators have proved that carbohydrates can be synthesized from formaldehyde under laboratory conditions. These experiments leave much to be desired because they do not even approximate the conditions under which carbohydrates are formed in green leaves.

Considerable advancement has been made in this field of research by Baly and his co-workers. Baly has found that it was possible to polymerize formaldehyde to a sugar under the influence of the longer wave-lengths of light. He postulates, therefore, that the function of chlorophyll is to act as a transformer of energy so that the longer wave-lengths of light can effect the synthesis of formaldehyde from carbon dioxide and water, and this is followed by the polymerization of this product into carbohydrates. Baly has now extended his original observations and announces the synthesis of carbohydrates from carbonic acid by means of visible light, thus approximating natural conditions The presence of finely divided materials in the solution promoted the rate of synthesis. It would appear, therefore, that the great amount of energy required to convert the carbonic acid to carbohydrates is supplied to it in two portions, one by the surface and the other by the visible light

The Assimilation of Formaldehyde or Its Derivatives by Plants. — The results of any experiment which would go to show that plants possess the power of assimilating formaldehyde or its derivatives would go far toward establishing the validity of Von Baeyer's hypothesis. Probably the first investigator to make an extensive study of this possibility was Bokorney, who attempted to grow plants in a solution of formaldehyde of a concentration equal to 1 : 50,000, and who found that even in this concentration formaldehyde was toxic to the plant. Later, he found that methylal, $HCH(OCH_3)_2$, could be used very satisfactorily. It hydrolyzes slowly forming formaldehyde:

$$HCH(OCH_3)_2 + HOH \rightleftarrows H \cdot CHO + 2\,CH_3OH$$

Bokorney found that, in the quantities of methylal employed, the formaldehyde liberated was utilized in the synthesis of carbohydrates in a carbon dioxide-free atmosphere and without any toxic action on the plant itself. These results have been substantiated by others, but some are inclined to the belief that utilization of the formaldehyde is possible

only in the presence of light. Later Spoehr proved that formic acid, an oxidation product of formaldehyde, could be readily assimilated by plants in the manufacture of carbohydrates and in the absence of any other source of carbon.

On the whole, therefore, the results obtained in the experiments mentioned above appear to corroborate the hypothesis of Von Baeyer. The formation of formaldehyde from carbon dioxide and water and its subsequent polymerization into carbohydrates have been accomplished, the presence of free formaldehyde has been proved, and likewise the fact that formaldehyde may be directly assimilated by plants grown in the absence of carbon dioxide. At the present time, therefore, the main points of the hypothesis are quite generally accepted.

Factors Influencing the Synthesis of Carbohydrates by Plants

Several of the more important factors which have more or less bearing on photosynthesis will now be mentioned.

Light. — That light is necessary for the normal synthesis of carbohydrates is recognized. Although a considerable amount of work has been done relating to this subject, many of the results obtained are of a doubtful value on account of faulty methods of attack. On the whole, however, the results show invariably that active photosynthesis is correlated with low light intensities. Under conditions of high light intensities there is usually apparent a lowered photosynthetic activity of the plant, owing to the presence of a number of interfering factors.

Only a small quantity of the sunlight striking the plant appears to be used in photosynthetic activity, the greater portion being lost by reflection, absorption, or transmission. The light that is available for this process includes the greater part of the ultraviolet. Carbohydrates may be synthesized, however, in the absence of ultraviolet light and under the influence of any light of a definite wave-length. In a general way, however, it is recognized that, under normal conditions, some wave-lengths are more efficient for photosynthesis than others. It is entirely possible that a general relationship may be found to exist between the quantity of energy absorbed from the light of different wave-lengths and the rate of photosynthesis.

Oxygen. — Statements in the literature as to the necessity of oxygen for photosynthesis are somewhat conflicting, but the general conclusion seems to be that oxygen is necessary, although different plants may vary in their resistance to lack of oxygen. According to Willstatter and Stoll, however, photosynthesis can proceed only in the presence of oxygen. They found that reducing the oxygen pressure of an artificial air mixture

to one one-hundredth of the normal atmosphere did not reduce the efficiency of the photosynthetic process, but that photosynthesis was found to decrease by further reduction of the oxygen pressure, the decrease in rate varying according to the species used.

Spoehr and McGee found that the leaves of a very low carbohydrate content are more dependent on the presence of free oxygen than those of a high carbohydrate content, and that the rate of photosynthesis closely correlated the rate of respiration. Owing to the relatively high oxygen content of the atmosphere, and to the fact that an evolution of gaseous oxygen always accompanies the photosynthetic process, the lack of oxygen rarely, if every, constitutes a limiting factor in this particular activity of the plant.

Temperature. — The rate of photosynthesis, all other things being equal, is found to depend to a great extent on the temperature of the environment. The effect of temperature, according to Willstätter and Stoll, is modified somewhat by the chlorophyll content of the leaves, the low-chlorophyll leaves exhibiting a lower acceleration rate, with increasing high temperatures, than the high-chlorophyll leaves The formation of starch is likewise a controlling factor, the accumulation of which, following the employment of high temperatures, generally lowers the rate of photosynthesis

That photosynthesis can take place at what is ordinarily looked upon as a subnormal temperature, is borne out by the fact that, in the case of certain Alpine plants, carbohydrate formation may take place at a temperature of 0° C. In these plants, however, there appears to be no accumulation of starch, the sugars remaining in solution producing a sap of sufficiently high concentration to lower the freezing point to a marked degree.

Increasing the temperature of the environment surrounding the plant from 5° C. to 25° C yields results, as a rule, which conform to van't Hoff's law. At higher and lower temperatures, however, this does not necessarily hold true, since many other factors may play a part, some of which may tend to bring about the death of the plant.

Moisture. — Normal photosynthesis can take place only when the cells are turgid. If the cells of the green leaf lose their turgidity, a closing of the stomata may occur and thus interfere with the absorption of carbon dioxide. According to Garner and his co-workers, the partial wilting of the green leaf has a decided effect in stimulating the enzymatic processes involved in conversion of starch into reducing sugars. On the other hand, it is likewise true that increases in water content cause reformation of starch. Furthermore, it may be said that, in the presence of starch, saccharose is formed as the water content decreases, so that the

saccharose content in the tissues is a function of the water content. This relation is said to hold true in darkness and light. In darkness the hexose content of the tissues also increases with reduction in water content. It seems clear, therefore, that these transformations in relation to water content are of fundamental importance in the photosynthetic process of the plant. Any factor that serves to bring about a greater concentration of dissolved substances in the sap of the guard cells and adjoining cells may lead to the closing of the stomata and the temporary cessation of the process of photosynthesis.

Mineral Nutrients. — Some mineral nutrients and non-nutrients appear to have a pronounced effect on the rate of photosynthesis. This is true, especially, of nitrate nitrogen. Boze found that free nitric acid in the nutrient medium caused an increase in photosynthetic activity anounting to as much as 200 per cent. He found likewise that the effect of iodine was almost as striking. Other salts, among which may be mentioned those of copper, zinc, mercury and cobalt, appear to lower the rate of this process. For normal photosynthesis all the mineral nutrients necessary for plant growth must be present in the proper concentrations.

Electrical Conditions. — Electrical conditions alone can never completely duplicate the effect of light. According to Henrici, however, the influence of electricity has been known to increase the rate of photosynthesis, owing to the fact that under such conditions the oxygen of the atmosphere becomes ionized, which renders it more reactive in the process of oxidation, which is so necessary for photosynthesis. Moreover, it is known that, if the soil on which plants are grown is treated electrically, there is a definite stimulation of photosynthetic activity — an effect that is probably correlated with increased availability of the soil nutrients through the process of electrolysis.

Chlorophyll Content. — Chlorophyll, the green pigment of plants, consists of the blue-green component *a*, and the yellowish-green component *b*, whose magnesium-free derivative is red-brown in neutral solutions. Their empirical formulae are as follows:

Chlorophyll *a*, $C_{55}H_{72}O_5N_4Mg$
Chlorophyll *b*, $C_{55}H_{70}O_6N_4Mg$

The relationship between the components *a* and *b* as regards composition and constitution is significant in explaining the chemical function of chlorophyll. It would appear from these formulae that *b* is an oxidation product of *a*. The function of chlorophyll is to aid in the assimilation of carbon. Some are inclined to the belief that carbon dioxide is attracted by the affinity of the magnesium compounds and that its reduc-

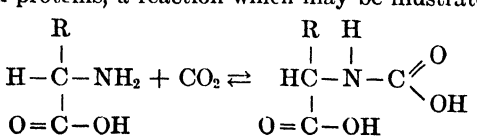
tion is brought about by the *a* component in a process which involves the utilization of absorbed light energy. In this process *a* is supposed to be oxidized to *b*, and this is again transformed into *a* by the splitting off of oxygen, a state of equilibrium appearing to exist between the two components. Accompanying chlorophylls *a* and *b* are the yellow carotinoid pigments, carotin, $C_{40}H_{56}$, and xanthophyll, $C_{40}H_{56}O_2$.

Since these yellow (carotinoid) pigments constantly accompany the green pigments, it is thought that they are fundamentally related. Perhaps their purpose is to regulate the ratio of the chlorophylls through the withdrawal of oxygen by carotin from component *b*, with the formation of xanthophyll, and this oxygen is later set free from xanthophyll, reforming carotin.

The necessity of chlorophyll for photosynthesis is recognized. Etiolated leaves of plants normally green in color do not, as a rule, possess any photosynthetic activity, but as they are allowed to synthesize chlorophyll they may become extremely active in this respect, even if but a small quantity is present. Considerable differences of opinion exist, however, regarding the relation of the quantity of chlorophyll present, to the rate of photosynthesis. It is recognized, however, that old leaves may contain considerable quantities of chlorophyll and yet have a less intense photosynthetic activity than a younger plant containing a smaller quantity of chlorophyll. The influence of the quantity of chlorophyll on the rate of photosynthesis, therefore, may be overshadowed by other factors

The Absorption of Carbon Dioxide. — Most of the carbon dioxide used by green plants for photosynthesis comes from the atmosphere The diffusion of this gas occurs through the stomata and, ordinarily, should be a rather slow process, owing to the facts that it occurs in small quantities in the atmosphere and that it has a limited solubility in plant sap Carbohydrate formation does not necessarily parallel the quantity of carbon dioxide absorbed in any given interval of time, owing to the fact that there may be an accumulation of carbon dioxide in the plant in different forms, which may be available during active photosynthesis.

According to Spoehr, the first step in photosynthesis is not the absorption of carbon dioxide, followed by an immediate splitting of the compound, but rather a union between carbon dioxide and certain of the leaf compounds. In this connection it may be stated that carbon dioxide combines with proteins, a reaction which may be illustrated as follows:



The effect of such a reaction is to increase the concentration of carbon dioxide in the cell sap as a result of the dissociation of this complex, and the reestablishment of the equilibrium of carbon dioxide concentration just as fast as the carbon dioxide is utilized for assimilatory purposes. According to Willstatter and Stoll, colloidal solutions of chlorophyll react with carbon dioxide in a manner somewhat analogous to that of the leaf proteins.

The carbon dioxide concentration of the atmosphere may be increased, resulting in an increased rate of photosynthesis. When the concentration exceeds 25 per cent of the total atmosphere, a profound lowering of the rate of carbohydrate formation is apparent.

Energy Requirements for Photosynthesis. — According to Gortner, the equation for photosynthesis should be written:

$$6\ CO_2 + 6\ H_2O + 677.2\ \text{Cal.} \rightarrow C_6H_{12}O_6 + 6\ O_2$$

and the reverse of this equation is true for respiration.

The energy of the sunlight fixed in the process of photosynthesis is later released to a considerable extent, for vital activities, through the process of respiration. It is generally recognized that the efficiency of photosynthesis is relatively low. According to Transeau, the total energy of sunlight falling on an acre of ground during the growing season is approximately 2,043 million calories Less than 2 per cent of this total energy is utilized for photosynthesis Transeau, in his study of the corn plant, states that only a small amount of the carbon assimilated during the growth of the plant is present in the mature plant, enormous quantities of the assimilated carbon being lost through the process of respiration. He further notes that the average rate of photosynthesis must be about eight times greater than the rate of respiration.

The Products of Photosynthesis

The main products of photosynthesis appear to be oxygen and carbohydrates. Of the carbohydrates, glucose, fructose, sucrose and starch are produced, either directly or indirectly, in all cases. Considerable attention has been devoted in the past to the question as to which carbohydrate is formed first, since it is recognized that these products are directly or indirectly synthesized from one another. The mere presence of any particular carbohydrate, therefore, does not necessarily indicate that it was synthesized in the photosynthetic process proper

The question as to the first carbohydrate formed in the process of photosynthesis still remains unanswered. According to Spoehr, the present state of our knowledge warrants the assumption that glucose is the first product formed. Owing to the relationship existing among the different

carbohydrates, it is entirely possible to visualize the formation of glucose which on dehydration yields starch, or through molecular rearrangement forms fructose, which may unite with another molecule of glucose forming sucrose.

Chemical Interrelations of the Carbohydrates

Attention has been called to the fact that glucose, in solution, may undergo a molecular rearrangement giving rise to fructose. According to Nef, glucose solutions, if rendered slightly alkaline, yield various mono-, di-, and polysaccharides. Under other environmental conditions they may give rise to a pentose sugar. For example, if the oxime of glucose is heated with acetic anhydride and zinc chloride, a vigorous reaction is brought about, resulting in the formation of pentacetate of gluconic nitrile. On treatment with ammonia and silver oxide, HCN splits off of this compound leaving arabinose.

On the other hand, glucose may be treated with HCN forming a seven-carbon nitrile, which may undergo hydrolysis yielding the corresponding acid On reduction, this yields a heptose sugar. Furthermore, on mild oxidation, glucosone is formed, which may be reduced to fructose. In this way it is possible for a ketose to be formed from an aldose sugar.

It is commonly stated that glucose, on dehydration, gives rise to starch. The mechanism involved in this transformation is not clearly understood. It is not a true dehydration, however, since the reaction cannot proceed in an atmosphere of either hydrogen or oxygen but will take place where both elements are present. According to Matthews, such a reaction is brought about by a reduction followed by an oxidation. Brown and Morris, however, have found that sucrose surpasses glucose in the formation of starch

The conditions under which the interrelations of the carbohydrates have been studied in the laboratory may or may not hold true in the plant. The reaction between an aldose sugar and HCN which is sometimes a product of normal metabolism, forming a compound containing one more carbon atom, is quite possible. On the other hand, it is clearly indicated that aldehyde groups on oxidation to carboxyl groups may lose carbon dioxide under the influence of the carboxylase enzyme. This yields a derivative containing one less carbon atom which may, in the course of a series of reactions, be changed into a pentose sugar.

The Formation of Carbohydrates from Fats

In the germination of oil-bearing seeds, the carbohydrate content is found to increase as the oil content decreases. Many other instances are known where carbohydrates are derived from fatty compounds. The

nature of the carbohydrate or carbohydrates formed from fats depends upon the kind of plant and in all probability upon the kind of fat and especially on its degree of saturation. It is generally believed that saturated fats or fatty acids are less efficient in carbohydrate manufacture than are the unsaturated fats and fatty acids. On the whole, however, very little is known as to the mechanism involved in the production of carbohydrates from fatty substances.

The Formation of Carbohydrates from Proteins

Proteins, on hydrolysis, yield amino acids and other products, some of which are readily converted into carbohydrates. According to Dakin, glycine, alanine, serine, cysteine, aspartic acid, glutamic acid, hydroxyglutamic acid, proline, and ornithine may give rise to glucose under the appropriate conditions.

The nitrogen of an amino acid may be removed during metabolism in the form of ammonia which may be utilized by the plant organism in the re-synthesis of amino acids or for many other purposes. The decomposition of the amino acid may cease after the liberation of ammonia, or the residual compound may be used for the synthesis of carbohydrates or as a source of carbon for normal reactions. Especially is this true for lower forms of plant life. The synthesis of carbohydrates from proteins is not as important as the synthesis of proteins from carbohydrates.

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CHAPTER XVIII

THE PLANT LIPIDS

Glycerides, such as the fats, oils, phospholipids, and related materials are found in all plants. They are present in those tissues which are active in metabolism but are rarely present in a large quantity. They tend to accumulate, however, in regions of less intense metabolism, as in seeds, where they may serve as a reserve food supply for the embryo.

The Synthesis of Lipids in the Vegetable Kingdom. — The synthesis of lipids by higher plants does not bear any direct relation to the process of photosynthesis. It has been noted, however, that oil droplets appear in the cells of lower plants during periods of illumination, but there is no direct evidence to show that these are formed photosynthetically. The characteristic lipid of a plant appears only in relatively large quantities as the period of maturation approaches, and it is generally accepted that it originates from organic compounds existing in the surrounding tissue.

The Synthesis of Glycerides from Carbohydrates. — Glycerides are thought to arise, primarily, from carbohydrates, and therefore their synthesis may be indirectly related to photosynthesis.

The fattening of animals on a high-carbohydrate ration, the development of fats in the germination of oil-bearing seeds, the formation of fats from carbohydrates as a result of low-temperature conditions, all show a rather close physiological relationship between these two important groups of organic compounds. The most striking proof that such a conversion does exist is brought out in studies dealing with seed formation and seed germination. Immature seeds show a minimum of glyceride accumulation, but as they become more mature there is an increase in glyceride content, followed by a more or less corresponding decrease in the carbohydrate content. Since no substantial proof is at hand showing that glycerides are carried as such into the seed, it appears evident that they are found in place and at the expense of carbohydrates. Moreover, an immature seed may be separated from the host plant and found later to become higher in glyceride content and lower in carbohydrates.

Many plants, especially those of a lower order, appear to synthesize a considerable quantity of these fatty compounds. Not much is known, however, of the mechanism of fat metabolism in these lower organisms, although a considerable amount of work has been conducted on the syn-

thesis of glycerides by the yeast plant, since this plant can be grown readily in large quantities and the environmental conditions surrounding its development can be rather easily controlled. During the World War the question of using yeast and similar lower plant organisms as sources of fat became one of practical importance, especially in Germany. According to Linder, a yeast (*Endomyces vernalis*), containing 18 per cent of fat could be obtained when grown in a well-aerated medium containing a considerable quantity of carbohydrate or related materials.

MacLean and Hoffert found that when yeast was incubated in a well-aerated medium of fermentable sugars the fat content was increased. In a solution of lactose, a non-fermentable sugar, no fat was formed. When glucose, fructose, sucrose or maltose was present, the fat content was increased, the quantity varying according to the sugar used. Maltose solutions, however, show themselves, on the whole, to be rather less effective in producing an increase of fat than are solutions of either glucose, fructose, or sucrose.

In the synthesis of fats from carbohydrates by yeasts and similar plants, the mineral constituents may play important rôles. This is true, especially, in the case of phosphorus. One of the effects of phosphate additions is to bring about the formation of a hexose phosphate which aids materially in furthering the process of fermentation Harden and Young regard the formation of hexose phosphate as an essential reaction in the fermentation of sugar brought about by living yeast plants.

The synthesis of fats from carbohydrates by living plants involves changes of the sugar into fatty acids and glycerol and the union of these compounds to form a triglyceride, containing different or identical fatty acid radicals. The latter process may be brought about by the agency of a lipolytic enzyme.

A hypothetical equation for the formation of glycerol from a carbohydrate may be given as follows:

$$\underset{\text{glucose}}{C_6H_{12}O_6} \rightarrow \underset{\text{glyceric aldehyde}}{2\,CHOH(CH_2OH)(CHO)} \xrightarrow{-2\,H_2O} \underset{\text{pyruvic aldehyde}}{2\,C(=O)(CH_3)(CHO)} \xrightarrow{+2\,H_2O} \underset{\text{lactic acid}}{2\,CHOH(CH_3)(COOH)}$$

In this equation, glyceric aldehyde is represented as an intermediate product between glucose and pyruvic aldehyde. Although its production has not been proved, it is a well-known fact that a solution of glucose in caustic alkalies undergoes complex changes, leading to the formation of lactic acid, and that under the same conditions both glyceric aldehyde and pyruvic aldehyde give rise to lactic acid.

In studies dealing with the fermentation of sugar, Pasteur was able to show that only about 3 per cent of the fermented sugar was utilized in the synthesis of glycerol.

The addition of sodium sulfite to a fermenting mixture of yeast and sugar results in an enormous increase in the yield of glycerol. According to Neuberg and his colleagues, the effect of sodium sulfite is to prevent acetaldehyde, which may be formed in the course of the fermentation process, from taking part in reactions normal to this process. These investigators say that pyruvic aldehyde may be oxidized to pyruvic acid, and that pyruvic acid may lose carbon dioxide under the influence of the carboxylase enzyme and form acetaldehyde which may be reduced to ethyl alcohol. In the absence of active acetaldehyde, glyceric aldehyde may be reduced instead, forming glycerol

Whether this theory as to the mechanism of glycerol formation is applicable to the living plant is not known, but the formation of acetaldehyde by lower plant organisms has been observed It is known, however, that both oxidative and reductive processes are involved, and when the reduction processes prevail to a greater extent, the fat accumulation is at a maximum.

The Synthesis of Fats from Proteins. — In 1879, Naegeli and Loew published the results of an experiment in which they stated that fats were formed by yeasts and other lower plant forms, from substances existing in the plasma of the cell, and they argued, therefore, that the fat could not be derived from the nitrogen-free compounds since they were present only in very small quantities in the cell. They believed that all materials for fat formation were built first into protoplasmic protein and in this way were utilized for the formation of fat, the fat being stored subsequently as reserve material.

According to MacLean, however, when yeast is grown in aerated glucose solutions, where no external supply of nitrogen was available, the loss in protein material could account for only a small quantity of the fat synthesized.

There is good reason to believe, however, that some of the amino acids, contained in the protein molecule, are convertible into glucose, and that glucose is an excellent source of material in fat synthesis. Many bacteria are able to accumulate relatively large quantities of fats when they are supplied with material made up almost wholly from protein materials. From the standpoint of higher plants, the synthesis of fats from proteins is not a reaction of great importance.

The Influence of Environment on the Synthesis of Fats. — The production of glycerides by the plant requires favorable conditions for the accumulation of carbohydrates during the vegetative period and for the

transformation of carbohydrate into glycerides during the reproductive phase of growth.

In a study dealing with the effect of environmental conditions on the oil content of soybeans, Garner and his colleagues found that the size of the seeds of plants grown in various parts of the United States varied more than 100 per cent. It is evident, therefore, that environment may affect tremendously the size of the seed and the quantity of the oil stored therein. In further tests, which included a wide range of soil types and climatic conditions, it was found that the relative effects of soil types were not specific and constant, but depended largely on seasonal conditions.

Within ordinary limits the relative fertility of the soil appears to be a minor factor in influencing the oil content of a seed. In fertilizer tests with cotton, the additions of a complete fertilizer to an unproductive soil gave a larger seed, however, which contained a higher percentage of oil. Applications of nitrogen in increasing quantities did not affect the size of the seed but lowered the percentage of oil, whereas increasing applications of phosphorus or potassium did not affect either character.

Plants growing in temperate or cold climates generally contain unsaturated fats which are characterized by low melting points. On the other hand, plants grown in tropical climates contain saturated fats. Cultivated plants, as a rule, produce glycerides which are less saturated than those produced by wild plants. There is thus some relation between the composition of the naturally occurring fats and the conditions of the environment in which they are formed.

The starch content of a plant is found to disappear slowly when subjected to low temperatures, resulting in an increased concentration of sugars within the cells. This change enables a plant to become more resistant to low temperatures as the freezing point of the cell sap is increased. In the course of time, however, the low temperature results in a change of the sugars to fats and oils. The effect of such a change is to increase the percentage of unfree water in the cell through the lowering of the surface tension of the plant sap, thereby materially increasing the resistance of the plant. The explanation of these changes cannot be confined to any one factor. It is evident, however, that fats are formed in nature by a series of reactions, the end-points of which may vary according to the temperature at which they occur. At higher temperatures the chemical reactions leading to the formation of highly unsaturated fatty acids reach completion, while at lower temperatures, possibly because reduction processes do not take place so readily, the same end-point is not reached, the unsaturated fatty acids being in this case the final products. Attention must be given, however, to the possibility of

fat synthesis, and the degree of unsaturation being controlled by the degree of respiration. Under cold temperatures there is not the opportunity for a rapid oxidation of carbohydrates to take place and under these conditions carbohydrates may become reduced, giving rise to fats.

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CHAPTER XIX

THE VEGETABLE PROTEINS AND RELATED COMPOUNDS

Proteins are widely distributed throughout the vegetable kingdom. As colloidal materials, they are found in the protoplasm, forming the most vital constituent. As insoluble compounds, they constitute a reserve supply of nitrogenous material and are localized in the cells of roots, bulbs, buds, tubers and seeds. In the latter form, in most instances, they constitute a very important source of food for higher animals.

The leaves constitute the most important centers of protein formation, although it is entirely possible that they may be synthesized to a limited extent in other plant parts as well. Protein synthesis depends largely upon the quantity and form of the nitrogen supplied to the plant. Thus among the non-chlorophyllous plants are found some which are unable to utilize nitrate nitrogen to advantage, and but few are able to utilize amines. On the other hand, these plants may utilize urea to advantage. In general, however, the non-chlorophyllous plants utilize peptones, although a few exceptions have been noted Both higher and lower forms of chlorophyllous plants utilize both organic and inorganic forms of nitrogen.

For crop plants, the nitrogen should be supplied in nitrate form, or the soil should be in such a condition that both ammonium salts and organic nitrogenous nutrient materials may be changed quite readily to the nitrate form. It is recognized, however, that some crop plants appear to utilize ammonium salts more efficiently than any other form of nitrogen.

In addition to nitrogen, potassium is a conditioning factor in the synthesis of proteins by green plants. Plants growing in sunlight and in a nutrient medium containing all the necessary mineral nutrients excepting potassium will synthesize proteins, but in darkness, no protein synthesis will take place. In a complete nutrient solution, however, protein synthesis will take place in the dark, though not to any considerable extent.

The Mechanism of Protein Synthesis. — If a solution of nitrate nitrogen is exposed to ultraviolet light, a reduction occurs whereby the nitrate is reduced to nitrite and hyponitrite, oxygen being liberated in the process. Baudisch found that a mixture of potassium nitrite and

methanol, in aqueous solution, would undergo decomposition under the influence of ultraviolet light to form potassium hyponitrite and formaldehyde, which was followed by a condensation of these products to form the potassium salt of formhydroxamic acid:

$$KNO_2 + CH_3OH = KNO + HCHO + H_2O$$

$$KNO + HCHO = H \cdot C \cdot OH \quad (\text{with } \| \text{ NOK})$$

On hydrolysis, followed by reduction, this product was found to yield a compound having the formula:

$$H-C(=N-H)-OH$$

which, uniting with nascent formaldehyde, formed a compound having the formula:

$$H-C(OH)(-N-H)-C(OH)-H$$

and which on molecular rearrangement yielded the amino acid, glycine:

$$H-C(-NH_2)(=O)-OH$$

Photosynthesis, through the formation of *nascent formaldehyde*, $H-C\equiv O-H$, may likewise bring about the formation of amino acids. For example, Klages found that, under laboratory conditions, formaldehyde and ammonium cyanide would unite to form methylene-amino acetonitrile, which on hydrolysis yielded glycine:

$$2\,H \cdot CHO + NH_4CN \rightarrow CH_2 : N \cdot CH_2 \cdot CN + 2\,H_2O$$

$$CH_2 \cdot N \cdot CH_2 \cdot CN + 2\,H_2O \rightarrow CH_2 \cdot NH_2 \cdot COOH + NH_3$$

Amino acids may be formed from nitrate nitrogen by certain plants and in the absence of light. Thomas, of the Pennsylvania State College, in his studies dealing with the nitrogen metabolism of the apple tree, found that no trace of nitrates or nitrites was obtainable in the upper plant tissues, except in fruit buds just previous to opening, although these studies were made on trees receiving relatively heavy applications

of nitrate nitrogen. The finer roots, however, showed the presence of nitrates, but less indicative tests were obtained in older root parts. Tests likewise showed considerably more amino acids to be present in older root parts than in the newer tissues. As a result of these investigations, Thomas naturally concludes that nitrates are reduced and amino acids are formed in the absence of light, and in the main part of the root system rather than in the leaves.

A number of plant compounds have the power of reducing nitrates. Ekerson found that leaves of nitrogen-high tomato plants yielded a juice from which a snow-white powder could be obtained by appropriate methods, and this powder had the power of reducing nitrates when added to an alkaline solution of nitrates and easily oxidizable carbohydrates. There was little or no reduction in acid solutions. The most favorable reaction for this reduction corresponded to a *p*H 7.6. Light was not necessary for the reduction process, but free oxygen was required. Plant enzymes are known which have the power of reducing nitrates, and Baudisch found that the reduction of nitrates could be accomplished in the presence of glucose and a trace of iron.

After amino acids are formed they may unite to form proteins under the influence of specific enzymes. The opinion is expressed, however, that protein synthesis must be especially difficult because the hydrolysis of proteins goes to practical completion. It is apparent, therefore, that for synthesis to take place the concentration of water must be diminished to a considerable extent. Living cells appear to possess this power, perhaps by surface condensation or imbibition on the part of the cell colloids

The quantity of water appears to be but a single factor in the synthesis of proteins from amino acids by plants. Many investigators have found that the reduction of nitrates to intermediate nitrogenous compounds takes place in darkness, but the synthesis of proteins takes place only in the light.

The Synthesis of Proteins from Carbohydrates. — Studies dealing with protein synthesis in the vegetable kingdom usually include those plants which contain a minimum amount of nitrogen and a maximum of carbohydrate material. This tends to prevent the possibility of protein decomposition products being mistaken for newly synthesized products. If such plants are supplied with small quantities of nitrate nitrogen, it is soon found that protein synthesis occurs in the green leaves and at the expense of the carbohydrates present. It would appear, therefore, that such plants have all the substances essential for protein synthesis with the exception of nitrogen.

In her work on protein synthesis, Ekerson used tomato plants which were grown under conditions of low available nitrogen supplies. These

were found to contain considerable quantities of glucose, fructose, sucrose and starch with but little nitrogen. Within 24 hours after these plants were supplied with calcium nitrate, tests were obtained readily for nitrates in all tissues, the tops of a few plants showing the presence of nitrites but no ammonia. No such tests were obtainable preceding the addition of the nitrate.

Within 36 hours considerable quantities of nitrites and ammonia were found in all plant parts. Within 48 hours, less nitrite but more ammonia was present, and a few crystals of asparagine were found. At the same time there was a profound decrease in starch concentration, especially in the cortical cells of the plant tips. Within a period of three to five days there was less ammonia, but a considerable quantity of amino acids. Simultaneously with the appearance of amino acids and the disappearance of starch, it was noted that the plastids became greener in color and the acidity of the tissues increased. Ekerson found that, as the starch became hydrolyzed into glucose, fructose was formed also, both of which were used for respiration and amino acid synthesis, and that malic, succinic and other organic acids were intermediate products in both processes.

The Synthesis of Proteins from Fats. — Fats may give rise to carbohydrates in living plants and carbohydrates may be utilized in the synthesis of proteins. From the standpoint of plant metabolism, the process of protein formation from fats is not usually considered to be of importance.

The Relation of Environmental Conditions to Protein Formation. — Light has already been mentioned as an environmental factor in the synthesis of proteins Any environmental factor such as light, which has to do with the synthesis of carbohydrates, likewise aids in the synthesis of proteins, provided, of course, that sufficient supplies of nitrogen are available

Climatic conditions are an important factor in determining the average protein content of many crops. Mangels has found that temperatures above the normal in June and July increase the protein content of wheat, but the climatic conditions which produce high-protein wheat, unfortunately, are not favorable to high yields.

Soil has likewise an important influence on the protein content of plants, and the use of the proper rotation systems may enable a wheat grower to produce high-protein wheat in seasons when climatic conditions tend to favor the production of low-protein wheat. Such factors as variety, pathological conditions or stage of maturity at harvesting may likewise be of importance in this connection, although, possibly, not so important as the soil and climatic factors.

A high moisture content of a soil containing sufficient available nitrogen for the maximum growth and the development of the wheat plant, according to Neidig and Snyder, results in high-yielding wheat containing a high percentage of protein. Results reported in the literature indicate that protein accumulation is closely associated with plant growth. It is generally believed that amino nitrogen increases during the early stages of growth and later gradually decreases as synthesis of proteins takes place. Any factor having to do with plant growth, therefore, would likewise influence protein synthesis.

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CHAPTER XX

RESPIRATION

Considerable attention has been given to the absorption of mineral nutrients and the rôles they play in plant metabolism. Some attention has been given to a study of the more important plant constituents, their formation and derivation. Reactions vital to the activities of the plant have been mentioned but not stressed. It is proper, therefore, to center our attention at this time upon some of those reactions which deal with the living processes of the cell and, especially, those which are involved in the process of *respiration.*

The term respiration is used to include all biochemical reactions which deal with the production of energy for the various plant processes. All plant organs involved in this process, or doing work with the energy so produced, may be regarded as organs of respiration

Normally, the presence of free oxygen is considered necessary for respiration, although some of the lower forms of plant life appear to be able to respire in the absence of free oxygen, whereas others respire both in its presence and in its absence. It is possible, therefore, to distinguish between three forms of respiration, namely, *aerobic, anaerobic* and *facultative-anaerobic.* Aerobic, or normal respiration, is the most important from the standpoint of higher plants, and it may be regarded as a slow oxidation of biological materials, accompanied by the production of carbon dioxide and water and various intermediate products, with the liberation of energy. In order to understand the chemistry of respiration, it is necessary for us to consider, among other things, the fundamentals of biological oxidations.

THE MECHANISMS OF RESPIRATION AND THE SYSTEMS INVOLVED

The compounds used ordinarily as fuel by living plants are not, as a rule, acted upon readily by molecular oxygen under laboratory conditions. They are not efficient reducing agents, and moreover, molecular oxygen is at best but a mild oxidizing agent In living plant tissues, however, these compounds are readily oxidized by oxygen under the influence of a number of biochemical catalysts. It is apparent, therefore, that the efficiency of these catalysts must be remarkable, and this is found to be true. Some organic compounds which are but slowly

oxidized by boiling nitric acid are broken down rapidly in the presence of tissue extracts in an environment of atmospheric oxygen.

Not all biochemical oxidation catalysts are of the same type, nor do they act in similar ways, and it is quite difficult for us, at the present time, to understand their mode of action, although considerable progress has been made in recent years relative to the part they play in oxidation-reduction processes. In this chapter, mention will be made of those fundamental principles underlying oxidation and oxidase activity that are fairly well established.

The oxidation of an organic compound may involve a loss of hydrogen:

$$\underset{\text{methanol}}{CH_3OH} - 2\,H \rightarrow \underset{\text{formaldehyde}}{H \cdot CHO}$$

or a gain in oxygen atoms:

$$\underset{\text{formaldehyde}}{H \cdot CHO} + O \rightarrow \underset{\text{formic acid}}{H \cdot COOH}$$

On the other hand, reduction involves a gain in hydrogen atoms or a loss of oxygen atoms

According to Wieland, oxidation-reduction reactions actually take place by a direct transfer of hydrogen atoms from one molecule to another, oxidations being, therefore, but *dehydrogenations*. In support of Wieland's theory, it may be said that in the oxidation of organic compounds, there is actually a loss of hydrogen atoms, while in very few cases is there noted a direct addition of oxygen atoms.

In those cases where there is a gain of oxygen atoms, Wieland assumes a hydrate formation:

$$R - \underset{\displaystyle O}{\overset{\displaystyle H}{C}} + HOH \rightarrow R - \underset{\displaystyle OH}{\overset{\displaystyle H}{C}} - OH$$

and shows that this compound may lose hydrogen atoms forming an acid:

$$R - \underset{\displaystyle OH}{\overset{\displaystyle H}{C}} - OH - 2\,H \rightarrow R - \underset{\displaystyle OH}{C} = O$$

Colloidal platinum and palladium have a strong affinity for, and absorb considerable quantities of, hydrogen. By making use of palladium-black, Wieland found that many oxidizable organic compounds in aqueous solution give up part of their hydrogen to this colloidal substance, forming a loosely combined hydride, PdH_2, and become oxidized.

When the hydrogenated palladium was removed and placed in aqueous solutions of reducible substances, the latter became reduced. On adding palladium-black to an aldehyde solution, however, Wieland found that the aldehyde became oxidized to an acid, and the palladium became charged with hydrogen as before. It is clear, therefore, that water takes part in oxidations, either through the formation of hydrates or in the following manner:

$$R-\underset{\displaystyle H}{\overset{|}{C}}=O + H\cdot OH + Pd \rightarrow R-\underset{\displaystyle OH}{\overset{|}{C}}=O + PdH_2$$

Hydrogen, as it occurs on the surface of palladium and on many other surfaces, is extremely active and may react readily with methylene blue, giving rise to the reduced form which is colorless:

$$PdH_2 + MB \rightarrow Pd + MBH_2$$

or it may unite with molecular oxygen as follows:

$$PdH_2 + O_2 \rightarrow Pd + H_2O_2$$

By the use of palladium and a substance such as methylene blue that is capable of removing active hydrogen from the surface of the metal, it is found that a solution of glucose may become oxidized in the absence of free oxygen. It is apparent, however, that, if free oxygen was used instead of methylene blue, the reaction would take place more quickly owing to the formation of hydrogen peroxide, which is itself an efficient oxidizing agent.

Considerable attention has been given in the past, especially by Traube and Bach, to what is now known as the "hydrolysis theory" of oxidation. This theory assumes that the reaction takes place through the addition of the elements of water to the oxidizer and reducer, the hydroxyl groups going to the latter and the hydrogen to the former. This theory has much to recommend it. If a solution of sodium sulfite is aerated it becomes oxidized to sodium sulfate:

1. $$\begin{matrix}NaO\\NaO\end{matrix}\!\!\diagdown\!\!S=O + 2\,HOH + O_2 \rightarrow \begin{matrix}NaO\\NaO\end{matrix}\!\!\diagdown\!\!\underset{\displaystyle OH}{\overset{OH}{\overset{|}{S}}}=O + H_2O_2$$

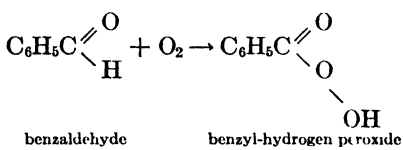
2. $$\begin{matrix}NaO\\NaO\end{matrix}\!\!\diagdown\!\!\underset{\displaystyle OH}{\overset{OH}{\overset{|}{S}}}=O - H_2O \rightarrow \begin{matrix}NaO\\NaO\end{matrix}\!\!\diagdown\!\!S\overset{O}{\underset{O}{\diagup\!\!\diagdown}}$$

In order that such a reaction may be brought about, the substance must permit of ready oxidation. A solution of sodium arsenite, which is more stable, will not become oxidized in like manner. If sodium sulfite is present, however, the arsenite becomes oxidized to the arsenate, which is brought about, in all probability, by H_2O_2.

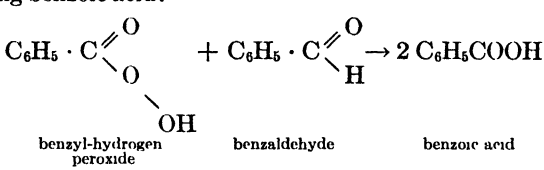
It is likewise possible that an aqueous solution of glucose may become oxidized through the influence of atmospheric oxygen, the OH groups of the water molecules breaking the bonds between the carbon atoms, the by-product H_2O_2 likewise entering into the reaction.

A surprisingly large number of the most diverse kinds of inorganic and organic compounds are found to yield hydrogen peroxide as a result of slow oxidation. In addition to some metals, these include alcohols, acids, terpenes, carbohydrates, phenols, alkaloids and many other compounds. A number of investigators who advocate the theory that both inorganic and organic peroxides play a major rôle in biological oxidations proper, call attention to the fact that, where peroxides are used under laboratory conditions as oxidizing agents of ordinary plant compounds, the products so derived compare favorably with the by-products of tissue oxidations

Proof that hydrogen peroxide and organic peroxides are found in plants is not lacking. It is thought by some workers that aldehydes, such as benzaldehyde, may unite directly with oxygen yielding a peroxide:



and that this is capable of uniting with another molecule of benzaldehyde forming benzoic acid:



If instead of benzaldehyde, another compound, capable of becoming oxidized, was present, the benzyl-hydrogen peroxide would likewise have acted as an " oxygen-donator " and would have catalyzed the oxidation reaction.

From what has already been stated, it is clear that practically any oxidation can be written in terms of any mechanism by making simple

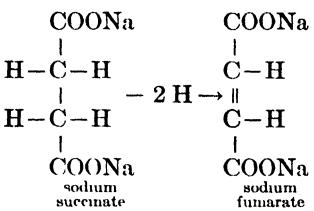
assumptions. The " hydrogen-transfer " theory of Wieland is one of the most popular theories at the present time, although it is clearly understood that other theories could explain the same thing. Moreover, as we shall see later, Warburg postulates the activation of oxygen rather than the activation of hydrogen. It is altogether possible that we should consider the activation of both hydrogen and oxygen.

THE RESPIRATORY ENZYMES OF PLANTS

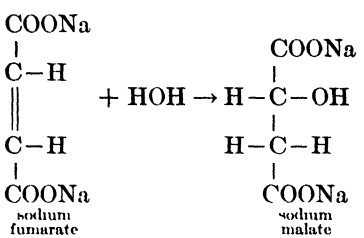
Dehydrases

Enzymes which appear to have the power of activating organic substances through the removal of hydrogen, rather than through the activation of atmospheric oxygen, are known as *dehydrases*. These are widely distributed throughout the vegetable and animal kingdoms. A few of these will now be mentioned with reference to the respiration processes of plant and animal organisms.

Succinoxidase. — This enzyme may catalyze the oxidation of succinic acid or its salts:

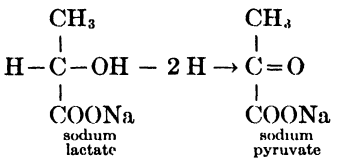


Associated with this enzyme is another known as *fumarase* which has the power of oxidizing fumaric acid or fumarates to malic acid or malates, compounds which are easily oxidizable:



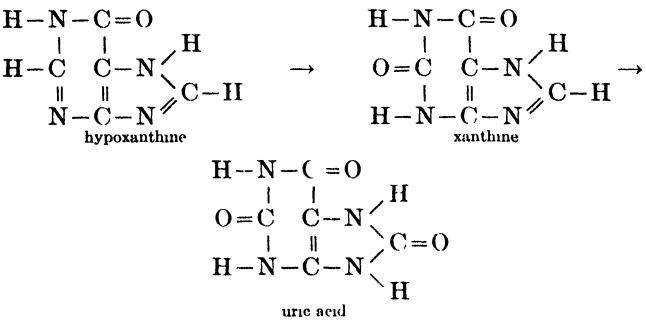
An extract containing succinoxidase will not necessarily reduce methylene blue to the colorless base or absorb oxygen. If sodium succinate is present, however, a rapid absorption of oxygen or a reduction of methylene blue is found to take place.

Lactic Oxidase. — In the presence of methylene blue or other easily reduced compounds, lactates are oxidized to pyruvates by this enzyme:



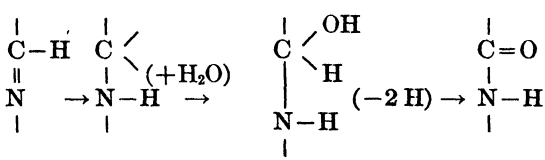
This enzyme appears to be more or less specific for the hydroxy acids. It is not able to use oxygen as an oxidizer, although it easily reduces methylene blue, and is more common to animals than to plants.

Xanthine Oxidase. — This enzyme oxidizes purine bases:



On the addition of hypoxanthine or xanthine to the enzyme extract, the system rapidly absorbs oxygen in amounts corresponding to the extent of oxidation. The system likewise reduces methylene blue, corresponding to the amount of purine added.

The net result of the above reactions is a gain in oxygen atoms; therefore, according to Wieland, we must assume the formation of a hydrate. According to Dixon, however, this is not necessary. It may be that the activation itself enables the molecule to become hydrated, the change being represented by the first two steps in the following equations:



In the oxidation of purines, activation by xanthine oxidase is sufficient to render them readily oxidizable by molecular oxygen and no special

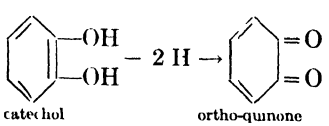
oxygen-activating catalyst is necessary for the reaction. In the case of the lactic oxidase, and others, this is not true. Here the activated molecules are readily oxidized by methylene blue, but they are quite unable to react with molecular oxygen The addition of methylene blue will enable the system to react with oxygen, because this substance acts as a " carrier " between the activated molecules and the oxygen.

Peroxidases

The extracts of many plants are known to give what is called the " guaiacum reaction." Guaiacum contains *guaiaconic acid*, which on oxidation yields a blue pigment which serves as a test for the activation of the oxidizer rather than as a test for the activation of the reducer, as in the case of methylene blue.

As a result of extended studies in this particular field of research, Bach and Chodat came to the conclusion that the blueing of guaiacum was due to the cooperation of two distinct enzymes, namely, a *peroxidase*, and a peroxide-forming enzyme which they termed *oxygenase*. The latter was supposed to combine with molecular oxygen forming an organic peroxide which was activated by the peroxidase to bring about the oxidation. Later, Onslow was able to show that the oxygenase of plants could be resolved into two factors: an enzyme, which she in turn termed *oxygenase*; and an auto-oxidizable substance which appeared to be catechol or one of its derivatives. She found that, on the removal of this compound, the enzyme was no longer able to produce peroxide-like substances in the presence of oxygen, but on the addition of catechol this became rapidly oxidized by oxygen with the production of such substances. It is apparent, therefore, that the action of this enzyme is to bring about the oxidation of catechol by atmospheric oxygen. According to Dixon, the term used to designate this enzyme should be " catechol oxidase " rather than " oxygenase."

The most probable oxidation product of catechol under these conditions is ortho-quinone:



catechol ortho-quinone

In this reaction it must be recognized that it is entirely possible for hydrogen peroxide to be formed, as well as the quinone:

$$C_6H_4(OH)_2 + O_2 = C_6H_4O_2 + H_2O_2$$

The evidence available at the present time shows that hydrogen peroxide is formed and that the activation of this substance by peroxidase must not be ignored. It likewise shows that the greater part of the oxidation is due to the quinone without the action of peroxidase functioning as a hydrogen acceptor.

The catechol-oxidase system appears, at first sight, to have very little in common with the dehydrase system which has just been discussed. As a matter of fact, they are closely related. In dealing with the xanthine oxidase, with methylene blue and xanthine, the reaction may be given as follows:

$$E + X + HOH + MB \rightarrow E + XO + MBH_2$$

and the reaction with oxygen may be written as follows:

$$E + X + HOH + O_2 \rightarrow E + XO + H_2O_2$$

(E = enzyme, X = xanthine, XO = uric acid)

Hydrogen adsorbed on an active surface, such as colloidal palladium, may be regarded as active hydrogen *par excellence*. The same may be said of nascent hydrogen. In those cases where the reaction takes place at the metal surface, the possibility that the surface may have an activating effect on the oxygen cannot, however, be ignored. In like manner it may be possible for colloidal organic materials to activate hydrogen on their surfaces, but the information available tends to indicate that active hydrogen is combined in the molecule rather than adsorbed physically on the surface. Such molecules are usually referred to as being "auto-oxidizable," and the production of hydrogen peroxide, during the autoxidation of organic substances, appears to be of quite general occurrence.

The catechol oxidase is but a member of a larger class of enzymes found in the vegetable kingdom, which include laccases, tyrosinase, polyphenolases, and similar enzymes. These are not well known, owing to the fact that they have not been sufficiently separated. As a matter of fact, it is not possible at the present time to determine whether the tyrosinase system represents a single enzyme or group of enzymes. The tyrosinase system seems to be connected with pigment formation from tyrosine. Like the peroxidases, but unlike the dehydrases, these enzymes are quite sensitive to cyanide The addition of a very small amount of cyanide is sufficient to inhibit their activity completely.

Hydrogen peroxide is quite toxic to enzymes when it is present in relatively large concentrations. If, therefore, peroxide-producing systems are common in living cells, it is apparent that some control mechanism must be present to prevent large accumulations of this substance.

This appears to be the true function of the enzyme *catalase*, which decomposes hydrogen peroxide into molecular oxygen:

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

This action is quite unlike the action of peroxidase or hydrogen peroxide. In the latter case, the decomposition gives rise to nascent oxygen:

$$H_2O_2 = H_2O + O$$

The action of catalase is inhibited by the presence of cyanide.

Iron as a Respiratory Catalyst

A theory of cell respiration that has been given considerable prominence in recent years is that which has been advocated by Warburg and which assumes that iron is the catalyst of respiration. According to Warburg, the activation of oxygen by iron is the essential process, and the activation of organic molecules is therefore of secondary importance. Warburg states that the free ions of iron are inactive from the standpoint of respiratory activity and that the active iron is present in the form of a definite complex compound, which he designates by the term " respiratory enzyme " and which appears to be related to the hematin series of pigments. Oxygen is supposed to combine with this " respiratory enzyme " as follows:

$$X \cdot Fe + O_2 \rightleftarrows X \cdot FeO_2$$

where $X \cdot Fe$ represents the " respiratory enzyme " The oxidation of the organic substances then follows:

$$X \cdot FeO_2 + 2\,A \rightarrow X \cdot Fe + 2\,AO$$

In support of his views Warburg calls attention to the fact that those substances which inhibit most reactions which are catalyzed by iron strongly inhibit the respiration process.

Attention was called by Warburg to the fact that a suspension of blood charcoal in water was capable of oxidizing many substances, such as organic acids, by means of atmospheric oxygen. That is to say, a suspension of blood charcoal, in a solution of oxalic acid, " respires "

Blood charcoal contains iron, and this was supposed by Warburg to be the active catalyst in the reaction, since the action of those narcotics so detrimental to normal cell respiration were likewise detrimental to this system. To prove his point, Warburg prepared pure carbon from sugar which was found to be inactive as a catalyst of respiration, but if iron was added, an increase in activity was noted, which was further increased if nitrogen was present. Warburg concluded, therefore, that

the respiratory activity was not due merely to the charcoal surface itself but rather to *iron-nitrogen-carbon* complexes in the surface, and that HCN acted by poisoning these active centers by combining with the iron atoms.

The possibility of HCN poisoning an active surface, and thus interfering with oxygen uptake by living cells, is granted, but there is no ground for believing that the whole process of respiration is due to this form of iron alone, since examples may be cited where HCN does not always interfere with respiration. For example, Emerson found that Chlorella is unaffected by HCN and only becomes cyanide-sensitive when supplied with liberal quantities of glucose.

The Glutathione System

Glutathione, a compound isolated by Hopkins, is found to be widely distributed throughout both the vegetable and animal kingdoms. It is found to be a tri-peptide consisting of cysteine, glutaminic acid and glycine. As it appears that the sulfur group is the active constituent; its formula for convenience may be written RSH.

On oxidation, glutathione is changed to a disulfide form:

$$2\,RSH + O_2 \rightarrow R \cdot S \cdot S \cdot R + H_2O_2$$

RSH and R · S · S · R are referred to as *reduced* and *oxidized* glutathione, respectively.

Glutathione appears to be present in cells, mainly in the reduced condition, on account of the fact that the oxidized form is rapidly undergoing reduction at the same time. As a matter of fact, if oxidized glutathione is added to tissues, it becomes reduced rapidly. It therefore appears to be autoxidizable, but such is not the case. It is oxidizable only if a trace of iron in the proper form is present In addition to iron salts, and especially iron in the form of hematin, copper salts are also effective in this respect. Moreover, it seems to be apparent that the presence of iron or copper alone is not sufficient to make glutathione autoxidizable, but the presence of an unknown substance, according to Dixon, is also necessary.

The Cytochrome System

According to Keilin, a pigment exists in practically all living cells which may act as an oxidizing catalyst and which he termed "cytochrome." This pigment, like glutathione, can exist in an oxidized or reduced form. Reduced cytochrome, however, is not a single substance but is composed of three very closely related compounds, rather complex

in character and resembling hemochromogens in some ways and differing from them in others.

Oxidized cytochrome becomes reduced rapidly in cells, and the reduced cytochrome is rapidly oxidized in the cell in the presence of oxygen. In other words, cytochrome, like glutathione, may be referred to as a carrier of oxygen or as an " oxygenase " and, therefore, it may be expected to play an important part in tissue respiration.

Keilin refers to the three compounds of reduced cytochrome as *a*, *b*, and *c* and finds that if a small amount of *M*/10,000 HCN is added, to a well-aerated solution of these substances, *a* and *c* do not become oxidized. On the other hand, *b* may readily oxidize under these conditions. Keilin found that, if oxidized cytochrome is used, the addition of cyanide would not interfere with its reduction, since cyanides do not act on the reducing substances of cells.

All factors which inhibit the action of dehydrases, such as narcotics, likewise inhibit the reduction of cytochrome. On the other hand, all factors which inhibit the action of catechol oxidase, such as cyanide, likewise inhibit the oxidation of cytochrome *a* and *c*. It is therefore evident that cyanide and narcotics act in totally different manners — a conclusion of importance in relation to Warburg's work on inhibitors, which has been mentioned.

From what has already been stated, it is evident that, in the process of respiration, the various respiratory materials are activated by a series of enzymes — the dehydrases. One type of dehydrase, represented by the xanthine oxidase, is capable of oxidizing its substrate by direct reaction with molecular oxygen, and for this reason it is sometimes referred to as an " aerobic oxidase " Another type, represented by lactic oxidase, is not able to oxidize its substrate by direct reaction with molecular oxygen; and for this reason it is sometimes referred to as an " anaerobic oxidase."

The aerobic oxidases, in oxidizing their substrates, reduce molecular oxygen to hydrogen peroxide, which may later be activated by peroxidases or certain iron-bearing compounds and oxidize directly a number of organic compounds. Anaerobic oxidases may oxidize their substrates by reducing cytochrome, which may later become oxidized by catechol oxidases with uptake of oxygen. These systems play important rôles in respiration, but it is likewise apparent that other systems may contribute to this process as well, which adds to the complexity of this study.

RESPIRATORY MATERIALS

Carbohydrates serve as the respiratory materials of plants. In seed plants all living cells are normally provided with a supply of sugars and other carbohydrate materials such as hemicelluloses, starch, and other polysaccharides. These readily break down into monosaccharides which undergo oxidation in the protoplasm.

Fermentable sugars represent the usual kind of material used in respiration, but there may be a respiration at the expense of proteins of the protoplasm, a type found in starving cells. This occurs, however, only where there is a deficiency of sugars, as in etiolated leaves.

The Formation of Organic Acids During Respiration

Organic acids are normal products in the respiration process of plants and are found in all tissues. Among the acids commonly found are formic, oxalic, succinic, citric and malic. Formic acid is found to accumulate in nettle leaves and in those plant parts where there is an intense metabolism. Oxalic acid is found in relatively large quantities in plants like the rhubarb and sorrel.

Increasing the sugar content of a living plant invariably results in an increase in the organic acid content as well. According to Mayer, certain of the organic acids may be considered as final products of respiration. Other authorities, however, do not incline to this view. It has long been known that the malic acid content of a plant tissue is subject to wide variation, an accumulation usually taking place at night which is followed by a decrease during the day. It is thought that malic acid tends to undergo oxidation in the presence of sunlight but not in its absence, and therefore should not be looked upon as a final product of respiration.

The temperature of the environment has considerable to do with the accumulation of organic acids within the plant. According to Harvey, a temperature of 15° C. to 40° C. favors complete oxidation of carbohydrates to CO_2 and H_2O, if oxygen is not a limiting factor. At low temperatures, however, even if oxygen is present in relatively large quantities, there may be an accumulation of organic acids, which in this case represents incomplete combustion.

The respiration of young apples, according to Harvey, is extremely intense, but the rate of respiration falls off markedly as the apple matures. During active respiration the malic acid content is relatively high, but as respiration is decreased there is likewise a decrease in the malic acid content. The falling off of the respiratory rate is thought to be due largely to the exhaustion of malic acid in respiration.

A restricted oxygen supply, which may be induced by a thick impervious cuticle, may likewise lead to an accumulation of organic acids during respiration. In the Cactaceae, according to Harvey, malic is the principal acid formed, whereas in the Mesembryanthemeae oxalic acid predominates. This type of respiration may lead to a liberation of a considerable portion of the energy of the carbohydrates without the production of CO_2. A restricted oxygen supply not only leads to the production of organic acids but alcohols as well, which form esters, the presence of which is so apparent in ripe fruits. The production of organic acids may relieve the "flat" taste of fruits having little acidity.

THE RESPIRATORY RATIO

The amount of oxygen absorbed in plant respiration is usually closely correlated with the quantity of CO_2 produced, and their ratio, usually termed the *respiratory ratio*, may be used as a basis for respiration studies

When the carbohydrate undergoing respiration is a monosaccharide, such as glucose, the volume of CO_2 equals the volume of oxygen absorbed, hence the ratio is unity:

$$C_6H_{12}O_6 + 6\,O_2 = 6\,O_2 + 6\,H_2O$$

If, on the other hand, a fat is the main substance utilized in respiration, the value is less than unity:

$$C_3H_5(C_{17}H_{33} \cdot COO)_3 + 80\,O_2 = 57\,CO_2 + 52\,H_2O$$

Where organic acids constitute the primary source of respiration materials the ratio is greater than unity:

$$2\,H_2C_2O_4 + O_2 = 4\,CO_2 + 2\,H_2O$$

When the oxidation of glucose to oxalic acid or other organic acids takes place, the respiratory ratio may be quite low. There is a high rate of O_2 absorption but the oxidation does not proceed as far as gaseous CO_2, at least for the time being.

The respiratory ratio is, therefore, a measure of the kind of materials consumed rather than the intensity of respiration. In order to show the intensity of respiration, Kidd, West and Briggs employ what they term a "respiratory index" which involves the quantity of CO_2 produced in unit time per gram of plant material estimated on the dry basis at 10° C., when the respiratory material and atmospheric oxygen are not limiting factors.

FACTORS AFFECTING THE PROCESS OF PLANT RESPIRATION

A number of factors are found to influence the nature and extent of plant respiration, a few of which will be mentioned:

Water. — Since water is necessary for oxidation and since the general activity of the cell is closely correlated with the quantity of water it contains, it is quite evident that any condition involving a gain or loss in water content must be of considerable importance from the standpoint of tissue respiration

Different investigators have found that an increase in cell turgescence above the normal is usually followed by increased respiratory activity. On the other hand, a slight decrease is likewise accompanied by an increase in the rate of respiration. This may be attributed, in part, to a more rapid removal of by-products of respiration when the cells are turgid and to an increase in the concentration of respiratory materials when water is lost. Eventually, a lowering of the water content of the cells is followed by a decrease in the rate of respiration. Some plant parts, however, are found to respire under conditions of extreme desiccation, but at a low rate. This is particularly true of seeds.

A slight increase in moisture content results in a profound increase in the rate of seed respiration. In this connection, Bailey and his coworkers found that the spontaneous heating of stored grain was due indirectly to the moisture content. Gradually increasing the moisture content to 14 5 per cent resulted in a gradual increase in the rate of respiration. Above this figure, however, a profound increase was noted in the respiratory activity. This was attributed to the fact that the viscosity of the gluten was lowered, which resulted in an increase of total surface for enzyme action and the absorption of atmospheric oxygen, and likewise a better contact with the embryo, the seat of respiratory activity.

Temperature. — Some plants which are low in moisture content are able to respire at exceedingly low temperatures. Mosses, lichens and other plants or seeds are found to retain their vitality on exposure to a temperature of $-250°$ C. Since respiration is necessary for life, it is evident that this process must continue, even under such environmental conditions. In general terms, however, an increase in temperature results in an increase in the respiratory activity until death takes place.

An increased temperature must be in operation for some time before the stimulation of the respiratory process is noticeable; a sharp variation in temperature is without effect, and a change in the respiration intensity after transference from one temperature to another is

gradual. So far as is known, there is no optimum temperature for respiration.

In the experiments of Bailey and his co-workers previously mentioned, it was found that the heat liberated, in the respiration process of the stored grains, accumulated, and when a temperature was obtained at 55° there was a stimulated diastatic activity which had the effect of rendering more materials available for respiration. Such a change may result in a permanent increase in respiration, which has so often been reported where plants have been maintained under conditions of high temperature for an appreciable period of time

Light. — Plants respire in the presence and absence of light. In darkness, however, there is a gradual falling off in the rate of respiration. Light has a tendency to ionize the oxygen of the air, rendering it more efficient for oxidation purposes, but the main effect of light is due to its influence on the manufacture of respiratory materials. Too intense an illumination, however, may tend to decrease the rate of respiration, as it may seriously interfere with chlorophyll production and hence interfere with photosynthesis. It may likewise interfere with normal enzyme action as enzymes are destroyed under these conditions According to Kastle and Armstrong, the tissues of a normally illuminated plant have less peroxidase than one kept in darkness.

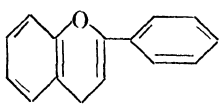
Acidity. — The respiratory rate is increased by an increase in alkalinity. For this reason, subjecting some plants to a relatively high concentration of ammonia gas may result in an increased production of CO_2. According to Haas and Hill, *Nitrosomonas* shows the greatest rate of respiration in a medium in which the *p*H value is between 8 4 and 8 8. In *Penicillium chrysogenum*, variations in the *p*H values between 4 and 8 do not affect the normal rate of this process to any appreciable extent.

Oxygen. — For normal respiration free oxygen is necessary, although it is possible for respiration to proceed to a limited extent in its absence. Ordinarily, the oxygen content of the atmosphere more than suffices for respiration and the concentration may be lowered materially without effect. In some instances, however, plants cannot absorb this element very readily and therefore the atmospheric concentration may be of importance.

Plants may suffer from a lack of oxygen because of a high concentration of CO_2 in the tissues. For example, the interior of a pumpkin, according to Kostychev, has been found to have an atmosphere containing 2.52 per cent CO_2 and 18.29 per cent O_2. Under these conditions, respiration would be considerably less than if this atmosphere were more nearly the composition of the ordinary atmosphere. On the other hand, plants cannot thrive in an atmosphere of pure oxygen.

Carbon Dioxide. — Green plants are found to respire under conditions where the carbon dioxide concentration of the atmosphere is increased above normal. Where the concentration of CO_2 of the air exceeds 25 per cent, however, the rate of respiration is materially lowered. In the case of germinating seeds, a CO_2 content slightly above normal may seriously interfere with this process.

Anthocyanin Pigments. — These red, blue and violet pigments are present in most plants. It is to the anthocyanins that we owe the brilliant colors of our flowers, our foliage plants and our autumn landscapes. These compounds contain the *anthocyan nucleus*:

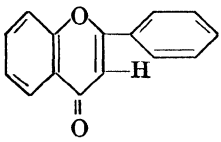


Anthocyanins are glucosides. If hydrolyzed, a derivative of anthocyan called an anthocyanidin is formed in addition to glucose. Anthocyanidins, according to Willstatter, are purple in color. On combination with potassium a blue salt is formed. If treated with a mineral acid, a red oxonium compound is formed.

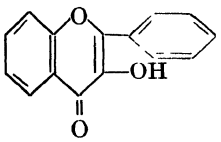
These pigments are produced to a marked extent in those tissues where organic compounds are present in large quantities. Any factor which leads to an accumulation of carbohydrates, such as relatively low temperatures following intense illumination, leads to the synthesis of these compounds. Where chlorophyll is present and the tissues are active photosynthetically, these pigments are never present to any appreciable extent. Some plants produce anthocyanin pigments in the dark, but such cases are rather exceptional. Grape pigments may be found in the absence of light, but the red color of apples does not form except in the presence of light.

Anthocyanin pigments are thought to shield the chloroplasts and diastatic enzymes from too high a degree of light intensity. Moreover, it has been proved that these pigments have the power of converting light rays into heat rays, thereby raising the temperature of their immediate environment. The main function of anthocyanins in plant metabolism, as is believed by a great number of investigators, is that they aid in the respiration process. They are easily reduced and oxidized, and are thought to act as oxygen carriers, assisting in cell oxidations and reductions.

Flavone and Flavonol Pigments. — The pigments which are yellow in color are widely distributed throughout the vegetable kingdom. They are derived from the two compounds, *flavone* and *flavonol*:



Flavone



Flavonol

This group of pigments, like the anthocyanins, occurs in plants as glucosides, one or more of the hydroxyl groups, of which there may be several, being combined with a sugar molecule. Such compounds are colorless, or practically so, but when hydrolyzed the yellow color appears. Almost any white flower, according to Gortner, turns yellow when exposed to the vapors of ammonia, owing, in part, to hydrolysis, and in part to salt formation of ammonia with the colored compounds.

The flavone and flavonol pigments are quite closely related to the anthocyanin pigments from a chemical standpoint. Like the anthocyanins, they are easily oxidized and reduced and are thought to act as oxygen carriers in the process of plant respiration.

Factors influencing the respiration of animals will be discussed in Chapter XXV.

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PART III. THE ANIMAL

CHAPTER XXI

FOODS AND FEEDING STUFFS

The animal body must receive, in the food, all the nutrients necessary for the construction and repair of metabolizing tissues, and, in addition, sufficient oxidizable substances to furnish the various forms of energy required by the body. Although the animal body is often compared with an internal-combustion engine or similar machine, there is at least one essential difference. When fuel is burned in an internal-combustion engine, heat and mechanical energy are produced from the chemical energy of the fuel. When food is eaten by the animal the same phenomenon occurs but with this essential difference, i.e., that the food also contributes to the manufacture of new tissues and to the repair of old ones, whereas fuel cannot contribute to new cylinders and pistons in the engine. The engine, therefore, wears out and deteriorates; the living machine is continuously being remade with new materials.

Foods may be defined in a number of ways, depending upon the viewpoint of the person creating the definition. For our purpose, we may consider any substance a food if it is capable of repairing metabolizing tissues. A *complete food* might be defined as one that will contribute to every need of the body when this food is the sole article of diet for an appreciable period of time It is absolutely impossible to find a single foodstuff which will meet the exacting requirements of a healthy body Perhaps milk most nearly approaches this ideal, but we shall find later that milk may be lacking in a number of essential factors.

Most of the foodstuffs that we appreciate and enjoy are woefully deficient in many respects. It is for this reason that man has found by experience that dietary diversity encourages health and growth, whereas the reverse is true if " one-sided " diets are ingested for appreciable periods of time. We shall learn, in Chapter XXVI, the scientific reasons for the superiority of diversified diets. In this chapter we shall devote our attention, primarily, to the chemical composition of some of our most important foodstuffs. In a later chapter we shall consider the fate of foods in metabolism, with reference, particularly, to the biological value of the various ingredients.

Water is one of the most important components of the diet, and all living organisms must ingest relatively large amounts of it daily. This can be accomplished by the ingestion of water itself or by obtaining it in milk, vegetables, fruits, and similar foods. Water aids in digestion, and the work of Hawk and his co-workers has shown that the drinking of relatively large amounts of water with meals is highly desirable. These researches show that the foods are not only more completely digested when water is taken at meal time, but that the hydrolytic products of digestion are utilized more completely by the body.

Not all waters are of the same biological value, however, on account of the variation in their chemical composition. Some waters are very rich in inorganic salts, which will vary in different localities In some of the western states, for example, the waters are laden with salts of various kinds, most of which possess definite physiological effects. Some waters are rich in the sulfates of sodium and magnesium and, on that account, are markedly laxative Some waters are extremely " hard," possessing relatively large quantities of calcium and magnesium. These waters, as a rule, are deficient in iodine, and in such regions goiter is often quite prevalent. Similar conditions exist in those regions which depend upon the very " soft " snow-waters of the mountains. Such waters are practically free from inorganic salts.

Although the fame of " water cures " at various mineral springs is well known, it is doubtful if the waters possess the magical properties attributed to them It is true, of course, that the laxative effects are beneficial, cleansing the system of toxins produced by constipation. Most authorities agree, however, that much of the benefit obtained is derived from the correct daily routine of the diet, moderate exercise, and regular hours of rest required of all patients at such sanitariums. It is true undoubtedly that most people do not drink as much water as they should.

In general, the diet of the average human adult should consist of 60 to 150 grams of protein per day and, in addition, sufficient fat and carbohydrate to take care of the energy requirements of the individual. The latter will depend upon the size and weight of the person concerned as well as upon the amount of physical work done. In addition, the food should contain sufficient mineral salts and vitamins to keep the body functioning normally. According to Sherman, man should obtain daily in his food about 0.9 gram of phosphorus and 0.6 gram of calcium. Although all mineral elements are important, the last-mentioned elements seem to be most liable to be lacking in most ordinary diets.

The following table, taken from Bulletin 28 of the Office of Experiment Stations, U. S. Department of Agriculture, gives the composition of some common food materials.

TABLE 52

Approximate Chemical Composition of Some Typical Foods

Foods	Refuse	Water	Protein N×6 25	Fat	Total carbohydrates	Ash	Fuel value per lb., cal.
Beef							
Chuck, including shoulder Medium fat As purchased Average	15 2	57 9	16 6	10 1		0 8	735
Flank All analyses Average As purchased	5 5	56 1	18 6	19 9		0 8	1185
Round. Fat As purchased	12 0	54 0	17 5	16 1		0 8	1005
Veal Fresh							
Leg, lean As purchased	9 1	66 8	19 4	3 7		1 1	520
Leg Cutlets As purchased	3 4	68 3	20 1	7 5		1 0	690
Mutton Fresh							
Leg Medium fat As purchased	18 4	51 2	15 1	14 7	. .	0 8	900
Loin, without kidney or tallow As purchased	14 8	40 4	13 1	31 5		0 6	1575
Pork Fresh							
Ham Medium fat As purchased	10 7	48 0	13 5	25 9		0 8	1345
Ham Smoked	12 2	35 8	14 5	33 2		4 2	1670
Salt pork, clear fat		7 9	1 9	86 2		3 9	3670
Bacon, smoked	8 7	10 4	9 5	59 4		4 5	2685
Pork sausage		39 8	13 0	44 2	1 1	2 2	2125
Chickens Broilers As purchased	41 6	43 7	12 5	1 4		0 7	295
Mackerel Edible portion		73 4	18 7	7 1		1 2	645
Eggs Uncooked Edible portion		73 7	14 8	10 5	0 3	1 0	720
Eggs Uncooked As purchased	11 2	65 5	13 9	9 3		0 9	635
Eggs Boiled whites		86 2	13 0	0 2	0 5	0 6	250
Eggs Boiled yolks		49 5	16 1	33 3		1 1	1705
Butter As purchased		11 0	1 0	85 0		3 0	3605
Buttermilk As purchased		91 0	3 0	0 5	4 8	0 7	165
Cheese American Pale		31 6	28 8	35 9	0 3	3 4	2055
Milk Whole As purchased		87 0	3 3	4 0	5 0	0 7	325
Milk Evaporated Unsweetened		68 2	9 6	9 3	11 2	1 7	780
Milk Condensed Sweetened		26 9	8 8	8 3	54 1	1 9	1520
Oleomargarine		9 5	1 2	83 0		6 3	3525
Vegetable Foods							
Corn meal Granular		12 5	9 2	1 9	75 4	1 0	1655
Oatmeal		7 3	16 1	7 2	67 5	1 9	1860
Wheat flour Entire wheat		11 4	13 8	1 9	71 9	1 0	1675
Wheat flour Patent roller. Family grade Spring wheat		11 9	10 9	1 1	75 6	0 5	1655
Wheat flour Winter wheat		13 1	12 3	1 1	73 0	0 5	1635
Wheat Breakfast Foods							
Farina	.	10 9	11 0	1 4	76 3	0 4	1685
Shredded	.	8 1	10 5	1 4	77 9	2 1	1700
Wheat bread As purchased Home made		35 0	9 1	1 6	53 3	1 0	1225
Wheat bread Whole wheat		38 4	8 7	0 9	49 7	1 3	1140
Crackers Oyster crackers		4 8	11 3	10 5	70 5	2 9	1965
Honey		18 2	0 4		81 2	0 2	1520

TABLE 52 — *Continued*

APPROXIMATE CHEMICAL COMPOSITION OF SOME TYPICAL FOODS

Foods	Refuse	Water	Protein N×6 25	Fat	Total carbohydrates	Ash	Fuel value per lb., cal
Vegetables							
Asparagus Fresh		94 0	1 8	0 2	3 3	0 7	105
Beans, butter, green Edible portion		58 9	9 4	0 6	29 1	2 0	740
Beets Cooked		88 6	2 3	0 1	7 4	1 6	185
Cabbage Edible portion Fresh		91 5	1 6	0 3	5 6	1 0	145
Carrots Cooked		3 5	7 7	3 6	80 3	4 9	1790
Corn Green Edible portion		75 4	3 1	1 1	19 7	0 7	470
Cucumbers Edible portion		95 4	0 8	0 2	3 1	0 5	80
Peas, green Cooked Edible portion		74 6	7 0	0 5	16 9	1 0	465
Potatoes Cooked Boiled		75 5	2 5	0 1	20 9	1 0	440
Potatoes Cooked Mashed and creamed		75 1	2 6	3 0	17 8	1 5	505
Potatoes Sweet Raw Edible portion		69 0	1 8	0 7	27 4	1 1	570
Squash Edible portion		88 3	1 4	0 5	9 0	0 8	215
Tomatoes Fresh		94 3	0 9	0 4	3 9	0 5	105
Fruits							
Apples Edible portion		84 6	0 4	0 5	14 2	0 3	290
Bananas Edible portion		75 3	1 3	0 6	22 0	0 8	460
Oranges Edible portion		86 9	0 8	0 2	11 6	0 5	240
Pears Edible portion		84 4	0 6	0 5	14 1	0 4	295
Nuts							
Almonds Edible portion		4 8	21 0	54 9	17 3	2 0	3030
Hickory nuts		3 7	15 4	67 4	11 4	2 1	3345
Peanuts		9 2	25 8	38 6	24 4	2 0	2560

The Composition of Milk

Space will not permit a detailed discussion of foods in general, but it is necessary that we consider some of the outstanding characteristics of milk since it is the most complete food known to science. Milk is the normal secretion of the mammary gland and, although there are many kinds of milk, all references, unless otherwise indicated, will refer to cow's milk:

TABLE 53

COMPOSITION OF MILK FROM DIFFERENT SPECIES

	Water, per cent	Casein, per cent	Albumin, per cent	Fat, per cent	Lactose, per cent	Ash, per cent
Human	87 41	0 91	1 23	3 76	6 29	0 31
Cow	87 27	2 95	0 52	3 66	4 91	0 69
Goat .	84 14	3 04	0 99	6 00	5 02	0 81
Sheep .	81 90	4 57	1 26	6 52	4 82	0 93
Buffalo	82 14	4 29	0 49	7 44	4 81	0 83
Camel	87 04	3 49	0 40	2 76	5 57	0 74
Horse	90 68	1 27	0 75	1 17	5 77	0 36
Ass	89 88	0 73	1 31	1 50	6 09	0 49
Reindeer	68 20	8 40	2 00	17 10	2 08	1 50

Cow's milk varies in composition, particularly in fat, depending on the breed of cow from which the milk is obtained Other factors, such as inheritance, individuality, time of milking and period of lactation, also influence the composition of milk.

TABLE 54

EFFECT OF BREED ON MILK COMPOSITION

Breed	Water, per cent	Protein, per cent	Fat, per cent	Lactose, per cent	Ash, per cent	Total solids, per cent
Jersey	85 27	3 80	5 14	5 04	0 75	14 75
Guernsey	85 45	3 84	4 98	4 98	0 75	14 55
Ayrshire	87 10	3 34	3 85	5 02	0 69	12 90
Shorthorn	87 43	3 32	3 63	4 89	0 73	12 57
Holstein	88 01	3 15	3 45	4 65	0 68	11 93

Although it is possible to affect the amount of milk produced by underfeeding and overfeeding, it is not possible to produce marked changes in

the chemical composition of milk by changes in feeding practice. It is possible, however, within narrow limits, to change the nature of some of the ingredients. For example, the feeding of certain feeds tends to produce butterfat which varies in its melting point, but efforts to increase the percentage of butterfat by feeding methods have not met with success.

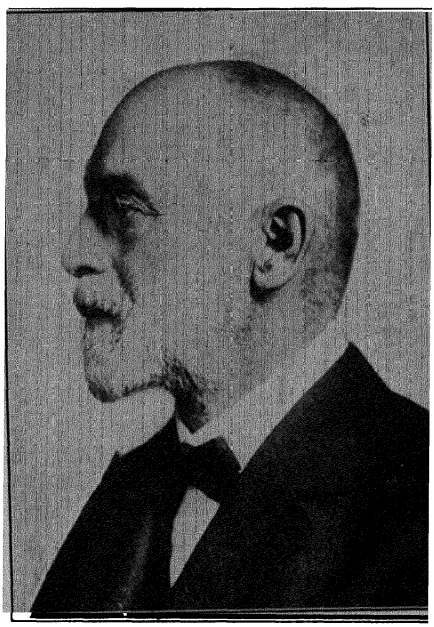

FIG. 44. Dr. S. M. Babcock (1843–1931), famous as the inventor of the Babcock test for fat in milk. He is equally well known for his researches in dairy chemistry and animal nutrition.

Mineral Salts. — One of the outstanding characteristics of milk is the fact that it contains mineral salts in approximately the same proportions as they exist in the body of a new-born animal. Milk ash contains potassium, calcium, sodium, magnesium, iron, phosphorus and chlorine as well as traces of copper, zinc, silicon, aluminum, manganese and iodine. Citric acid and carbonic acid are important acids, combining with bases to form salts. Other forms in which salts are thought to exist in milk are chlorides and phosphates.

The following tables, by Van Slyke and Bosworth and by Söldner, are given to show the reader that authorities are not agreed as to the exact forms in which the salts of milk may exist:

TABLE 55

MILK SALTS (ACCORDING TO VAN SLYKE AND BOSWORTH)

	Of milk, per cent	Of total salt combinations, per cent
Protein combined with calcium	3 200	78 02
Dicalcium phosphate, $CaHPO_4$	0 175	4 27
Calcium chloride, $CaCl_2$	0 119	2 90
Monomagnesium phosphate, $MgH_4P_2O_8$.	0 103	2 51
Trisodium citrate, $Na_3C_6H_5O_7$	0 222	5 41
Tripotassium citrate, $K_3C_6H_5O_7$	0 052	1 26
Dipotassium phosphate, K_2HPO_4	0 230	5 61

TABLE 56

MILK SALTS (ACCORDING TO SOLDNER)

	Of total salt combinations, per cent
Protein combined with calcium	5 13
Tricalcium citrate, $Ca_3(C_6H_5O_7)_2$	23 55
Tricalcium phosphate, $Ca_3(PO_4)_2$	8 90
Dicalcium phosphate, $CaHPO_4$	7 42
Trimagnesium citrate, $Mg_3(C_6H_5O_7)_2$ . .	4 05
Dimagnesium phosphate, $MgHPO_4$.	3 71
Tripotassium citrate, $K_3C_6H_5O_7$	5 47
Dipotassium phosphate, K_2HPO_4	9 22
Monopotassium phosphate, KH_2PO_4	12 77
Potassium chloride, KCl .	9 16
Sodium chloride, NaCl	10 62

It would appear that the chemical constituents of milk are in complex equilibrium, and much is yet to be learned regarding the forms and combinations in which they exist. It is thought by many authorities that potassium, sodium, chlorine and lactose are entirely in solution whereas the casein and the fat are present entirely as emulsoids and colloidal sols. Some writers are of the opinion that calcium exists in milk as calcium caseinate; others believe that calcium phosphate has the power to combine with this protein. Still others are of the belief that calcium caseinate and calcium phosphate may combine in chemical or physical combinations. When milk is heated, very small amounts of the salts,

including calcium and phosphorus, may be lost in a sludge or residue that settles out. There is little evidence that the soluble calcium and phosphorus salts are changed to insoluble salts by heat. It is possible that the coagulation of albumin by heat may trap salts mechanically and carry them out of solution. Lactalbumin, calcium, magnesium and phosphorus are found partly in true solution and partly in suspension.

Other Constituents. — Although casein, lactose, fat and mineral salts are present in the greatest quantity, in milk, a number of other normal

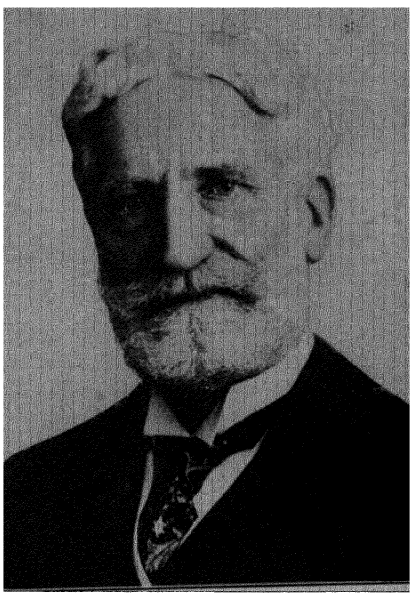

FIG. 45. Dr. L. L. Van Slyke (1859–1931), pioneer worker and eminent authority in the field of dairy chemistry.

constituents seem to be present in small amounts. For example, market milk always contains dissolved gases such as carbon dioxide, nitrogen and oxygen, which have been introduced subsequent to milking. These may be introduced by aeration or by the action of bacteria, although milk drawn from the udder contains about 10 per cent (by volume) of carbon dioxide which usually diminishes during subsequent handling and pasteurization. Van Slyke and co-workers are of the belief that carbon dioxide exists in milk as H_2CO_3 and $NaHCO_3$.

Lactalbumin is an important milk protein, although it rarely exceeds

0.05 per cent of the milk. In relation to other proteins, it constitutes about one-sixth of the total protein material present. Lactoglobulin is another protein that is present in very small amounts in normal market milk (about 0.05 per cent), but in colostral milk (colostrum) it may occur to the extent of 15 per cent. Recent research work seems to indicate that lactoglobulin is identical with the serum globulin of the blood, but so far as can be ascertained there is no marked similarity so far as lactalbumin and serum albumin of blood are concerned. Lecithin and cholesterol are also normal milk constituents, the former ranging from 0 03 to 0.05 per cent, whereas the latter fluctuates with the fat and may vary from 105 to 176 parts per million of raw milk. Other constituents are enzymes, of which protease, lipase, diastase, catalase, peroxidase, reductase and a lactose-fermenting enzyme are the most important.

Milk Products. — The following table shows the approximate chemical composition of some of the more important products made from milk.

TABLE 57

APPROXIMATE COMPOSITION OF SOME IMPORTANT MILK PRODUCTS

Product	Water	Protein	Fat	Lactose	Ash	Sucrose	Salt	Lactic acid	Curd
Powdered whole milk	2 00	26 91	28 65	36 50	5 94				
Evaporated whole milk	73 63	6 71	8 22	10 13	1 55				
Sweetened condensed milk	27 03	7 85	8 99	12 65	1 76	41 65			
Whole milk	87 30	3 55	3 62	4 82	71				
Light cream	72 46	2 95	20 00	4 00	59				
Heavy cream	63 41	2 58	30 00	3 50	52				
Whipping cream	54 35	2 21	40 00	3 00	44				
Skim milk	90 35	3 72	15	4 98	80				
Evaporated skim milk	71 05	11 16	45	14 94	2 40				
Powdered skim milk	3 89	35 42	1 71	48 74	8 08				
Butter	13 90		82 41				2 51		1 18
Buttermilk	91 61	3 30	50	3 40	65			50	
Condensed buttermilk	60-64	12-15	1-2	16-20	2 5-3 5			2-3	
Dried buttermilk	1 93	38 74	5 87	39 91	7 68			5 87	
Cheddar cheese	37 33	23 39	33 41		7 02				
Domestic Swiss cheese	30-34	26-30	30-34		3-5		1-14		

STOCK FEEDS

What has been said about foods in general, so far as their functions and utilization are concerned, will also apply to feeding stuffs used in livestock feeding. Owing to the nature and source, however, of feeding stuffs, they differ in chemical composition from the foods commonly used for human consumption. This is due, in the main, to the fact that domestic animals are capable of using as food many products which the human digestive tract could not tolerate.

For convenience of classification we shall consider feeding stuffs, very briefly, from the standpoint of source of material. Considering first the feeds that are obtained from plant sources, we have:

1. Seeds or cereals (concentrates).
2. Stem and leaf crops (fodders).
3. Vegetables (succulent feeds).

Seeds or Cereals. — In general, it may be said that seeds used for animal feeding can be divided roughly into three classes: (1) the cereal grains, (2) the oily seeds, and (3) leguminous seeds.

The Cereal Grains — From the standpoint of tonnage this group is probably of the greatest importance; it consists of such cereals as wheat, corn, barley, oats and similar seeds. In addition to these are to be considered the factory by-products such as brans, middlings, gluten feeds, hulls, etc., that are sold on the market as products to be used as ration ingredients. The chemical composition of typical cereals and cereal by-products is indicated in the table at the end of this chapter

In a general way, the cereal grains may be said to be characterized by a relatively high content of carbohydrates (nitrogen-free extract) which is largely starch Although they are not poor in protein, they cannot be considered protein-rich. The protein of cereals is not considered to be equal, in biological value, to proteins obtained from legumes or from animal products. As a rule, the cereals are not rich in fiber, fat or mineral salts. They are lacking particularly in calcium.

The Oil-containing Seeds. — Cottonseed and linseed are two important oil-containing seeds used in stock feeding. These feeds are by-products obtained in the manufacture of cottonseed and linseed oil. They are characterized by a relatively high content of protein and are important as concentrates in raising the protein content of a grain ration Cottonseed meal cannot be fed in excessive quantities owing to toxic effects known as "cottonseed poisoning" This toxic action was thought to be due to an alcohol known as "gossypol" At the time this chapter is being written (1931) Huffman and Moore of Michigan and Halverson and Sherwood of North Carolina have announced the results of several years' work on the feeding of cottonseed meal to dairy cattle in which they question the gossypol theory of "cottonseed meal poisoning." These investigators, working independently, have arrived at the conclusion that the injurious effect of cottonseed meal is due to the lack of dietary factors which can be furnished by a good grade of alfalfa hay or similar roughage. The North Carolina workers are of the opinion that the trouble is due to an insufficient supply of vitamin A and calcium. These workers were able to obtain typical symptoms of "cottonseed

meal poisoning" by feeding peanut meal, linseed meal or soybean meal with a mineral mixture and a poor grade of roughage, which confirms their conclusion that the condition is not due, primarily, to the presence of a toxic substance. This work does not discredit the existence or toxicity of gossypol, however. Further work is necessary before this question can be settled definitely. Peanut cake and sunflower cake are also products that may be classed in the group of feeds made from oil-rich seeds.

The Leguminous Seeds. — The seeds of the leguminous plants such as the soybean and cowpea are rich in protein, and their use is increasing every year, as their value becomes appreciated.

Stem and Leaf Crops or Fodder Crops. — In the fresh green state these feeds contain relatively large amounts of water and, consequently, small amounts of total solids or dry matter. After drying they are characterized by a relatively high amount of crude fiber and ash, but the amount of carbohydrates is relatively low. The protein will vary, depending on the type of plant under consideration. Dry alfalfa hay or other legume hays, for example, are quite rich in protein, whereas the grass hays are relatively poor in this regard.

Silage, most commonly made from corn, is another member of this group. It is a most important feed and varies in composition, depending on the maturity of the corn and on the presence of other crops, such as clover or soybeans, which are used, with corn, in silage manufacture.

Vegetables Used as Feeds. — In many parts of this country and Europe root crops are raised as feed for livestock. Most of these, such as the mangel, sugar beet, rutabagas and turnips, are root crops, but other types of vegetables, such as pumpkins, squash, cabbage and kale, are raised for this purpose. These feeds are valued for their succulent qualities. They are fed mainly for their vitamins, mineral salts and carbohydrates, although they do contribute to some degree to the protein intake.

Feeds from Animal Sources

Feeds of this type can be classed in two large groups: (1) those consisting of milk or milk by-products, and (2) those consisting of packing house by-products.

Milk and Milk By-products. — Whole milk, skim milk and buttermilk are important feeds for growing live stock. They are now obtainable also in evaporated and powdered form. Whey, as a by-product of cheese making, is also a feeding material but its use is confined mainly to hog-feeding.

Packing House By-products. — Tankage, meat meal, meat scraps, blood meal and pork cracklings are valuable sources of protein with which to supplement a cereal ration.

Fish meal or ground dried fish is also an important protein concentrate. Fish meals obtained from marine fish contain appreciable amounts of iodine, a fact which becomes of extreme importance in goitrous regions. In addition, the fish meals contain, in the ground fish bones, a valuable source of easily assimilated calcium and phosphorus. All these animal products, including milk, are valued for the biological value of their proteins, i e., they contain the amino acids of the right type necessary for tissue building and in adequate amounts

Definition of Terms

When feeding stuffs are purchased on the market, most state laws require that such feeds be sold on the basis of chemical composition As a result, it is customary to make the following chemical analyses on all feeding stuffs.

1. Moisture.
2. Crude protein.
3. Crude fat.
4. Crude fiber.
5. Ash.
6. Nitrogen-free extract.

Moisture. — It is necessary to determine the moisture content of foods and feeding materials in order that we may have more accurate methods of comparing one feed with another. As a rule, feeds are usually fed and compared on what is known as a " moisture-free " basis in order that comparisons may be made on the basis of dry matter or total solids. Certain feeds deteriorate rapidly if the moisture content exceeds certain limits which vary for different feeds. For these reasons, knowledge of the water content is highly desirable. Furthermore, a feed-buyer cannot afford to buy expensive feeds and pay feed prices for water As a result, the buyers of such commodities often prescribe the maximum water-content the material may contain.

Crude Protein. — Since all proteins contain about the same amount of nitrogen (16 per cent), it has become the practice of all laboratories to determine the total nitrogen present in the feed and multiply this amount by the factor 6.25. This general factor is used for most proteins, although there are occasionally exceptions to this rule. For example, the factor 5.7 is used for the calculations of crude protein in wheat and its products, since the proportion of nitrogen in wheat proteins is greater

(about 17.5 per cent) than in most proteins. The nitrogen is determined by the Kjeldahl method, oxidizing the feed completely by boiling in sulfuric acid. This liberates the nitrogen in the form of ammonia, which combines with the acid, forming ammonium sulfate. By the addition of a strong alkali (NaOH) the ammonia is liberated and is distilled into an acid of known strength, from which the amount of ammonia present may be calculated, following the titration of the excess acid. By further calculation the amount of nitrogen is determined, which, when multiplied by the factor 6.25, gives the amount of crude protein present. The term " crude " is used advisedly, since this method determines the total organic nitrogen regardless of source. There are, of course, small amounts of nitrogenous compounds present in feeds which are not proteins. For this reason the term " crude protein " is applied.

Crude Fat or Ether Extract. — When dry feeds are extracted with anhydrous ethyl ether all ether-soluble materials are extracted. This extract, consisting mainly of glycerides of the fatty acids, is weighed and the percentage computed. Since, however, the ether extract contains appreciable amounts of sterols, pigments and other ether-soluble materials, the residue has been given the name " crude fat " or " ether extract."

Crude Fiber. — When a feed is boiled, consecutively, with sulfuric acid (1.25 per cent) and sodium hydroxide (1 25 per cent), the proteins, fats and carbohydrates are hydrolyzed, leaving behind, after filtration, the more resistant woody material (fiber) This is dried, weighed and burned. The loss in weight, on burning, is assumed to be the indigestible portion of the feed and is known as " crude fiber " Although it is an index to the amount of indigestible matter in the feed, it is only approximately true, for the reason that the ruminating animals and the horse can utilize appreciable amounts of crude fiber. Nevertheless, it is the least valuable portion of the feed, and for this reason should be kept at a minimum in those feeds sold on the market as concentrates.

Ash. — A weighed portion of a feed is ignited in a muffle furnace or over a gas flame until the carbonaceous matter is completely oxidized and the mineral salts remain in the crucible or dish as a white or grayish-white ash. This ash does not truly represent the mineral salts in the feed, since many of the salts are changed during the heating process. Organic substances containing such elements as sulfur and iodine are destroyed, and a large proportion of these elements may be lost by volatilization. If the temperature of heating is sufficiently high, it is possible that other elements such as sodium and potassium may be lost also. Usually, however, the temperature is controlled to prevent the sublimation of these bases. The ash of feeds contains the basic elements com-

bined as oxides, carbonates, phosphates, etc., although these bases did not necessarily exist in these forms in the feed. The ash determination is, therefore, but a rough index of the mineral content of the feed.

Nitrogen-free Extract. — When the chemist has determined all the above-mentioned constituents and has calculated them in terms of percentage of the moisture-free feed, he adds the percentages and subtracts the total from 100. The difference is the so-called " nitrogen-free extract," which is composed almost entirely of carbohydrates, although certain organic acids and similar materials are included. For practical purposes, nitrogen-free extract may be considered synonymous with carbohydrates.

Table 58 gives the approximate composition of some of the common farm feeds and commercial feeding stuffs.

Federal and State Control of Commercial Feeds

Massachusetts and Connecticut were pioneer states in devising legislation to control the quality of commercial feeds sold for livestock feeding Prior to 1900 not many states had paid much attention to this problem, although a number of states had required the inspection and analysis of fertilizers. Subsequent to 1900, however, the various states gradually enacted laws for feed control until at the present time practically all the states have laws regulating the manufacture and sale of commercial feeding stuffs In 1906 the Federal Food and Drug Act was enacted, which embraced animal feeds as well as human foods. This law allowed for cooperation between the government and the states, particularly with reference to interstate shipments of feeds

Prior to the enactment of these laws it was most difficult for the livestock feeder to be certain that he was obtaining what he was paying for. In fact, farmers were defrauded of thousands of dollars by dishonest manufacturers who mixed large amounts of useless waste materials of low feeding value and sold them at relatively high prices. Such materials included weed seeds, mill screenings, oat and rice hulls, ground stems, peanut hulls, ground cocoanut shells, corn cob meal and similar materials.

As the result of the employment of chemists and microscopists in the various feed control laboratories, the spurious feeds have been ascertained, the manufacturers and dealers who persisted in such practices have found themselves involved in litigation and, in many cases, such individuals have been forced to go out of business. Most manufacturers, however, have been glad to cooperate with the federal and state agencies, with the result that today very few feeds on the market do not meet the requirements of the law. Although the methods and require-

TABLE 58

CHEMICAL COMPOSITION OF FEEDS

Feeding stuff	Water, per cent	Ash, per cent	Crude protein, per cent	Carbohydrates		Fat, per cent
				Fiber, per cent	N-free extract, per cent	
CONCENTRATES						
Grains and seeds						
Barley, common	9 3	2 7	11 5	4 6	69 8	2 1
Corn, dent, grade No 1	12 0	1 5	9 9	2 0	69 7	4 9
Cottonseed	9 4	4 6	19 5	22 6	21 9	19 0
Cowpea seed	11 6	3 4	23 6	4 1	55 8	1 5
Flaxseed	9 2	4 3	22 6	7 1	23 2	33 7
Oats	9 2	3 5	12 4	10 9	59 6	4 4
Wheat, all analyses	10 2	1 9	12 4	2 2	71 2	2 1
By-products of grains and seeds; miscellaneous concentrates						
Barley shorts, or barley feed, high grade	10 2	4 2	12 8	8 6	60 6	3 6
Beet pulp, dried	8 2	3 5	8 9	18 9	59 6	0 9
Brewers' grains, dried	7 5	3 5	26 5	14 6	41 0	6 9
Corn bran	10 0	2 4	9 7	9 8	62 4	5 7
Corn cob	10 0	1 5	2 0	31 8	54 3	0 4
Corn gluten meal	9 1	1 1	35 5	2 1	47 5	4 7
Cottonseed meal, choice	7 5	6 2	44 1	8 1	25 0	9 1
Cottonseed hulls	9 7	2 7	4 6	43 8	37 3	1 9
Hominy feed	10 1	2 6	10 6	4 4	64 3	8 0
Linseed meal, new process	9 6	5 6	36 9	8 7	36 3	2 9
Linseed meal, old process	9 1	5 4	33 9	8 4	35 7	7 5
Oat hulls	6 8	6 0	4 0	29 2	52 3	1 7
Oat kernel	6 9	2 2	14 3	1 4	67 1	8 1
Peanut oil meal or cake, from shelled nuts	6 6	4 8	44 8	7 6	26 0	10 2
Peanut shells, commercial	9 1	5 5	7 3	56 6	18 9	2 6
Red dog flour	11 1	2 5	16 8	2 2	63 3	4 1
Rice bran, high grade	10 1	9 7	12 1	12 4	44 3	11 4
Rice hulls	9 3	16 9	3 3	35 4	34 0	1 1
Sunflower seed cake	10 0	4 2	34 8	10 9	21 8	18 3
Wheat bran, all analyses	10 1	6 3	16 0	9 5	53 7	4 4
Wheat middlings, standard (shorts)	10 5	4 4	17 4	6 0	56 8	4 9

TABLE 58

CHEMICAL COMPOSITION OF FEEDS — (*Continued*)

Feeding stuff	Water, per cent	Ash, per cent	Crude protein, per cent	Carbohydrates		Fat, per cent
				Fiber, per cent	N-free extract, per cent	
CONCENTRATES — *Cont.*						
Milk and its products						
Cow's milk	87 2	0 7	3 5		4 9	3 7
Buttermilk	90 6	0 7	3 6	.	5 0	0 1
Buttermilk, dried	4 5	8 1	34 6		50 9	1 9
Buttermilk, semi-solid	65 0	2 7	13 4		15 9	3 0
Skim milk, centrifugal	90 1	0 7	3 8	.	5 2	0 2
Slaughter-house by-products						
Dried blood	9 7	3 3	82 3	.	3 8	0 9
Fish meal	10 5	28 1	51 4			8 3
Fresh bone	30 4	21 1	19 7		3 8	25 0
Tankage, guaranteed 60 per cent protein	7 9	15 3	60 4	5 3	3 7	7 4
Tankage, below 40 per cent protein	9 1	24 7	36 9	4 1	10 8	14 4
DRIED ROUGHAGE						
Cured corn and sorghum forage, etc.						
Corn fodder, medium in water	18 3	5 0	6 7	22 0	45 8	2 2
Corn stover, medium in water	19 0	5 5	5 7	27 7	40 9	1 2
Sorghum fodder, dry	9 7	7 8	7 4	26 1	45 9	3 1
Hay from the grasses, etc.						
Bermuda grass	9 7	7 6	7 1	25 6	48 2	1 8
Bluegrass, Kentucky, all analyses	13 2	6 6	8 3	28 3	40 7	2 9
Red top, all analyses	9 8	6 8	7 4	28 7	45 0	2 3
Sudan grass	11 6	7 0	8 2	26 9	44 7	1 6
Timothy, all analyses	11 6	4 9	6 2	29 8	45 0	2 5
Hay from the legumes						
Alfalfa, all analyses	8 6	8 6	14 9	28 3	37 3	2 3
Alfalfa meal	8 8	9 0	14 3	30 1	35 8	2 0
Alfalfa leaves	6 6	13 6	22 5	12 7	41 2	3 4
Alfalfa stems	5 6	4 9	6 3	54 4	27 9	0 9
Clover, alsike, all analyses	12 3	8 3	12 8	25 7	38 4	2 5
Clover, red, all analyses	12 9	7 1	12 8	25 5	38 7	3 1
Clover, sweet, white	8 6	7 2	14 5	27 4	40 1	2 2

TABLE 58

CHEMICAL COMPOSITION OF FEEDS — (*Continued*)

Feeding stuff	Water, per cent	Ash, per cent	Crude protein, per cent	Carbohydrates		Fat, per cent
				Fiber, per cent	N-free extract, per cent	
DRIED ROUGHAGE — *Cont*						
Hay from the legumes — Cont.						
Cowpea, all analyses	9 7	11 9	19 3	22 5	34 0	2 6
Soybean hay	8 6	8 6	16 0	24 9	39 1	2 8
Straw and chaff from the cereals						
Barley straw	14 2	5 7	3 5	36 0	39 1	1 5
Oat straw	11 5	5 1	3 6	36 3	40 8	2 4
Oat chaff	8 2	11 5	5 9	25 7	46 3	2 4
Wheat straw	8 4	5 2	3 1	37 4	44 4	1 5
FRESH GREEN ROUGHAGE						
Corn and the sorghums						
Corn fodder, all analyses	78 1	1 2	1 9	5 2	13 0	0 6
Fresh green grass						
Bluegrass, Kentucky, all analyses	68 4	2 8	4 1	8 7	14 8	1 2
Timothy, all analyses	62 5	2 2	3 1	11 7	19 3	1 2
Green legumes						
Alfalfa, all analyses	74 7	2 4	4 5	7 0	10 4	1 0
Clover, red, all analyses	73 8	2 1	4 1	7 3	11 7	1 0
Clover, sweet	75 6	2 1	4 4	7 0	10 2	0 7
Soybeans, all analyses	76 4	2 4	4 1	6 3	9 8	1 0
Roots, tubers, etc.						
Beet, common	87 0	1 5	1 6	0 9	8 9	0 1
Beet, sugar	83 6	1 1	1 6	1 0	12 6	0 1
Mangel	90 6	1 0	1 4	0 8	6 1	0 1
Potato	78 8	1 1	2 2	0 4	17 4	0 1
Rutabaga	89 1	1 0	1 2	1 4	7 0	0 3
Turnip	90 5	0 9	1 4	1 1	5 9	0 2
MISCELLANEOUS GREEN FORAGES						
Cabbage	91 1	0 8	2 2	0 9	4 7	0 3
Kale	88 7	1 9	2 4	1 5	5 0	0 5
SILAGE						
Silage from corn, the sorghums, etc						
Corn, well matured, recent analyses	73 7	1 7	2.1	6 3	15 4	0.8

ments differ from state to state, the following copy of the Pennsylvania Feeding Stuffs Law may be considered representative.

PENNSYLVANIA FEEDING STUFFS LAW

Act of May 3, 1909, No 224, as Amended May 11, 1921, No. 229; and March 19, 1923, No 10

Section 1. — Be it enacted, &c., that every barrel, bag, pail, parcel or other package of concentrated commercial feeding-stuffs, as defined in section two of this act, used for feeding domestic animals, also condimental stock and poultry-food, and patented, proprietary, or trade-mark stock and poultry-feed, possessing nutritive value combined with medicinal properties, sold, offered, or exposed for sale, within this State, shall have affixed, thereto, in a conspicuous place on the outside thereof, a legible and plainly-printed statement in the English language clearly and truly certifying the number of net pounds of feeding-stuff contained therein, the name, brand, or trade-mark under which the article is sold, the name and address of the manufacturer or importer, and a statement of the percentage of crude protein, the percentage of crude fat, and the maximum percentage of crude fiber which it contains; these constituents to be determined by the latest methods adopted by the Association of Official Agricultural Chemists of North America Every concentrated commercial feeding-stuff, as defined in section two of this act, intended for domestic animals, that is compounded from two or more substances, in addition to the above requirements shall have affixed thereto, in a conspicuous place on the outside thereof, a statement, in the English language giving the true and correct names of all of the ingredients of which it is composed Whenever any concentrated commercial feeding-stuff is kept for sale, stored in bins, the dealer or dealers keeping the same for sale in bulk, or if it is put up in packages belonging to the purchaser, the dealer or dealers shall, upon request of the purchaser, furnish him with a card upon which is written, or printed the brand name, guaranteed percentage of crude protein, crude fat and crude fiber, and a statement showing the true and correct names of all the ingredients of which it is composed, and also the name and address of the manufacturer or importer, whenever possible Provided, that when any manufacturer or purchaser, located within the State of Pennsylvania, of any concentrated commercial feeding-stuff, as defined in section two of this act, shall send samples of the same to the Secretary of Agriculture for analysis, the chief chemist of the Department of Agriculture shall furnish such analysis, showing the percentage of crude protein, the percentage of crude fat, and the percentage of crude fiber which it contains, and shall charge a fee of one dollar for each such analysis, which analysis shall be made within fifteen days after the sample is received by the chief chemist, and all moneys so received shall, from time to time, be covered into the State Treasury by the Secretary of Agriculture.

Section 2. – The term "concentrated commercial feeding-*stuffs*," as used in this act, shall include cottonseed meals, cottonseed feeds, linseed meals, *linseed-oil meals, cocoanut oil meals,* peanut meals, *corn oil meals, corn gluten feeds, corn gluten meals,* corn bran, maize feeds, starch feeds, *barley middlings, barley feeds,* pea meals, bean-meals, sugar feeds, dried distillers' grains, dried brewers' grains, malt sprouts, *dried malt grains, dried vinegar grains, wheat bran, wheat middlings, wheat mixed feeds, rye bran, rye middlings, rye mixed feeds, buckwheat brans, buckwheat middlings, buckwheat feeds,* hominy feeds, cerealine feeds, maizeline feeds, rice meals, dried beet pulp, dried molasses beet pulp, *feed molasses,* molasses grains, molasses feeds, clover meal, alfalfa

meal and feeds, *semi-solid or dried buttermilk*, ground beef scraps, ground fish scraps, *oil meals or residues from extracted seeds and grains, mixtures* of whole grains, and all other mixtures bearing distinctive *brand or trade names*, and all mixtures of similar nature used for feeding domestic animals, including poultry, condimental stock and poultry-*foods*, and patented, proprietary or trade-mark, stock and poultry-*foods*, possessing nutritive value combined with medicinal properties, and *all* mixed feeds other than pure grains *which are not sold as mixtures bearing distinctive names, but which are sold as pure grains. It shall not* include hays, straws and corn stover, *ensilage*, whole grains, nor the unmixed meals made directly from the entire grains of wheat, rye, barley, oats, Indian corn, *ear corn, Kaffir corn*, broom corn, sugar cane, sorghum, *rice*, buckwheat, and flax-seed, not mixed with other substances, but sold separately as distinct articles of commerce; nor pure grains *mixed* together, not *being* mixed with any other substance, *nor having distinctive brand or trade names, but sold as mixtures of pure whole grains.* The Secretary of Agriculture, by himself or his agents, is fully empowered to take samples of these articles when found, and, if upon analysis, they prove to be adulterated or misbranded, the vender shall be in all respects subject to the penalties hereinafter set forth for the adulteration or misbranding of concentrated commercial feeding-stuffs

Section 3. — No foreign mineral substance or substances injurious to the health of domestic animals, nor oat hulls, *oat feed, oat meal mill by-product, clipped oat by-product*, ground corn cobs, flax plant refuse, elevator chaff, cottonseed hulls, ground corn stalks, *cocoa shells, cocoanut shells*, weed seeds, *humus, peat, sand, sawdust, coffee hulls* or other similar adulterants shall be mixed with any feeding-stuff sold, offered, or exposed for sale in this State. Provided, that nothing in this section shall be construed as prohibiting the use of mineral substances known to possess medicinal properties and that are not injurious to the health of domestic animals in compounding stock and poultry-*food* and patented, proprietary or trade-marked stock and poultry-foods And provided further, that any feeding-stuff the crude fiber content of which does not exceed *eleven* per centum, that may be found, upon analysis, to contain either oat hulls, *oat feed, oat meal mill by-product, clipped oat by-product*, flax plant refuse, elevator chaff, cottonseed hulls, ground cornstalks, *cocoa shells, cocoanut shells*, or all of these ingredients, shall not be considered adulterated, with oat hulls, *oat feed, oat meal mill by-products, clipped oat by-product, flax* plant refuse elevator chaff, cottonseed hulls, ground cornstalks, *cocoa shells, or cocoanut shells*, within the meaning of this act. And provided further, that nothing contained in this act shall prohibit the *mixing* of whole corn without being shelled with other *whole* grains or the admixture of ground corn cobs with exclusively corn feeds, in such proportion as to keep the *crude* fiber content from exceeding *eleven* per centum

The use of any or all metal fasteners in fastening or attaching tags or cards to packages of feeding-stuffs is hereby prohibited

In addition to the requirements to section one of this act a statement shall be affixed to all packages or other containers of feeding-stuffs, showing, in the case of feed molasses, the minimum percentage of total sugars which it contains, and, in the case of semi-solid buttermilk, the minimum percentage of total solids which it contains.

Section 4. — Each and every manufacturer, importer, agent, person, corporation, or firm, on or before the first day of January of each year, or before selling, offering, or exposing for sale, in this Commonwealth, any concentrated commercial feeding-stuffs, as defined in section two of this act, shall register and file annually with the Secretary of Agriculture a certified statement of the names and number of each kind or brand of such concentrated commercial feeding-stuffs that he shall offer for sale

during the next ensuing year, and a copy of the analysis of each one of such brands as required by section one of this act; and he or they shall pay to the Secretary of Agriculture the sum of twenty-five dollars for each and every brand of such concentrated commercial feeding-stuffs to be sold or offered for sale within the Commonwealth, *except brands of* (a) *pure wheat bran,* (b) *pure wheat middlings,* (c) *pure wheat mixed feed,* (d) *pure rye bran,* (e) *pure rye middlings,* (f) *pure rye mixed feed,* (g) *pure buckwheat bran,* (h) *pure buckwheat middlings,* (i) *pure buckwheat feed, being by-products resulting from the milling of pure wheat, rye, and buckwheat in the manufacture of wheat, rye, and buckwheat flour, and* (j) *pure corn meal made by grinding and bolting corn; and* (k) *pure corn and oats chop made by grinding together, or by grinding separately and mixing together, pure corn and oats; standards for which brands shall be fixed by the Secretary of Agriculture, in which cases he shall pay to the Secretary of Agriculture the sum of six dollars instead of twenty-five dollars for each and every such brand to be sold or offered for sale within the Commonwealth.* All moneys so received shall be immediately paid by the Secretary of Agriculture to the State Treasury for the use of the Commonwealth.

Section 5. — Each and every manufacturer, importer, agent, person, corporation or firm, selling, offering, or exposing for sale, in this State, any concentrated commercial feeding-stuffs, as defined in section two of this act, without the statement or statements required by section one of this act, or to which is affixed a statement or guarantee which is false in any particular, or in relation to which the provisions of all of the fore-going sections have not been fully complied with, shall, for every such offense, forfeit and pay the sum of fifty dollars, which shall be recoverable, with costs, including the cost of analysis, by any person suing in the name of the Commonwealth, as debts of like amounts are by law recoverable Provided, that the Secretary of Agriculture shall, together with his deputies, agents and assistants, be charged with the enforcement of this act, and shall have free access to all places of business, mills, buildings, carriages, cars, vessels, and packages of whatsoever kind, used in the manufacture, importation or sale of any concentrated commercial feeding-stuffs, and shall also have power and authority to open any package containing, or supposed to contain, any concentrated commercial feeding-stuff, and take therefrom samples for analysis, upon tendering the value of said samples; and the party or parties interested, or their representative, shall be given an opportunity to be present when said samples are taken. Said samples shall be thoroughly mixed and then divided into two samples, and put in glass vessels, and carefully sealed; and a label placed upon each vessel, stating the name or brand of the feeding-stuff or material sampled, the name of the manufacturer when possible, the name of the party from whose stock the sample was taken, the time and the place of taking, the guaranteed analysis, and other information relevant thereto; said labels to be signed by the Secretary of Agriculture or his agent, and by the party or parties interested, or their representative if present at the taking of the samples. One of said duplicate samples shall be retained by the Secretary of Agriculture or his agent, and the other shall be left with the party whose stock was sampled. The Secretary of Agriculture shall cause such samples as are secured by him under the provisions of this act, as may seem to him proper, to be analyzed within sixty days after said samples are received by the chief chemist; and the results of the analysis of such samples, together with such additional information as circumstances advise, shall, by his authority, be published in reports or bulletins from time to time: Provided, that when the materials sampled are in packages, the samples shall be taken from four separate packages. And provided further, that if the analysis of any sample does not differ from the guarantee more than ten per centum of

the protein, fat or fiber guaranteed, the manufacturer shall be considered as having complied with the requirements of this act; except when the percentage of either protein or fiber shall exceed twenty per centum, and shall not be present in excess of thirty per centum, in which case the variation allowed shall not exceed seven per centum of the guaranteed amount, and also except when the percentage of either protein or fiber shall exceed thirty per centum, in which case the variation allowed shall not exceed five per centum of the guaranteed amount.

Section 6. — In addition to the above penalty, every person, corporation, or firm, and every officer, agent, servant, or employee of such person, corporation, or firm, violating any of the provisions of this act, shall also be guilty of a misdemeanor, and, upon conviction thereof, shall be punished by a fine of not less than fifty dollars, nor more than one hundred dollars, for the first offense, and a fine of not less than one hundred dollars, nor more than two hundred dollars, for every subsequent offense Provided, that all penalties, fines, and costs, including the cost of analysis, imposed and recovered under the provisions of this act, shall be paid to the Secretary of Agriculture, or his agents, and by him immediately covered into the State Treasury And provided also, that all necessary expenses under the provisions of this act shall, after approval in writing by the Governor and the Secretary of Agriculture, be paid by the State Treasurer, upon the warrant of the Auditor General, in the manner now provided by law.

Section 7. — This act shall take effect on the first day of August, one thousand nine hundred and nine

Section 8. — The act of Assembly, entitled "An act regulating the sale of wheat, rye, corn, and buckwheat-bran, and middlings, or any admixture thereof, also of condimental stock and poultry-food, and patented, proprietary, or trade-marked stock and poultry-food possessing nutritive value combined with medicinal properties, and mixed feeds, including mixtures bearing distinctive names, used for feeding poultry and other domestic animals, and also of concentrated commercial feeding-stuffs; defining concentrated commercial feeding-stuffs, prohibiting their adulteration, providing for the collection of samples, and analysis thereof, by the Department of Agriculture and the publication of information concerning the same; providing also for the expenses of the enforcement of the law, and fixing penalties for its violation," approved the twenty-eighth day of May, Anno Domini one thousand nine hundred and seven, is hereby repealed; and all acts or parts of acts, inconsistent with the provisions of this act, are also hereby repealed.

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CHAPTER XXII

DIGESTION OF FOODS

In the previous chapter we considered, in a brief way, the outstanding characteristics of foods and feeding stuffs with regard to chemical composition. We observed that some foods are fed mainly for the protein they contain; others are valuable for their content of energy-producing nutrients (fats and carbohydrates); other foods are characterized by containing mineral elements in abundance; and others contain relatively large amounts of cellulose, which is digested less readily than the other nutrients. We shall now direct our attention to the fate of the various nutrients as they undergo digestion in the alimentary tract. *Digestion may be defined as the process whereby the inorganic and organic nutrients are made soluble and diffusible in order that the nutrients may be utilized by the animal body for the repair and building of tissue and for the transformation of energy.*

In Chapter I we referred to the willow-tree experiment of Van Helmont, the versatile Belgian physician, who is well known for his early attempt to find the "Principle of Vegetation." Van Helmont is best known, however, for his work in medicine, and it was he who first suggested that the processes involved in digestion might be analogous to those of fermentation Following Van Helmont's explanation, many theories were advanced to explain the mechanics of the digestive process. One theory, advanced by Dr Archibald Pitcairn in the seventeenth century, explained the digestive process on a purely mechanistic basis. Dr. Pitcairn was of the belief that no "Stygian" liquor was involved in stomachic digestion but that the process was largely mechanical in character, the enormous power of the stomach walls being sufficient to grind foods to particles of infinitesimal fineness, in which form they were utilized by the body.

This theory was overthrown, however, when an investigator by the name of Stevens, using a man who was able to regurgitate his food at will, fed to the experimental subject food which had been packed in hollow metal balls which were perforated with many holes. When the balls were regurgitated, it was found that the food had disappeared and the balls were empty Stevens and Spallanzani were able to show, independently, that gastric juice, after removal from the body, was capable of digesting food.

In the early part of the nineteenth century (1822) the entire matter was settled in a most conclusive manner by the classical experiments of an American army surgeon, Dr. William Beaumont. The doctor was called to treat a Canadian soldier, named Alexis St. Martin, who had been wounded in the left side by the accidental discharge of a musket. On healing, the wall of the stomach adhered to the abdominal wall in such a manner that a permanent opening, known as a gastric fistula, re-

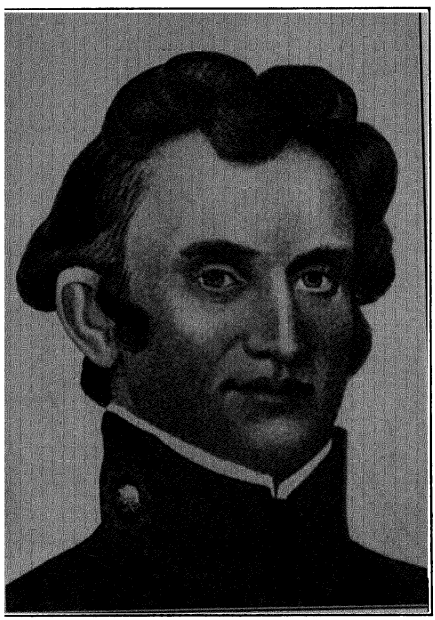


FIG. 46. William Beaumont (1785–1853), pioneer American physiologist whose classical discoveries concerning gastric digestion may well serve as a model and inspiration for present day research workers.

mained. It was possible for Dr. Beaumont to introduce food, and extract gastric juice from the opening, and, recognizing this unusual opportunity, he persuaded the authorities to allow him to use St. Martin as an experimental subject.

As a result, Dr. Beaumont established the true facts regarding gastric digestion, and most of his observations have been confirmed many times by subsequent research. He described the appearance of the stomach walls during gastric secretion; he described the gastric juice and noted its acid properties; he studied the excitation of the stomach by various methods and found that the gastric juice, removed from the stomach,

would digest meat quite rapidly. He made many other interesting and valuable observations regarding the factors influencing the time required for various types of meals to digest and be expelled from the stomach into the intestinal tract.

In recognition of Dr. Beaumont's classical researches, the Thirteenth International Physiological Congress, at its meeting in Boston in August, 1929, published a facsimile of Dr. Beaumont's 1833 edition of his book, entitled " Experiments and Observations of the Gastric Juice and the Physiology of Digestion." A medallion, containing the bust of Beaumont, was also struck in honor of his memory.

Salivary Digestion

When food is ingested, the first step in the process of digestion is *mastication*. The lips, teeth and tongue all play a part in the process, the object of which is to seize and break the food into small particles, which offer a greater surface for subsequent action of digestive juices. The first of these juices with which the food comes in contact is the *saliva*, which is secreted by the salivary glands and the cells of the mucous membranes of the mouth. The salivary glands are three in number, viz., the parotid glands, the sub-lingual glands and the sub-maxillary glands.

Saliva. — When food is eaten, the salivary glands are stimulated and saliva pours into the mouth and becomes intimately mixed with the food. For chemical studies, human saliva may be collected in large amounts by stimulating salivary activity by chewing paraffin wax. Armsby states that the horse may secrete, in a day, 84 pounds of saliva, and in the same period an ox may secrete as much as 112 pounds; Mathews is authority for the statement that man may secrete about one and one-half quarts.

Human saliva is a viscous, colorless liquid which has a specific gravity of 1.002 to 1.008. Chemical analysis shows that saliva is composed of water (99 41 per cent) and solids (0.59 per cent). The solids consist of mucin, epithelium and soluble organic matter (0.35 per cent), and inorganic salts (0.219 per cent) The ash of saliva is rich in potassium and contains the bases sodium, calcium and magnesium in appreciable quantities. In addition, phosphorus, chlorine and sulfur are invariably present. Saliva also contains the enzymes ptyalin and maltase. Saliva may be slightly acid or slightly alkaline, depending upon the indicator used. It is never far from neutral (pH 7), however, since the phosphates and carbonates present evidently act as buffers, keeping the reaction at or near the neutral point.

In man and in many animals, saliva functions in two ways, i.e., it acts

as a lubricant which assists the process of deglutition (swallowing), and it assists in the digestion of starchy foods. The saliva of carnivora does not possess this property of starch digestion. When saliva is mixed with a viscous solution of starch-paste, the solution becomes water-clear and loses its viscosity. Upon treatment of the latter solution with Fehling's solution, reduction occurs, while the original starch failed to give this test, and the clear solution does not give a blue color with iodine, indicating that the starch has changed to sugar.

The amylolytic enzyme, ptyalin, begins to affect starch hydrolysis just as soon as the finely ground food is thoroughly mixed with the saliva. The thoroughness with which starch digestion takes place in the mouth depends upon the enzymatic activity of the saliva, the length of time the food remains in the mouth and the fineness of division or thoroughness of chewing. The length of time the food remains in the mouth is dependent, to some extent, on our habits of living and whether or not we are hungry. When we are in a hurry or are unusully hungry, there is a tendency for us to " bolt " our food, a practice that has become altogether too common. While we are discussing the subject of hunger, it may be of interest to make a brief résumé of the best opinions regarding the difference between appetite and hunger.

Cannon and Washburn of Harvard and Carlson of Chicago University are of the opinion that appetite and hunger are entirely different phenomena. Some physiologists have thought that the matter is one of a quantitative nature, viz , that hunger is merely an exaggerated appetite. Cannon and Washburn believe that appetite is largely a psychic phenomenon, being related to the taste and smell of food and the memory of delectable foods. Even as we eat a meal, appetite seems to increase, being stimulated, no doubt, by the pleasant experience. On the other hand, disgusting and unpleasant memories may affect many people to the point where nausea replaces appetite.

Hunger, on the other hand, is a strong desire for food, so strong in fact that it is often accompanied by pain. When this occurs people are not affected so much by the memories of good or bad food but they crave to satisfy the hunger pangs and under these conditions will eat distasteful and even nauseating foods. By inflating a toy balloon in the empty stomach of a hungry man and connecting it, by rubber tubing, to a manometer, which registers changes in pressure, physiologists have shown that there are pronounced stomach contractions in hunger, and the hunger pangs and stomach contractions seem to be correlated.

Returning to our discussion of salivary digestion, we may state that most authorities do not attach great importance to the chemical changes which take place in the mouth. The food is swallowed so quickly that

salivary digestion, though of importance, does not have time to produce many changes before the food leaves the mouth. Physiological research, however, has shown that the greater part of salivary digestion takes place after the food reaches the stomach and prior to the cessation of enzyme activity which is brought about by the accumulation of hydrochloric acid.

Stomachic Digestion

When the bolus of food, mixed with saliva, is swallowed, it passes through the esophagus or gullet to the stomach where, for a time, salivary digestion continues. Ptyalin, acting on starch, catalyzes the hydrolytic

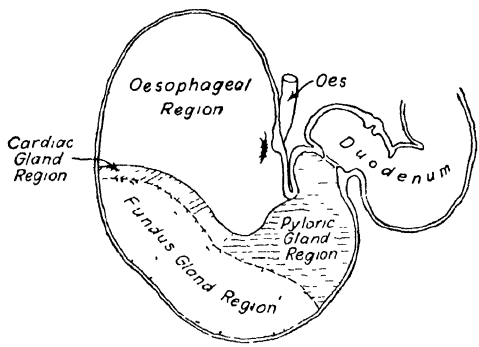


FIG 17. Diagram of the stomach of the horse (Courtesy of the W B Saunders Company)

process, and the disaccharide, maltose, is formed. Salivary maltase then continues the process, and the maltose changes rapidly to glucose, in which form it is later absorbed.

What has been said applies only to those animals possessing a single-sac stomach. The ruminant stomach will be discussed later. The portion of the single-sac stomach which is attached to the esophagus is known as the cardiac portion. The remainder of the stomach is shaped somewhat like a large lop-sided pear At the other end of the stomach is attached the small intestine. This portion of the stomach is called the pylorus or pyloric portion, and it is here that the pyloric valve is situated, which controls the flow of liquid or semi-liquid material from the stomach to the intestine and prevents the regurgitation of intestinal contents back into the stomach.

The large central portion is known as the fundus or fundic portion of the stomach. Most of the gastric pepsin (proteolytic enzyme) is thought to be secreted here. The cells which produce this enzyme are known as

" chief cells." In the pyloric portion, and extending toward the fundus, are cells of a different type, known as " parietal cells." It has been held by some physiologists that it is the parietal cells which produce the hydrochloric acid which characterizes gastric juice, although this fact has been questioned by recent workers.

The Ruminant Stomach

The type of stomach which has been discussed to this point is the type possessed by man and such animals as the horse, hog, and carnivorous animals, such as the cat and dog. Ruminants, such as cattle, sheep and goats, possess a compound stomach, consisting of four divisions or compartments which are known respectively as (1) the *rumen* or *paunch*, (2) the *reticulum* or *honeycomb*, (3) the *omasum* or *manyplies*, and (4) the *abomasum* or true stomach. It is only the abomasum that is truly comparable with the single stomach cavity of other animals.

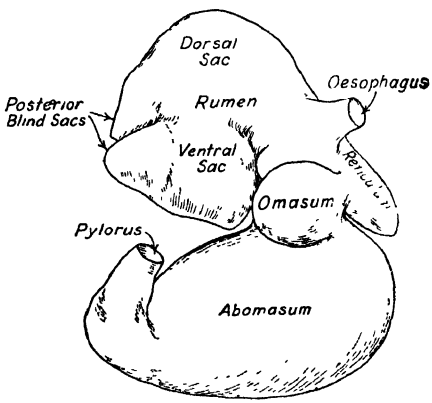


FIG. 48. Ruminant stomach (new born calf) Right view with rumen raised (Courtesy of the W B Saunders Company.)

The first three compartments of the ruminant stomach are regarded, anatomically and physiologically, as continuations and enlargements of the esophagus in which the feed is fermented and softened preparatory to gastric digestion in the abomasum. In domestic fowls a similar situation exists, the crop being an enlargement of the esophagus which acts as a storage organ and does not secrete digestive juices.

Rumination. — As a rule the ruminant masticates its food very imperfectly at the time of eating The liquid, soluble and finely divided portions of the feed pass down the esophagus and through the esophageal canal directly to the true stomach (abomasum). The esophageal canal, which is a continuation of the esophagus, is constructed somewhat like a rubber hose which has been cut lengthwise so that it can be opened. So long as the contents are of a liquid nature, they pass through the closed tube which is closed by muscular contraction. However, when large masses of fibrous hay and similar materials are swallowed, the esophageal canal opens and drops the coarse material into the capacious rumen. Here the feed is brought into intimate contact with saliva and various

types of enzymes and bacteria (introduced in the feed) and fermentation occurs. The rumen functions, primarily, as a storage compartment, and no digestive juices are secreted in this portion of the stomach

When the ruminant has completed feeding, it has the power to regurgitate this coarse material and masticate it at its leisure This process is known as "chewing the cud." From the rumen the finely comminuted food passes to the other (grinding) stomachs or compartments and, eventually, reaches the abomasum where true digestion takes place During the rumination process, methane, acids, alcohols, etc, are formed in small amounts by fermentation. Recent work of Bechdel, Honeywell, Dutcher and Knutsen indicates that at least one organism (*Bacterium vitarumen*) has a beneficial action on growth, by synthesizing, in the rumen, the vitamin B complex. This will be discussed in greater detail in a subsequent chapter.

Stomachic Characteristics of the Fowl

While we are discussing the stomachic characteristics of the various types of animals, a brief statement should be made regarding the characteristics of the domestic fowl, for the reason that the structure of its digestive tract differs from that of other animals to a marked degree.

The chicken, like other animals, possesses an esophagus which acts as a transfer organ, but it differs from that of other animals in that it is divided into two parts, known as the *first* and *second* portions These are separated by an enlargement of the esophagus which is known as the *crop* and which functions as a storage organ during the hours of feeding. It should be pointed out that no mastication has taken place, to this point, since fowls do not possess teeth

From the crop the food passes through the second portion of the esophagus to the *proventriculus*, which is a small expansion of the esophagus, measuring (in the hen) about one and one-half inches in length and nearly an inch in diameter. The proventriculus functions, primarily, as a secretory organ, furnishing gastric juice, but it differs from the true stomach of other animals in that the food does not remain long enough for gastric digestion to take place. From the proventriculus, the food, which has been moistened with gastric juice, passes to the *gizzard*, a large muscular organ which, by muscular action and with the aid of grit, grinds the food material to a fine state of division. During this period considerable gastric digestion takes place, and then the contents are passed on to the small intestine

We will now return to a more detailed discussion of gastric digestion in animals possessing the single-sac type of stomach. The changes

which are typical for humans and animals, such as the horse, hog, dog and cat, will apply in a large measure to other animals.

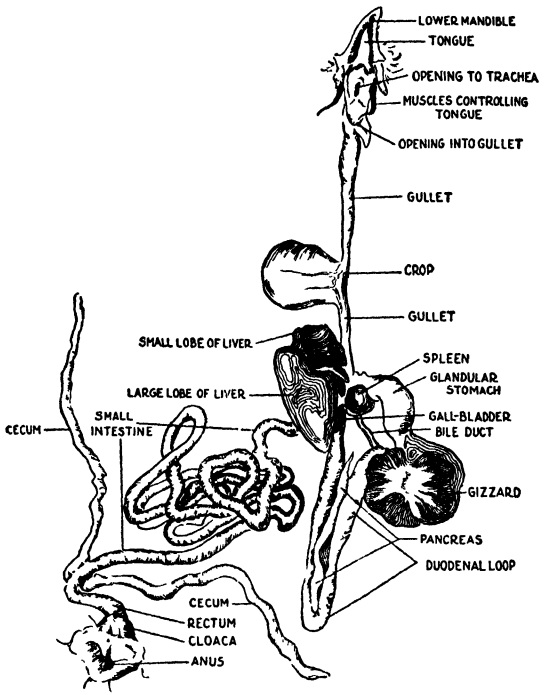


FIG. 49. Showing the various portions of the digestive tract of the domestic fowl. (After Lippincott)

Gastric Digestion

When the bolus of food reaches the cardiac portion of the stomach, the latter, because of its collapsible nature, becomes completely filled with food. As additional food is swallowed the successive portions find the stomach walls occupied, as the stomach expands, and they are, therefore, received into the interior of the stomach while some of the food remains at the cardiac portion, near the esophagus. As the muscular movements (peristalsis) or "churning" movements occur, there is a tendency for the food to become mixed, but this does not occur at once. As a result, the gastric juice secreted by the cells in the stomach wall cannot mix with the interior portion for a time. Consequently, salivary digestion continues in the stomach for various lengths of time and until it is stopped by the accumulated acid of the gastric juice. The foregoing

statements explain why salivary digestion in the stomach is considered to be of greater importance than salivary digestion in the mouth. At this point, varying quantities of starch have been changed to the soluble sugars, maltose and glucose, although relatively large amounts of starch may still remain unchanged. During the process just described the gastric juice is increasing in volume and the gastric contents are becoming more thoroughly mixed with the acid gastric juice, which initiates other chemical changes which will be discussed in a subsequent paragraph.

Gastric Juice. — Normal gastric juice is a thin, colorless liquid, acid in reaction, and possessing a specific gravity of 1.001 to 1.010 The solid matter comprises but about 0 5 per cent and is composed principally of sodium chloride, potassium chloride, phosphates and the enzymes, gastric pepsin, gastric rennin and gastric lipase The acidity of gastric juice is due to free hydrochloric acid, which has the power to destroy the amylolytic activity of the salivary enzyme, ptyalin, but which is necessary for the maximum activity of the gastric protease, pepsin. The normal acidity of gastric juice, in man, is about 0 4 to 0 5 per cent. Proteins and other substances may combine with hydrochloric acid, lowering gastric acidity. This is differentiated from free hydrochloric acid and is called " combined acid." The antiseptic action of gastric juice, which normally prevents fermentation in the stomach, is thought to be due, primarily, to hydrochloric acid.

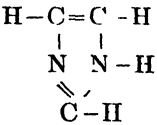
Pepsin is undoubtedly the most important enzyme of the gastric juice. It is not secreted as active pepsin but is first produced in an inactive form called *pepsinogen*. This zymogen, as we learned in a previous chapter, is the precursor or mother-substance of pepsin. Not until pepsinogen comes in contact with hydrochloric acid (which acts as an activator) does it become changed to active pepsin. Pepsin is most active in acid solutions, and it has been shown that the proteolytic activity of pepsin is not due to hydrochloric acid, per se, for many acids, at proper concentration, will produce most satisfactory results. The concentration of hydrochloric acid at which pepsin seems to act best ranges from 0.1 to 0 25 per cent. Like many other enzymes, pepsin is very active at about 38 to 40° C At high temperatures its activity is retarded until it is completely destroyed at 80 to 100° C.

Gastric Rennin. — This is a protein-coagulating enzyme which has the property of curdling milk, thereby holding the milk proteins in the stomach until peptic digestion can take place. According to Van Slyke and Bosworth, rennin owes its curdling or coagulating action to the formation of *paracasein* which combines with calcium, forming insoluble calcium paracaseinate, which possesses a molecule about one-half as large as that

of the original casein molecule; but the work of these two investigators has been questioned, especially as it relates to the molecular weight of casein and paracasein. Rennin probably shows its greatest activity in slightly acid solutions, although it will coagulate casein in neutral and even slightly alkaline solutions. Commercial rennin, known as "rennet," is obtained from the stomach of the calf and is used in creamery practice in the manufacture of cheese.

Gastric lipase is a fat-splitting enzyme of relatively small importance in gastric digestion and rarely functions unless gastric acidity, for some reason, falls to a very low level.

Gastric Secretion. — In terminating our brief discussion of gastric juice, our mental picture of gastric secretion would not be complete without reference to the work of Edkins, and Koch and co-workers. These scientists believe that the tissues contain a chemical hormone called "gastrin" which stimulates gastric secretion In some manner, not yet ascertained, the presence of food liberates gastrin, which, in turn, stimulates the flow of gastric juice. This hormone can be extracted, and, when it is injected into other animals, gastric secretion occurs almost immediately. It does not affect salivary secretion. Chemical investigations indicate that it is probably related to imidazole:



Imidazole

Chemical Changes in Gastric Digestion. — We have discussed the changes through which starches may pass in early gastric digestion, due to the continued action of salivary amylase. We shall now turn our attention to the changes occurring in foods as the result of true gastric digestion.

So far as chemical changes are concerned, gastric digestion may be considered to be primarily proteolytic in nature. In other words, the main function of the gastric juice is to digest protein materials. Pepsin, which is responsible for the hydrolytic catalysis of proteins, splits the protein molecules to proteoses and peptones. Some peptids and amino acids are found in the digestion mixture but the bulk of the hydrolysate is composed of peptones, which are soluble in water.

The acid contents of the stomach (sometimes called chyme) do not seem to increase appreciably in soluble carbohydrates or simple sugars during gastric digestion, nor does there appear to be appreciable fat hydrolysis, unless the fat is well emulsified. Some inversion of sucrose

may be noted in gastric digestion, and most authorities are agreed that the hydrolysis of sucrose to fructose and glucose is brought about by the hydrochloric acid. It will be seen, therefore, that the acid chyme may consist of proteins, fats and carbohydrates which may have resisted digestion and, in addition, proteoses, peptones, peptids, amino acids, simple sugars, glycerol and fatty acids.

Intestinal Digestion

When the chyme has reached the proper state of fluidity and acidity, the pyloric valve opens and allows the stomach contents to flow into the *duodenum*, which is the portion of the small intestine (about 11 inches long in man) attached to the pyloric end of the stomach. The first spurt of acid chyme, as it comes in contact with the intestinal mucosa, causes the secretion of duodenal juice, an albuminous secretion with an alkaline reaction. In the duodenal juice is a heat-labile substance called *enterokinase* which activates the proteolytic activity of pancreatic juice. Three digestive juices play an important rôle in intestinal digestion, viz., pancreatic juice, bile and intestinal juice. These will be discussed in the order named

Pancreatic Secretion. — Lying along the duodenum is a most important organ, the pancreas, which pours its secretion (pancreatic juice) into the intestine The pancreas is a rather small tubular gland which delivers its digestive juice into the intestine by intermittent rather than by continuous flow. The discovery by Bayliss and Starling of the mechanism of pancreatic secretion is one of the romances of modern physiology. These workers proved that the mucosa of the duodenum contains a chemical hormone, which they have named *secretin*. The introduction of acid chyme, from the stomach, releases the hormone, which, by way of the blood stream, stimulates the pancreatic gland to pour forth its digestive juice. The release of secretin from the intestinal tissue is brought about, in all probability, by the contact of the acid stomach contents with the wall of the intestine The basis for this statement lies in the fact that the maceration of duodenal tissue with hydrochloric acid produces an extract which stimulates the flow of pancreatic juice. When this juice is injected into the blood stream of another animal, the flow of pancreatic juice follows immediately When intestinal extract is injected which has not been preceded by hydrochloric acid, no pancreatic stimulation can be noted. Furthermore, acid extracts of other organs do not have this effect. Further proof of the transfer of secretin by the blood was shown by a most ingenious experiment. The blood streams of two dogs were connected in such a manner that they possessed one

blood circulatory system. Tubes were placed in the pancreatic ducts of both animals, and a dilute acid solution was introduced into the duodenum of one of the dogs. The effect of the acid was to cause pancreatic secretion, simultaneously, in both dogs — proving that secretion of secretin took place in one dog and was transferred, by the blood, to the other animal. Secretin is not coagulable nor is it a protein. It is soluble in hot acid solution and in alcohol.

Pancreatic juice is a clear alkaline liquid which coagulates on heating. The alkalinity is due to the presence of sodium carbonate, and the coagulable portion consists of protein which, in some cases, is of sufficient quantity to form a solid coagulum on heating.

Enzymes of Pancreatic Juice. — Pancreatic juice owes its digestive properties to three enzymes, viz., trypsin (protease), amylopsin (amylase), and steapsin (lipase). When pancreatic juice is allowed to flow directly into a receptacle without coming in contact with the intestinal juices, it possesses no protein-splitting properties. If, however, the pancreatic juice is allowed to flow into the intestine and is then removed, it is found to be highly proteolytic. This has been explained by the presence in the intestinal juice of an activator called *enterokinase* which activates the zymogen *trypsinogen*, forming active *trypsin*. In other words, the enzyme trypsin is secreted by the pancreas in inactive form, from which it is changed to trypsin by contact with enterokinase. If the intestinal juice is boiled prior to mixing, trypsin is not formed, showing that enterokinase is heat-labile. The mechanism of this activation is far from clear. Some writers feel that considerable evidence exists to show that trypsin is, in reality, composed of at least two proteolytic enzymes.

The starch-splitting properties of pancreatic juice are due to the presence of the amylase *amylopsin*. When the juice is boiled the amylolytic properties are destroyed. Carnivora, which possess no salivary ptyalin, must depend upon amylopsin to digest any starches which may be ingested in the food. This enzyme, like ptyalin, hydrolyzes starches and dextrins to the maltose stage, at which point invertases must be present if the maltose is to be hydrolyzed to simple sugars.

Pancreatic juice not only assists in the emulsification of fats and oils but it also contains a very active lipase called *steapsin* which hydrolyzes the glycerides to glycerol and fatty acids. If pancreatic juice is boiled, these properties are lost. In the presence of bile, which will be discussed in a succeeding paragraph, steapsin acts much more rapidly and efficiently, hydrolyzing relatively larger amounts of fat per unit of time. Steapsin can be divided into two portions, neither of which possesses lipolytic activity in the absence of the other. When they are brought

together, fat-splitting is accomplished with ease. This is explained by assuming that steapsin possesses a co-enzyme, which is necessary for its normal lipolytic functions. Steapsin is very susceptible to acids, in which respect it differs markedly from trypsin.

Bile. — The second of the juices assisting in intestinal digestion is *bile* or gall, a bitter fluid secreted into the intestine by the liver. It may be green in color when fresh, but usually it is yellowish-brown. It is extremely bitter to the taste, possesses a specific gravity of about 1.020 and is characterized by containing bile pigments, bile salts, and the alcohol cholesterol. In the horse, the flow of bile is continuous, but in man and in animals such as cattle, sheep and swine, the bile is stored in the gall bladder, from which it pours, intermittently, through the bile duct to the duodenum. In man, the opening of the bile duct is very close to that of the pancreatic duct, which facilitates the intimate mixing of these important digestive fluids. In carnivora, the juices are delivered to the intestine through a common duct, whereas in the herbivora the ducts are separate and somewhat removed one from the other.

Bile does not possess digestive enzymes but plays a most important part in fat digestion. Because of its alkalinity and the presence of the bile salts (taurocholates and glycocholates) it assists in the emulsification of fats. In addition, the free fatty acids combine with the bile salts, in which form they seem to be able to pass through the intestinal wall. Many writers regard bile as nature's laxative, since it hastens peristalsis and absorption. By so doing, the chances for putrefaction of foods in the intestine are reduced to a minimum.

Bile contains several *pigments* of which *bilirubin* is considered the parent-substance. This pigment, which is yellowish-red or golden-red in color, may undergo chemical changes and form other pigments, the most important being *biliverdin*, which is green in color, and which may be formed from the parent pigment by oxidation. Biliverdin is changed to *stercobilin* by intestinal bacteria. This pigment is brown in color and gives to the feces their characteristic color. *Urobilin*, a urine pigment, is formed from biliverdin or stercobilin by reduction processes.

Hemoglobin, in the blood, is considered to be the parent-substance of bilirubin. This chemical transformation evidently takes place in the liver, the bile pigments arising from the iron-free portion of the hematin molecule. When the oxygenated red blood pigment, oxy-hemoglobin, is broken down, it yields a protein, *globin*, and a complex iron-containing ring compound called *hematin*. If the unoxygenated hemoglobin is decomposed it is called *hemochromogen*. From these compounds an iron-containing substance called *hemin* is prepared. When hemin, which is a hydrochloride, is decomposed — an iron-containing nucleus is obtained

which is called *heme*. The continental writers use the letters "ae" where we use the letter "e." As a result, these compounds are "haemoglobin," "haematin," "haemin," and "haem" in European literature. It has been shown that heme and hemin are present in plants and animals, and there is considerable evidence to show that these substances are closely related to respiratory changes in all living organisms.

It is impossible, in a book of this type, to discuss the structural relationships of the various pigments; however, the following diagram (Fig. 50) is instructive, inasmuch as it shows the relation of the animal pigments, one to another, as well as the relationship of plant compounds to animal compounds. The writers have not included formulae for the reason that much work remains to be done before we can discuss pigment structure with confidence

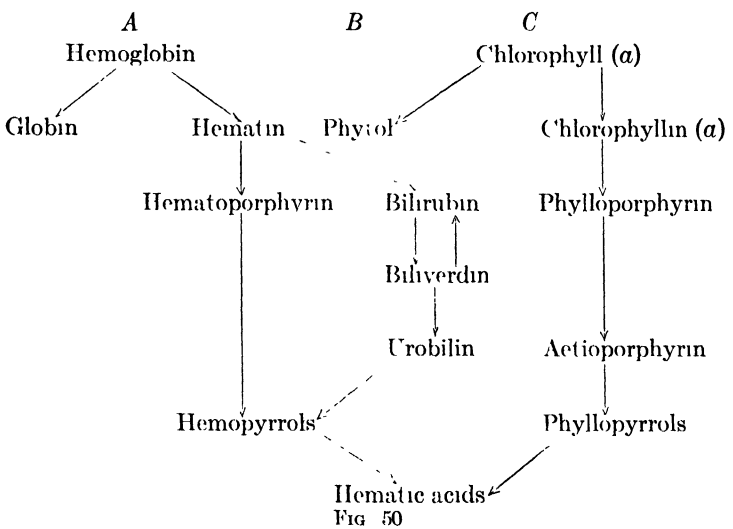


FIG 50

The bile salts are generally present as sodium salts of taurocholic and glycocholic acids. When taurocholic acid is decomposed it forms taurine (amino-ethyl-sulfonic acid) and cholic acid. When glycocholic acid is decomposed it yields glycocoll ($CH_2 \cdot NH_2 \cdot COOH$) and cholic acid. In other words, the bile acids are conjugated amino acids. Cholic acid ($C_{24}H_{10}O_5$) is probably somewhat similar to cholesterol in structure, but at this writing the structure of these compounds has not been ascertained with certainty.

Intestinal Juice. — This is the third digestive juice which aids in intestinal digestion; it is secreted by the glands which line the walls of the

small intestine. It is alkaline in reaction and contains at least four enzymes and a kinase, viz , *enterokinase* (activator of trypsinogen), *sucrase*, *maltase* and *lactase* (invertases which hydrolyze sucrose, maltose and lactose), and *erepsin*, a proteolytic enzyme

Chemical Changes in Intestinal Digestion. — When the acid chyme is introduced into the duodenum, some of the proteins, fats and carbohydrates are unchanged and some are incompletely hydrolyzed. Since nature strives to reduce these substances to the simplest and most soluble compounds, it is to be expected that intestinal digestion should finish what salivary and gastric digestion have started and initiate those changes that have not yet taken place.

The acid of the chyme is gradually neutralized by the alkaline intestinal contents, and the mass is mixed and passed on down the intestine by peristaltic muscular contractions which flow up and down the intestine. The pancreatic, bile and intestinal juices become mixed with the food, and proteins, fats and carbohydrates are hydrolyzed and prepared for absorption by the body.

Proteins. — Those proteins which have not been attacked are hydrolyzed, by trypsin and erepsin, to amino acids. The proteoses, peptones and peptids formed by gastric digestion are also hydrolyzed more or less completely by these enzymes.

Fats — In intestinal digestion, fats are emulsified and, because of the increased surface exposed, are caused to hydrolyze with much greater ease Steapsin, pancreatic lipase, splits the glycerides according to the following equation:

$$\begin{array}{l} CH_2 \cdot OOC \cdot (CH_2)_{16} \cdot CH_3 \\ | \\ CH \cdot OOC \cdot (CH_2)_{16} \cdot CH_3 \\ | \\ CH_2 \cdot OOC \cdot (CH_2)_{16} \cdot CH_3 \end{array} + 3\,HOH \rightarrow \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + 3\,CH_3 \cdot (CH_2)_{16} \cdot COOH$$

stearin — water — glycerol — stearic acid

Carbohydrates — Starch and the dextrins, which may have escaped hydrolysis in the stomach, are acted upon by *amylopsin* (pancreatic amylase), and the major portion of these polysaccharide molecules are changed to maltose. Maltose, sucrose and lactose are hydrolyzed to simple sugars, by the invertases, according to the following equations:

$$\underset{\text{maltose}}{C_{12}H_{22}O_{11}} + HOH \rightarrow \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{glucose}}{C_6H_{12}O_6}$$

$$\underset{\text{sucrose}}{C_{12}H_{22}O_{11}} + HOH \rightarrow \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{fructose}}{C_6H_{12}O_6}$$

$$\underset{\text{lactose}}{C_{12}H_{22}O_{11}} + HOH \rightarrow \underset{\text{glucose}}{C_6H_{12}O_6} + \underset{\text{galactose}}{C_6H_{12}O_6}$$

In ruminants and in the horse, cellulose is broken down by fermentation, and, in the case of straw and hay, some glucose, fructose and pentose sugars are found, although these may be destroyed to a considerable degree by the fermentive changes, since the rumen and the entire tract, with the exception of the stomach, are teeming with microorganisms. Methane, organic acids and alcohols are also by-products of these fermentive processes. It would appear, also, that the pentosans are digested in appreciable amounts. It can be seen, therefore, that all the complex food materials tend to break down, in digestion, to those simple substances which should be most soluble and most diffusible.

In terminating this brief discussion of the outstanding phases of intestinal digestion, we must not appear to have over-emphasized the alkaline reaction of the intestinal juices. To be sure, they are alkaline in reaction, but the amount of alkalinity is dependent on the type of indicator used. Recent work by McClendon and others, using electrometric methods, has shown that intestinal contents may be, often, neutral or even acid in reaction. In spite of this fact, it would appear that the normal chemical changes seem to go on unimpeded. This is to be expected, for many of the food materials are buffer substances which will tend to neutralize excessive amounts of acid or alkali. Furthermore, the general tendency, during digestion, would be for alkali to be used up and for acid substances to be formed.

The Divisions of the Intestinal Tract. — The small intestine is a rather narrow tube which starts at the stomach and ends at the *coecum*. At this latter point is a valve, known as the *ileo-coecal valve* through which the intestinal contents may pass to the coecum. The small intestine has been divided into three parts: (1) the *duodenum*, (2) the *jejunum*, and (3) the *ileum*. The length of the small intestine averages, in the horse, about nine times that of the body; in the ox and sheep, sixteen times; and in the hog, eleven times.

The *coecum* is an enlargement of the alimentary canal and serves as a storage organ for further digestion and fermentation. No digestive juices are secreted here, but the organ is of great importance in spite of this fact. In a way, it might be compared with the rumen or paunch of the ruminants, although it differs from the rumen in that digestive changes take place in the coecum owing to the presence of enzymes which originated in the small intestine The small intestine opens into the coecum, and not far from the intestinal inlet is another tube, somewhat larger, from which the coecal contents pass to the larger intestine. This large outlet tube is called the *colon* and just before it leaves the body it enlarges and becomes the *rectum*. Together, the colon and rectum are known as the large intestine.

Armsby states that, in a general way, the size of the coecum is inversely proportional to that of the stomach. In the horse, the coecum is very large and holds about 16 per cent of the total contents of the digestive tract. This organ allows the food to remain for long periods of time in contact with digestive juices which have come down from the small intestine, and as a result of these fermentive and putrefactive changes, the horse is able to digest and utilize crude fiber to a very appreciable extent. The coecum in ruminants is relatively small, with a capacity of about 3 per cent of the tract, and that of hogs is not much higher, being about 5 per cent.

In the horse, the large intestine is also highly developed and may comprise as high as 45 per cent of the total capacity of the digestive tract; like the coecum, it serves as a storage organ to continue chemical changes on refractory and indigestible foods.

In the domestic fowl two coeca connect with the intestine at the same point, forming a Y-shaped structure (See Fig 49)

Absorption. — The small intestine is lined with folds of tissue containing thousands of tiny finger-like projections known as *villi*, which increase the absorptive surface of the intestine enormously It was thought, at one time, that the proteins were absorbed, by the intestines, in the form of peptones This idea has been abandoned in recent years as the result of work by Folin and Denis, Van Slyke and co-workers and Abel and others. The evidence now points to the fact that proteins are absorbed mostly, if not entirely, in the form of amino acids These are absorbed in the small intestine and are either built into tissue or they are deaminized in the liver and the nitrogen is excreted as urea. These metabolic phases will be discussed in Chapter XXIV

At this point it might be well to emphasize that the villi occur in largest amount in the duodenum Absorption occurs in the jejunum and ileum in smaller amount per unit of length, but, on account of the fact that these portions of the intestine are much larger than the duodenum and possess more surface, it seems reasonable to believe that a greater portion of the absorption takes place in the jejunum and ileum.

The villi are supplied with two circulatory systems: (1) a blood system consisting of arterial and venous networks, and (2) a lacteal or single lymphatic capillary which communicates with the lymphatic system. From the villi, absorbed nutrients are carried via the mesenteric veins to the portal vein and thence to the liver and body tissues. A portion of the fats, however, are absorbed in the lacteals and reach the tissues via the lymphatic system, a rather poorly defined circulatory system, containing a fluid called lymph, which will be discussed in more detail in Chapter XXIII.

Fats are absorbed as glycerol and fatty acids and some of the fatty acids are absorbed as soaps and in combination with or in association with the bile salts. Although the mechanism of re-synthesis of fats is not clear, it is certain that fats are re-formed in the epithelial cells during absorption. As we have suggested in the previous paragraph, a large proportion of the fat is shunted around the liver by being carried to the tissues via the lymphatic system. This fat-rich lymph is carried by the thoracic duct, where it is poured into the blood stream at the juncture of the jugular and subclavian veins. This creamy lymph is called "chyle"

Carbohydrates are absorbed by the villi capillaries in the form of glucose, fructose and galactose and these simple sugars are carried by the blood stream to the liver where they are deposited as glycogen (animal starch) or they are sent to the tissues where they are utilized or deposited as glycogen Some disaccharide absorption may take place but it is considered of minor importance.

Absorption in the Large Intestine. — After the products of digestion have been absorbed in the small intestine the unabsorbed mass passes into the large intestine. The digestive changes which take place here are not of great importance. The mucous membrane of the large intestine contains gland cells, but the secretion is largely mucus and no digestive enzymes are secreted. The chemical changes which take place here are due largely to bacteria, but it is possible that enzymes may be carried through from the small intestine, and some hydrolytic changes may occur. Like the secretions in the small intestine, the secretions in the large intestine are usually alkaline. Putrefaction is a normal process, and the products of this change are such substances as proteoses, peptones, amino acids and other decomposition products such as indol, skatol, ammonia, etc. In addition to the putrefactive changes, carbohydrates, such as cellulose, are acted upon by fermentative organisms, leaving behind acids such as lactic, butyric and acetic acids, alcohols, and gases such as methane, carbon dioxide and hydrogen.

Water is absorbed in the large intestine, and the undigested mass gradually takes on the characteristics of feces. The feces are composed mainly of undigested food materials such as cellulose, keratin and other proteins, bacteria and fats. In addition to these are found the digested nutrients which have escaped absorption. Metabolic products such as indol and skatol are present, and it is to these substances that the odor of feces is largely due. In the next chapter we shall consider some of the more interesting and valuable facts regarding the chemical characteristics of some of the more important body tissues that are constructed from the nutrients which have just been discussed.

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CHAPTER XXIII

THE BODY TISSUES

After food materials have been digested and absorbed, the simple hydrolytic products are used in the repair and manufacture of tissues. Many of the typical body tissues differ markedly from one another in chemical composition, whereas others seem to be quite similar in this respect even though they may be very dissimilar so far as body functions are concerned. Because the products of absorption must pass to the tissues by way of the blood stream, it would seem profitable and advisable to discuss the chemistry of blood at this point.

BLOOD AND LYMPH

Blood. — Although it is not within the scope of this book to discuss anatomy or physiology, perhaps it would be helpful to refresh the reader's mind with the fact that the circulatory system consists of the heart, arteries, veins and capillaries. The heart, which is composed of muscle tissue, contains four compartments called the right auricle, left auricle, right ventricle and left ventricle. The nutrient-rich blood, returning from the tissues, enters the heart at the right auricle and passes to the right ventricle, which sends the blood to the lungs. Here the waste products (carbon dioxide and water) are eliminated in part and oxygen is inhaled and carried into the blood stream. The oxygen-rich blood is then carried to the left auricle, where it is forced into the left ventricle, and from here it leaves the heart to go to the body tissues, where the various nutrients are to be utilized. Blood is sometimes called the "liquid tissue," because of its importance and chemical characteristics.

Functions of Blood. — The main functions of blood may be classified as follows:

1. To carry nutrients (from the alimentary tract) and oxygen (from the lungs) to the body tissues.
2. To carry away waste products produced by the body cells during metabolism.
3. To transport internal secretions (hormones) from the ductless glands to other tissues of the body.
4. To serve in the distribution of heat and water.

The amount of blood in the bodies of different animal species (expressed as percentage of body weight) is given below:

Man	5 0 per cent
Swine	2 5 to 5 0 per cent
Fowls	7 7 to 10 0 per cent
Horse	10 0 per cent

The following table shows the composition of the blood of man as compared with that of a number of representative domestic animals.

TABLE 59

COMPOSITION OF BLOOD

Results expressed as mgm per 100 cc unless otherwise noted	Man	Albino rat	Dairy cattle	Horse	Sheep	Swine	Chickens
Non-protein nitrogen	25–35	15 2	30 1	34	32	35	44
Urea nitrogen	10–15	15 6	12 9	17 8	17 8	19 6	5 6
Uric acid	2–3 5	1 86	2 1	2 45	1 82	2 06	6 39
Creatinine	1–2	1 28	1 4	1 8	1 26	1 42	1 21
Creatine	3 7	6 03	4 3				3 26
Glucose	80–120	122 2	84 1	106	105	97 1	173
CO_2 capacity of plasma — Volume per cent	55–75		58 9				
Chlorides as NaCl	450–500	515 2	49 2				461
Calcium (serum)	9–11	10 0	12 6				9 9
Inorganic phosphorus (serum)	3–4	8–10	4 5				4 0
Hemoglobin (per cent normal haldane scale)	100		92 9				
Erythrocytes (per cu mm)	5,500,000		6,500,000	7,900,000			2,800,000
Leucocytes (per cu mm)	5,000–10,000		9,000	5,500			39,000

Constituents of the Blood. — The blood is composed of a large number of microscopical bodies known as blood corpuscles suspended in a straw-colored liquid called plasma. The plasma of blood may be separated into blood serum and an albuminous material known as fibrin. The corpuscles are divided into two classes: (1) the red blood corpuscles or erythrocytes, and (2) white blood corpuscles or leucocytes. A third group of microscopic bodies are known as blood platelets. It is from the red blood corpuscles that blood receives its red color. The liquid, or plasma, comprises about two-thirds of the blood, and the remaining third is composed of the substances in solution or suspension. When blood is whipped with a bundle of rods or twigs, the fibrin collects in a coagulated mass and is easily removed. The liquid which is left is known as defibrinated blood.

Properties of the Blood. — Blood shows an alkaline reaction to litmus, but recent physico-chemical methods have shown that it is practically

neutral. The specific gravity, which for human beings averages about 1.050, varies with the sex, type, and individual. After strenuous exercise the specific gravity is higher than normal, owing to the loss of water and the increase of waste products.

The Proteins of Blood. — The proteins of blood may be classified as follows:

1. Hemoglobin.
2. Serum albumin.
3. Serum globulin.
4. Fibrinogen.
5. Nucleo-protein.

Hemoglobin, the blood pigment, is the most important protein, from the standpoint of quantity, occurring in the blood. Fibrinogen is next in importance to hemoglobin; it is soluble, but becomes a stringy solid, known as fibrin, when clotting occurs

The Red Corpuscles. — The number of red corpuscles per cubic millimeter of blood in different animals varies with the type of animal and other conditions. In men the average number is about 5,000,000 per cubic millimeter, whereas in women it is somewhat lower, 4,500,000. The chief function of the red blood corpuscle is the carrying of oxygen. Hemoglobin is the most important constituent of red corpuscles, and it is to the hemoglobin that the oxygen-carrying properties are due. Blood which has been treated with certain substances becomes laked and is known as "laky" blood. This change is due to the rupturing of the red corpuscle membrane, allowing the hemoglobin to form a homogenous solution.

Hemoglobin. — This constituent is a conjugated protein and contains iron and sulfur in combination. It has been estimated that there is one atom of iron in each molecule of hemoglobin, and the molecular weights that have been given for hemoglobin vary from 14,000 to nearly 17,000. Oxygen forms a weak combination with hemoglobin (probably with the iron) known as oxyhemoglobin. Other gases, such as carbon dioxide, carbon monoxide, nitric oxide, etc., are thought to be capable of combining with hemoglobin to form similar compounds. Carbon monoxide poisoning seems to be merely a matter of oxygen starvation, the poison combining with the hemoglobin in place of oxygen. Other constituents of red blood corpuscles are water, salts, cholesterol, and lecithin.

White Blood Corpuscles. — These bodies, known as leucocytes, are not present in blood in such numbers as are the red corpuscles; for each colorless corpuscle there are 300 to 500 red ones. Leucocytes contain proteins, glycogen, lecithin, cholesterol and salts. Aside from aiding in absorption of fats, the function of leucocytes seems to be to act as blood

scavengers, affording protection against bacteria and foreign substances; there is also evidence that they cause chemical changes in protein and sugar. The blood platelets mentioned in the early discussion of blood are pale, round bodies whose chief function seems to be that of assisting in coagulation.

Blood Plasma. — This straw-colored liquid contains water, proteins, salts, fat, sugars, and other substances The important proteins are fibrin, serum globulin and serum albumin; the salts are mainly chlorides, sulfates, carbonates, and phosphates of sodium, potassium, calcium, magnesium and iron.

Coagulation or Clotting. — When a blood vessel is cut and nature has plugged it effectively, by means of a clot, we are curious, naturally, to see what the mechanism of this most important protective device may be. It is clear that the change which is most responsible for clot-formation is that of soluble *fibrinogen* changing to stringy, insoluble *fibrin.* This substance enmeshes the corpuscles, and the jelly-like clot is formed. The process is much more complicated than this, however. Why does the blood not coagulate or clot in the blood vessels? This is a question that has intrigued scientists for many years.

According to Howell, of the Johns Hopkins University, blood cannot coagulate in the absence of calcium salts. He postulates that the fibrinogen cannot change to fibrin until it is activated by a blood-coagulating enzyme called *thrombin* Thrombin seems to exist in an inactive form called *prothrombin* or *thrombinogen.* Howell is of the belief that, when blood is shed, the *blood platelets* disintegrate, liberating a lipid (quite similar to lecithin) called *cephalin.* This lipid (cephalin) combines with *antithrombin,* which liberates prothrombin, which immediately changes to active thrombin by the action of calcium salts. The thrombin then transforms the blood fibrinogen into elastic, stringy fibrin, which forms the clot

The blood of higher animals is heavier than water, possessing a specific gravity varying from 1 026 to 1 045, and contains about 20 per cent of total solids. In addition to the constituents already mentioned, the blood plasma and serum contain practically all the substances used and formed in all phases of the metabolic processes. Such substances are glucose, urea, uric acid and urates, amino acids, lecithin, cholesterol and its esters, fats, enzymes, carbamic acid, creatine, nucleo-proteins, acetone bodies, gases, ammonia, pigments, mineral constituents, hormones and possibly vitamins. All blood from the gastro-intestinal tract passes through the liver before entering the general circulation and, on the other hand, all blood returning from the capillaries of the tissues passes through the lungs before being sent into the general circulation.

Lymph. — In addition to the blood circulatory system there is another system, which is less well defined, known as the lymphatic system. This system contains a creamy colorless liquid called lymph, which resembles blood plasma. The lymph bathes all cells of the body and serves as a medium of transportation for food nutrients from the blood stream to the cells and waste products from the tissues to the blood stream. Considerable fat is absorbed from the intestinal villi via the lacteals and passes by way of the lymphatic system to the blood.

THE SUPPORTING TISSUES

In our discussion of digestion, absorption and the circulatory systems we have dealt with what might be termed the organs of alimentation. We shall now consider those tissues of the body that are able, because of their peculiar strength and elasticity, to support the softer body tissues. Such supporting tissues are bones, tendons, ligaments, and cartilages.

Bones. — Bones form the skeletal framework of the animal body and are composed largely of mineral matter. In the young embryo, bones appear first as cartilaginous structures consisting of cells imbedded in a homogeneous intercellular substance which is largely the albuminoid protein called collagen. When ossification takes place, the intercellular spaces begin to fill with mineral salts of which tricalcium phosphate, $Ca_3(PO_4)_2$, is the most important. The bone marrow contains a protein called ossein, which is very similar to collagen in its chemical characteristics, and fat is also present in appreciable amounts. Ossein may be hydrolyzed by steam to form gelatin.

The following analyses by Ingle give some idea of the gross composition of bone:

COMPOSITION OF FRESH BONE FROM MULES

Water	5 34 per cent
Organic matter	37 77 per cent
Ash . . .	56 89 per cent
The organic matter contained.	
Fat .	7 61 per cent
Nitrogen . .	3 99 per cent
The ash contained	
CaO	32 28 per cent
P_2O_5	21 86 per cent
SiO_2 .	0 09 per cent

Zalensky has analyzed the ash of bones, the results of which are given on the following page:

	Man	Cattle
$Ca_3(PO_4)_2$	83 89 per cent	86 09 per cent
$Mg_3(PO_4)_2$	1 04 per cent	1 02 per cent
Ca combined with		
CO_2, Cl, F	7 65 per cent	7 36 per cent
CO_2	5 73 per cent	6 20 per cent

In rickets, osteomalacia, osteoporosis, and similar bone diseases, the ossification process is interfered with. These conditions may be brought about by calcium and phosphorus starvation, by abnormal calcium-phosphorus ratios in the diet, by the absence of vitamin D or ultraviolet light, or by pathological conditions.

Lactating animals, producing relatively large amounts of milk, are prone to do so at the expense of their skeletons, unless the diet is adequate with regard to calcium and phosphorus. Investigators have found that it is quite difficult to keep high producing dairy cattle supplied with sufficient calcium in the proper form to prevent a negative calcium balance.

Teeth. — Teeth are composed of cement, dentine and enamel The cement seems to be identical with bone in its chemical characteristics. Dentine is present in the largest quantity; although it is similar to bone in chemical composition, its structure is different The enamel, which contains about 5 per cent of water, is the hardest tissue in the human body; it contains about 50 per cent of calcium oxide, CaO, and 40 per cent of phosphorus pentoxide, P_2O_5

Connective and Epidermal Tissues. — Connective and epidermal tissues serve to bind together the other body tissues and are characterized by great strength and elasticity The types of tissues generally classified under this heading are tendons, ligaments, cartilages and skin. The following table, taken from Hawk's " Practical Physiological Chemistry," shows the analysis of fresh tendon.

TABLE 60

	Per cent
Water	62 87
Solids	37 13
Inorganic matter	0 47
Organic matter	36 66
Fatty substance (ether-soluble)	1 04
Coagulable protein	0 22
Mucoid (a carbohydrate-protein combination)	1 28
Elastin	1 63
"Collagen"	31 59
Extractives, etc.	0.90

Collagen, elastin and keratin are the principal proteins in connective tissue. Elastin occurs principally in that type of connective tissue which must be strong and elastic. Keratin occurs in hair, hoofs, horn, fingernails, wool and feathers. Elastin contains no sulfur, whereas collagen contains about 0.6 per cent and keratin 4 to 5 per cent of this element. Collagen yields gelatin on long-continued boiling with water.

THE TISSUES OF MOTION

In this group we shall, arbitrarily, include only the muscle and nervous tissues.

Muscle Tissue. — This important tissue comprises about 42 per cent of the body weight in man, and from 50 to 75 per cent of the total body metabolism occurs in this tissue, depending, of course, on the physical activity of the individual. Muscle tissue is of two types, voluntary and involuntary. Voluntary muscle tissue represents the largest proportion of muscle tissue in the body and is subject to the will Involuntary muscle, over which we have little or no control, is found in such tissues as the arteries, uterus and bladder.

The reader is already familiar with the characteristics of muscle, and we shall not dwell on them other than to point out their unique properties of elasticity and contractility. Muscle cells are elongated structures composed of tiny threads or fibrils which have the power of shortening when properly stimulated. From a chemical standpoint, muscle cells are characterized by a very large amount of protein, although fats, carbohydrates, mineral salts and other substances are present also Fresh muscle contains about 75 per cent of water.

Muscle Protein. — It is almost impossible to describe, with any degree of accuracy, the types and forms of proteins in living muscle, for the reason that it is impossible for the chemist to work with living muscle tissue. All the work has been done upon dead muscle cells The most accurate work has been done upon muscle tissue which has been frozen the moment the animal is killed. This should prevent enzyme action and preserve the chemical constituents in a condition very similar to that in the living tissue. Such studies have shown that there are two types of muscle protein, *myosinogen* and *paramyosinogen.* The former comprises about 75 per cent of muscle protein; the other 25 per cent is largely paramyosinogen. When the muscle clots and rigor mortis sets in, both these proteins change in physical and chemical characteristics, and the resulting " clot " is known as *myosin.*

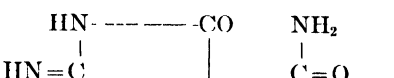
Muscle Carbohydrates. — Muscle tissue, like many plant tissues, tends to lay down or store a characteristic carbohydrate. This carbohydrate

is known as *animal starch* or *glycogen.* Glycogen is similar to starch in appearance and properties, being a white, amorphous solid which hydrolyzes to glucose. Chemical analysis shows that it has the same empirical formula as starch, $(C_6H_{10}O_5)_n$, and yields the same chemical products. The liver also has the power to store relatively large quantities of this carbohydrate Dextrin and glucose have also been identified as constituents of muscle tissue, but the quantities are exceedingly small

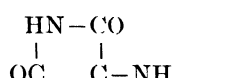
Muscle Extractives — When muscle tissue is treated with water, alcohol and ether, a number of characteristic chemical substances are removed These substances are known as *extractives* and are classified, with reference to their nitrogen content, into two groups: (*a*) non-nitrogenous extractives such as glycogen, glucose, inosite (a six-carbon alcohol having the formula $C_6H_{12}O_6$), lactic acid, and fatty compounds; and (*b*) nitrogenous extractives such as creatine, creatinine, xanthine, hypoxanthine, uric acid and urea.

The following are the structural formulae for the more important of the nitrogenous extractives:

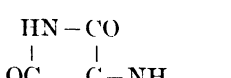
Creatine ($C_4H_9N_3O_2$)



Creatinine ($C_4H_7N_3O$) Urea (CON_2H_4)



Uric acid ($C_5H_4N_4O_3$)



Xanthine ($C_5H_4N_4O_2$)

Nervous Tissue. — This type of tissue is without doubt the most important tissue in the human body, for, by its functions, man and the lower animals are clearly differentiated Nerve tissues are scattered throughout the body. Those that end in the extremities and terminate at the body surface are small in size, but these join others, forming larger trunks, and these eventually join the spinal cord, which, in turn, is attached to the brain Many writers have shown that the survival of the fittest has been due in a large measure to superior nervous development It is this type of tissue that is irritable, receives responses, gives us memory, and controls all our psychic and motor activities

When we study the chemistry of the brain and nervous tissues, we find that they differ very materially from other tissues in one important

respect, viz., they contain relatively large amounts of alcohol-ether soluble materials. These lipids have been studied, and it has been found that neutral fats are present in traces only. Of the many substances present, phospholipids, glycolipids and cholesterol are probably the most important. Proteins are present, but they do not stand out as characteristic materials. The white matter of the brain is richer in water (85 per cent) than the gray matter (70 per cent) In youth the water content is high and gradually becomes lower as man becomes older.

Thudichum is responsible for a great deal of our early knowledge regarding brain-chemistry. His first work, published about 1884, is considered a classic in this field. He found an insoluble portion which contained proteins, nucleo-proteins and phospho-proteins. Examination of the " white substance " showed the following to be present: Cephalin, lecithin, cholesterol, phenosterol, glycolipids, sulfolipids, aminolipids, and similar substances. A water extract revealed inorganic salts, organic acids and salts, inosite, amino acids and purine derivatives.

The brain and nervous tissues, in general, possess very high respiratory or oxidative metabolism, which is not to be confused with growth or formative metabolism. Carbon dioxide production is high when nervous tissue is active and oxygen consumption is equally high. Theories of nerve impulse are most interesting. Most authorities are agreed that nervous impulses are physico-chemical in nature. Electrical disturbances, in addition to chemical changes, accompany nerve activity. It is not impossible that the time may come when we shall be able to interpret some phases of our psychic life in terms of chemistry and physics.

THE RESERVE TISSUES

Most of the tissues described in previous pages have had definite duties to perform, definite work to do. In other words, these tissues have comprised what Armsby calls " the working machinery " of the body. Another function of certain cells is the storing of reserve food supplies that may be called upon in time of stress.

Fatty Tissues. — Practically every tissue in the animal body contains fat in some form, although there are certain tissues which are characterized as fatty tissues. *Adipose tissue* is a representative fatty tissue and is usually laid down in the abdominal regions in humans. The nature of adipose tissue, kidney fat and intestinal fat is very largely dependent upon the kinds and amounts of fatty acids in combination. If stearic and palmitic acids predominate, the tissue is firm and hard, whereas the reverse is true if oleic acid is present in sufficient amounts. Body fats are composed, very largely, of stearin, palmitin and olein, although other

fats are present. Fatty tissues in hogs usually contain larger percentages of fat and lower percentages of water than those of sheep or cattle. Hog tissue may contain as much as 92 per cent of fat. As a rule, the subcutaneous fats (those immediately under the skin) are softer and possess more olein than the deeper fats. Although animal fats do not appear to differ appreciably in chemical composition from plant fats, they possess different nutritive properties, owing to the difference in concentration of fat-soluble vitamins. Beef and mutton tallow, representative of the hard fats, are characterized by large amounts of stearin and palmitin, whereas beef oil is rich in olein. Human fat falls between these extremes.

THE GLANDULAR TISSUES

Certain tissues in the body are capable of elaborating and discharging secretions which are of the utmost importance to the welfare of the animal body. These are known as glandular organs, and they possess peculiar cells known as "gland cells." The glandular tissues do not, of themselves, differ strikingly in chemical composition from other body tissues, but they are of interest to the student of agriculture because of the chemical substances which they elaborate.

These tissues may be classified in two groups: (1) Glands with ducts which secrete their chemical materials in such a manner that they may pass from the body, and (2) glands which possess no ducts and secrete their chemical materials directly into the blood stream where they are carried to other tissues. The latter are known as ductless glands, endocrine glands or glands of internal secretion.

The Glands of External Secretion

This group of glands is of great importance to the body since the secretions are used for digestion of food, the feeding of the young or the excretion of waste products. As we have said, these secretions are eliminated from the body eventually. The following list of glands and their secretions will give the reader some idea of this type of tissue and its function.

1. Salivary glands (secreting saliva) (*See* Salivary Digestion.)
2. Gastric and intestinal glands (secreting digestive juices) (*See* Stomachic and Intestinal Digestion)
3. Mammary glands (secreting milk).
4. Pancreas and liver glands (secreting pancreatic juice and bile, respectively). (*See* Intestinal Digestion.)
5. Sweat glands (secreting perspiration)

Petersen, Palmer and Eckles report a series of interesting experiments in which the udders of cows have been removed at the time of slaughter.

These were transferred quickly to a constant-temperature moist chamber, were hung in their natural positions and milked by hand in the regular manner. In some cases the right side of the udder was milked dry prior to slaughter and the post mortem milking of the left side was conducted as described above It was found that practically as much milk was obtained in the post mortem milkings as in the *in vivo* milkings. The authors conclude that milk secretion occurs, in the main, during the interim period between milkings, and that milk secretion does not occur at milking time, as many physiologists have believed.

The Glands of Internal Secretion

These glands, unlike those just discussed, have no ducts through which the secretion may flow. Instead, the chemical substances are taken directly into the blood stream. For the reasons just enumerated, these glands are also known as the "ductless glands" or "the glands of internal secretion." They are also known in medicine as the "endocrine glands" or "endocrine organs," and the division of medicine which deals with the study of these glands is known as endocrinology and the clinical application as opo-therapy.

These tissues are unique in that they manufacture, and secrete into the blood stream, chemical substances necessary for the normal functioning of the body tissues. Apparently, these chemical substances are necessary to stimulate other organs to function in a normal manner. These body regulators, manufactured by the ductless glands, are known as *hormones*, a term which means "chemical messengers." A few of these glands and their hormones will be discussed at this point. It might be well to state that extracts from some of these glands have been studied from which as yet no definite hormones, of known chemical structure, have been isolated. A few compounds have been isolated from some of the endocrine organs, however, and their chemical structure has been determined. In fact, in a few cases, it is no longer necessary to go to the animal organs for these curative hormones, for they are now being synthesized in much purer form by the manufacturing organic chemist. To give the reader some idea of the potency of these hormones, it should be pointed out that one part of adrenalin (from the adrenal glands) in 300,000,000 parts of water will cause physiological response in animals. Dr. Hoskins, of Harvard, has put this dilution idea into everyday language by explaining that, to obtain this dilution, it would require 40 miles of water carts (200 carts to the mile and 625 gallons to the cart) for each ounce of adrenalin.

The Thyroid Gland. — This gland consists of two lobes connected by an "isthmus" which is attached (in man) to the trachea at that point

of the throat or neck which is adjacent to the suprasternal notch. When this gland is removed from young growing animals they are stunted. A study of the heat output of such animals shows that the metabolic rate has become much slower than with normal animals of the same age and weight. In fact, within one week after the removal of the thyroid, the basal metabolism will be lowered as much as 40 per cent below normal. Occasionally, people or animals possess thyroid glands which fail to develop and symptoms appear which are much the same as those described above. When this disease (myxedema) develops in humans, the person is stunted mentally as well as physically. The skin becomes dry and thick and the hair becomes sparse.

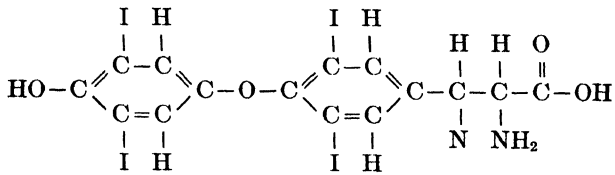
It was discovered that such cases responded and tended to become normal when thyroid glands of domestic animals were fed. Around 1895, it was discovered that the thyroid gland was about the only body tissue that contained appreciable amounts of iodine This observation led to the discovery that iodine-deficient diets were prone to cause myxedema and lowered metabolism, and that iodine-containing diets were curative in such disorders. Normal human thyroid glands may contain from 12 to 25 milligrams of iodine, whereas the same gland, in persons afflicted with goiter, may contain but traces of this element When children are afflicted with goiter of the type produced by iodine-deficiency, the disease is called *cretinism* and the stunted children are known as *cretins*. In adults the disease is called *myxedema*, and in both cases the disease is also designated as *simple goiter* by many writers. The disease is not common in sea-coast communities but is found to be quite common in inland districts, particularly in the Alps section in Europe and the Great Lakes region in this country. Goiter is not peculiar to man, but has been found to occur in cattle, horses, sheep and swine and even in fresh-water fish

McClendon and co-workers have endeavored to correlate the iodine content of waters (and in foods raised in the various regions) with the occurrence of goiter. In general, the correlations are quite marked — the disease being most common in iodine-poor regions and less common in iodine-rich regions.

In 1916, E. C. Kendall isolated a few grams of the iodine-containing hormone from several hundred pounds of the thyroid gland. It crystallized in sheaves and rosettes, melted at about 250° C , and was soluble in alkali but insoluble in acid. It contained carbon, hydrogen, oxygen, nitrogen and iodine, the latter comprising about 65 per cent of the molecule. Kendall and his co-workers found that this substance was the most active principle of the thyroid gland, and that its action in metabolism was identical with that of the gland itself. On account of its effect

on the oxidative or heat-producing mechanism of the body, Kendall named the hormone *thyroxin.*

It remained for Harington and his co-workers in 1926 to determine the chemical structure of thyroxin. They have shown it to possess the empirical formula $C_{15}H_{11}O_4NI_4$. Structurally, thyroxin was found to be beta-tetra-iodo, hydroxy-phenoxy-phenyl-alpha amino propionic acid:



Thyroxin

If the reader will examine the formula for thyroxin, as suggested by Harington and co-workers, he will note that this hormone is really an ether, which consists essentially of an oxygen linkage connecting a hydroxy-phenyl group with what was formerly the amino acid tyrosine. Recent work indicates that di-iodo-tyrosine is undoubtedly the precursor of thyroxin in the animal body.

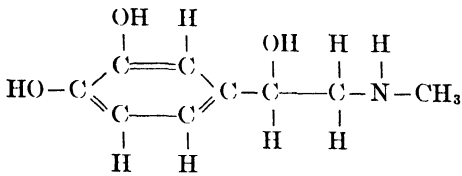
In 1927, Harington was able to synthesize this compound, and Lyon showed that its physiological action was identical with that of thyroxin obtained from the thyroid gland. This is one of the many triumphs of modern biochemistry, and the time is not far off when synthetic thyroxin will be available to the medical profession for the treatment of goiter at a price within the reach of every one, which was not the case when it had to be obtained from desiccated thyroid glands of domestic animals.

The Suprarenal Glands. — Fitting to the top of each kidney are two small endocrine glands called suprarenal or adrenal glands. These glands consist of an inner portion, the *medulla,* and an outer portion, the *cortex.* The latter is surrounded with a fibrous capsule. It is the medulla which possesses the property of synthesizing a hormone known as adrenalin or epinephrin.

In 1855 Addision described a disease which later became known as Addison's disease. In this disorder, which was often fatal, Addison finally traced the trouble to the diseased suprarenal glands, which subsequent research has shown were the cause of the disease. There is much clinical evidence that the cortex of the adrenal gland contains a hormone that has to with the normal development of the individual, particularly with regard to normal sex characteristics, but to date no hormone has been isolated.

An extract of the medulla has a definite effect on blood pressure, caus-

ing a strictor action on the blood vessels. The hormone was first isolated by Abel and Crawford in 1897, as a sulfate. It was found to contain carbon, hydrogen, oxygen and nitrogen It combined with acids to form salts and in the active form is levo-rotatory. Structurally, it was found to be di-hydroxy-methyl-amino-ethylol-benzene.



Epinephrin (or adrenalin)

Epinephrin causes contraction of the musculature of the circulatory system, with a consequent rise in blood pressure. In addition, this hormone has been used in the form of hydrochloride, in surgery, to check surface bleeding. It has also received considerable publicity in recent years through its use in stimulating the heart-beat in patients whose hearts have ceased to beat as a result of shock

The Pancreas. — We have already considered this organ as a producer of pancreatic juice. We shall now consider it from the standpoint of an endocrine organ.

When the pancreas is removed, animals develop a disease known as diabetes, a metabolic disorder in which the body loses its power to oxidize fats and sugars. In this disease, the sugar content of blood becomes high (hyperglycemia) until finally the sugar " spills over " into the urine. When this occurs the condition is known as " glycosuria." If the disease becomes sufficiently severe, acetone, acetoacetic acid and oxybutyric acid occur in the breath and in the urine Formerly, dietary treatment was about the only treatment that yielded satisfactory results — the patient being forced to subsist on a diet poor in glucose-forming carbohydrates and in fats. Modern endocrine chemistry has discovered the hormone the absence of which causes diabetes. It is called the *diabetic hormone* or *insulin*.

About 1869, an anatomist named Langerhans described aggregates of cells in the pancreas that did not appear to function in the secretion of pancreatic juice. These areas became known as the *Isles of Langerhans*. Twenty years later, Minkowski and Von Mering showed that complete removal of the pancreas produced diabetes, but that if a small fragment of the gland was allowed to remain the disease did not develop. It was suggested that the gland possessed a hormone that had to do with the control of carbohydrate oxidation. Later this suspicion was verified

when pathologists noted that the Isles of Langerhans were invariably affected in patients who had died from diabetes. In 1916, Schafer postulated an internal secretion produced by these Isles of Langerhans, and he suggested that the hypothetical hormone be called "insulin."

It is impossible in a book of this type to dwell on the historical development of this valuable and interesting study except to say that, after many years of discouraging research by many workers, Dr. Banting, of Toronto, Canada, finally succeeded in obtaining an extract of the pancreas which contained the hormone. Banting and Best ligated the pancreatic ducts, causing the cells which produce pancreatic juice to atrophy. The Isles of Langerhans, however, continued to function. An extract of the atrophied pancreas caused a lowering of blood sugar in diabetic dogs. At first the extracts were destructive to the tissues in which they were injected, but this difficulty was finally overcome. Insulin was finally developed to the point where it could be made on a commercial scale, and it has proved to be of great value in the prevention and cure of diabetes. The dietary treatment of the patient is not neglected, although diabetics can tolerate more carbohydrate when insulin is administered.

Although the chemical structure of insulin has not been determined, chemical research has made considerable advances with regard to its chemical properties. It is known that insulin preparations contain from 13 to 17 per cent of nitrogen. Sulfur is present, but no phosphorus can be detected. It is soluble in dilute solutions of hydrochloric acid, and the purest preparations possess an iso-electric point, at which they precipitate, of *p*H 4.3 to 5.7. This hormone does not dialyze through parchment or collodion membranes, and it can be adsorbed on charcoal, fuller's earth and Lloyd's reagent. It is stable in acid but unstable in alkaline solutions, and it can withstand boiling temperatures in the presence of dilute acid. It is of interest also to the student of organic chemistry to note that insulin responds to some of the so-called color reactions for proteins. Abel and Geiling have succeeded in preparing insulin in crystalline form, and it is hoped that the time is not far off when its structure and, subsequently, its synthesis will be accomplished.

The Parathyroid Glands. — These small glands are situated in the lower part of the neck close to the thyroid glands. Their presence and importance were lost sight of for many years, owing to the fact that they were usually removed with the thyroid glands in experimental operations. As a result, their separate existence and function were not recognized until about 1891, when Gley showed that they were separate organs possessing different functions. He showed that extirpation of the parathyroid glands brought about tetany in experimental dogs, and it was

found, subsequently, that this tetany was accompanied by a lowered blood calcium and that the tetany could be relieved by calcium administration.

Extracts can be made from the parathyroid glands of cattle which will keep parathyroidectomized animals in normal health. This is ample proof that a hormone-like substance is involved, but, to date, no one has been able to isolate and identify it. It is quite evident that this hormone functions in the body as a blood-calcium regulator It is in problems of this type that the biochemist has the privilege and opportunity to cooperate with the physician in the advancement of biological and medical science.

The Pituitary Gland. — This gland, which is also known as the "hypophysis," is a small structure situated near the base of the brain. It consists, anatomically, of three portions, namely, the anterior lobe, the intermediate lobe, and the posterior lobe. It is quite evident that more than one hormone is produced by this gland, and that the various lobes differ in function The anterior lobe contains a hormone-like substance which, when fed or injected in excessive amounts, produces abnormally rapid growth (gigantism), and, in females, this substance seems to have a definite action on the reproductive system, inhibiting ovulation and oestrum (heat).

The posterior lobe is thought to be a reservoir for hormones produced by the intermediate lobe. Until very recently, little was known about these hormones Oliver Kamm and associates announced in 1927 that, by precipitation, dialysis, extraction, and solution with organic solvents, they were able to identify two active principles — one which raises blood pressure and another which stimulates the contraction of uterine muscle. They state that these principles have been prepared as white, stable, water-soluble powders which are from 80 to 150 times more potent than the International Standard Powdered Pituitary Both principles are basic bodies and are believed to be amines With this difficult separation and purification accomplished, it should not be long before these hormones will be synthesized in the research laboratory.

The Sex Glands. — Space will not permit further discussion of the endocrine glands other than to say that potent extracts have been made from ovaries and testes, which possess definite physiological effects. No hormones have been isolated to date, but much progress has been made It is not unreasonable to believe that biochemistry, in cooperation with medicine, will, in the not far distant future, be able to identify and synthesize hormones and vitamins. This is undoubtedly the chemical era in medicine just as in industry Recently, Doisy and co-workers have reported the isolation of a follicular hormone, from the urine of

pregnant women, which they have named " folliculin." This hormone, when injected into non-pregnant females, possesses marked effects on oestrous function, and for this reason the substance is sometimes called the *oestrous hormone.*

In previous chapters we have studied the changes taking place in digestion and we have learned how these materials are absorbed. In this chapter we have endeavored to point out some of the chemical peculiarities and properties of the various body tissues. In the following chapter we shall consider the chemical changes which take place during metabolism.

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CHAPTER XXIV

METABOLIC CHANGES IN TISSUES

In Chapter VII we noted that the synthetic or constructive changes in plants and animals are known as anabolic changes, whereas those which are destructive in nature are designated as catabolic processes. In the living organism, these changes are going on simultaneously and continuously, and the total summation of such chemical transformations are known as metabolic changes.

In previous chapters we have studied the chemical compounds present in food and the changes they undergo in digestion We have considered the forms in which they are absorbed, and we have become acquainted with the more important chemical properties of the various body tissues which are synthesized from the above-mentioned products of digestion. In this chapter we shall consider a few of the important types of chemical transformations which take place as these tissues undergo metabolic changes.

PROTEIN METABOLISM

We shall start our discussion with a consideration of the utilization and elimination of the nitrogenous substances obtained from our food. The reader is aware that carbon dioxide and water are eliminated from the body in exhaled breath, and that the urine contains nitrogenous products produced by tissue breakdown. It shall be our objective to attempt to trace these changes from the time the amino acids are absorbed in the intestine until they are formed into tissue and, finally, until they are eliminated as waste products in the urine.

The solution of this problem is not so simple as it sounds, for many of the steps are unknown and cannot be ascertained until we know more about the nature of protoplasm. Since the tissues of the body consist largely of proteins, it is logical to postulate that the amino acids must be used, to a great extent, in the repair and synthesis of tissues This was suggested for the first time by the researches of Van Slyke and co-workers and by Folin and his colleagues. These investigators found that the amount of amino nitrogen in the blood stream increased rapidly, immediately following a meal or following amino acid injection. Later Abel, by means of diffusion studies, was able actually to separate amino

acids from the blood as it circulated. This marked a step in advance in our knowledge of protein metabolism, for, prior to this work, it was thought by most workers that protein digestion products were absorbed in larger aggregates. In fact, it was thought that proteins could be absorbed as such, or at least as simple peptids and polypeptids.

Van Slyke and Folin also made the observation that the rise in the amino nitrogen of the blood was but temporary, and that it disappeared rather rapidly following absorption. Further studies showed that the disappearance of amino nitrogen from the blood was accompanied by an immediate increase, in the tissues, and that this phenomenon was followed by an increase of ammonia in the muscles and an increase of urea in the liver and urine. In other words, it has been concluded that the amino acids are absorbed from the intestine and carried to the tissues, which are loaded, for a time, with more amino acids than they can use. These tissues appear to be able to pick and choose, from these acids, those that are needed, at the time, for repair and synthesis of tissues. This is followed by protein hydrolysis and deamination of free amino acids which have been liberated or which were not utilized by the cells. Simultaneously, with deamination, oxidative changes begin to take place. It will be necessary to discuss these changes, one by one, in order to visualize the probable mechanism of protein metabolism.

It does not seem to matter whether the changes occur in the muscle cells or in the liver, for the same fundamental changes are involved when the body attempts to prepare these amino acid molecules for oxidation. All tissue cells play a part in this process, but it is advisable to point out that the liver is undoubtedly the most important organ in disposing of the excess of nitrogenous nutrients.

Deaminization. — The removal of amino groups is termed "deaminization" or "deamination." It is thought that deamination is really a process of alpha oxidation, i.e., the original amino acid is oxidized at the alpha carbon, accompanied by the loss of the amino group and the subsequent formation of α-ketonic acids This can be explained best by the examples given in the following equation:

$$\underset{\substack{\alpha\text{-amino propionic acid}\\(\text{alanine})}}{CH_3 \cdot CH \cdot NH_2 \cdot COOH} \rightleftarrows \underset{\text{pyruvic acid}}{CH_3 \cdot CO \cdot COOH} + \underset{\text{ammonia}}{NH_3}$$

Similarly when γ-phenyl α-amino butyric acid is deaminized, the corresponding α-keto acid is formed.

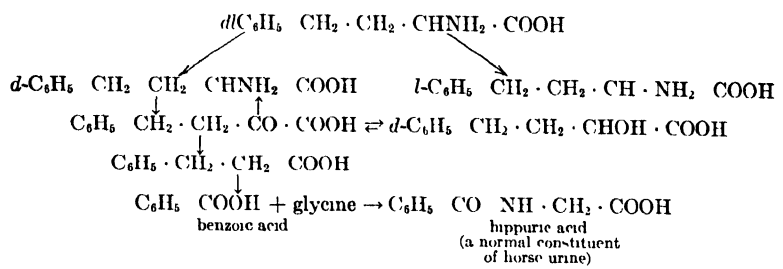
$$C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CH \cdot NH_2 \cdot COOH \rightleftarrows C_6H_5 \cdot CH_2 \cdot CH_2 \cdot CO \cdot COOH$$

Dakin has shown that the formation of the α-keto acid is undoubtedly the first step in the deamination, and that the second step is the formation of an α-hydroxy acid:

$$\underset{\text{pyruvic acid}}{CH_3 \cdot CO \cdot COOH} \rightleftarrows \underset{\alpha\text{-hydroxy propionic acid}}{CH_3CHOH \cdot COOH}$$

Research work has shown that the keto acids may undergo three types of change: (1) oxidize to lower fatty acids, carbon dioxide and water; (2) reduce to hydroxy acid; or (3) their ammonium salts may be reduced to amino acids. The first type of change, viz., oxidation to lower fatty acids, is known as beta-oxidation. This will be discussed when we consider fat metabolism, but we should call the reader's attention, in passing, to the fact that these fatty acids lose two carbon atoms when oxidation takes place, forming an acid containing two less carbons than the original acid. It appears that complex amino acids are oxidized less readily than the simpler ones. For example, cystine, tyrosine and tryptophane are not oxidized as readily as alanine and glycine.

The following schematic outline by Dakin will give the student the relationship of one product to another. It also emphasizes one point which we have failed to mention, viz., that dextro forms seem to be utilized whereas the levo forms do not:



The above outline also emphasizes another fundamental fact that should be kept in mind, viz., that benzene nuclei are not oxidized but are eliminated as such. In this particular case it shows how benzoic acid is eliminated as hippuric acid, a salt of benzoic acid and glycin. In other words, amino acetic acid acts as a "detoxicating" agent so far as benzoic acid is concerned

Urea. — The body protects itself from the alkaline ammonia formed by deamination of amino acids, by causing it to combine in harmless combinations with acids One of these acids is carbonic acid, formed by the action of carbon dioxide and water. Ammonium carbonate may lose a molecule of water to form ammonium carbamate. The latter sub-

stance may lose another molecule of water with the formation of urea, in which form the bulk of waste nitrogen is eliminated in the urine. These reactions, as they are normally given, may be represented as follows:

$$\underset{\text{water}}{H_2O} + \underset{\substack{\text{carbon}\\\text{dioxide}}}{CO_2} \leftrightarrows \underset{\substack{\text{carbonic}\\\text{acid}}}{H_2CO_3}$$

$$\underset{\substack{\text{carbonic}\\\text{acid}}}{H_2CO_3} + \underset{\text{ammonia}}{2\,NH_3} \rightarrow \underset{\substack{\text{ammonium}\\\text{carbonate}}}{(NH_4)_2CO_3}$$

$$\underset{\substack{\text{ammonium}\\\text{carbonate}}}{\begin{matrix} O-NH_4 \\ | \\ O=C \\ | \\ O-NH_4 \end{matrix}} \rightarrow \underset{\substack{\text{ammonium}\\\text{carbamate}}}{\begin{matrix} O-NH_4 \\ | \\ O=C \\ | \\ NH_2 \end{matrix}} \rightarrow \underset{\text{urea}}{\begin{matrix} NH_2 \\ | \\ O=C \\ | \\ NH_2 \end{matrix}}$$

In this and previous chapters we have represented urea as the diamide of carbonic acid. Evidence has accumulated to show that this is probably not the proper way to show the true structure of urea. For example, it is a well-known fact in organic chemistry that amino groups react readily with nitrous acid to form free nitrogen:

$$\underset{\substack{\text{amino}\\\text{compound}}}{R-NH_2} + \underset{\substack{\text{nitrous}\\\text{acid}}}{H-O-N=O} \rightarrow R-OH + N_2 + H_2O$$

It is clear that urea should give this reaction if it contains two amino groups. The fact remains that it does not react readily with nitrous acid unless a strong mineral acid is present. This and other chemical studies have led biochemists to believe that urea should be written as a cyclic structure:

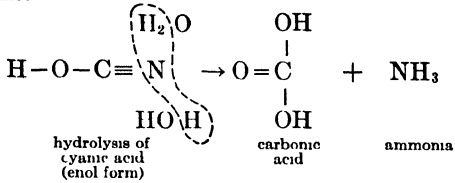
$$H-N=C\begin{matrix} \diagup NH_3 \\ \\ \diagdown O \end{matrix}$$

Urea (Werner's formula)

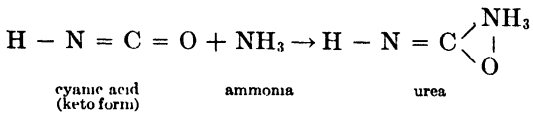
If this formula is correct for urea, the formation of this compound from amino acids is not possible by the dehydration of ammonium carbonate. Instead, it is postulated that *cyanic* acid is the precursor of urea. According to this theory, cyanic acid, which exists in two isomeric forms, may form urea as follows:

$$\underset{\substack{\text{Cyanic acid}\\\text{(enol form)}}}{H-O-C\equiv N} \qquad \underset{\substack{\text{Cyanic acid}\\\text{(keto form)}}}{H-N=C=O}$$

The enol form of cyanic acid may react with water to form carbonic acid and ammonia:

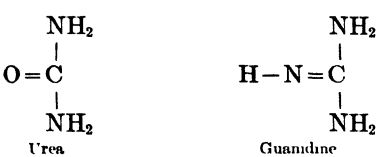


The keto form of cyanic acid is thought to unite with ammonia, forming urea:

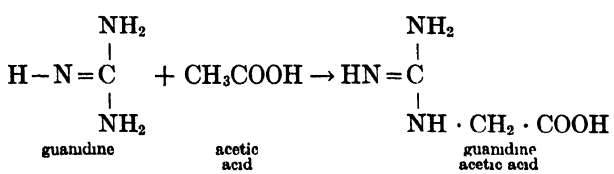


It must remain for future research to establish these facts definitely. In the meantime, it will suffice for our purpose to know that urea is formed in the liver and muscles and eliminated in the urine as the result of protein catabolism.

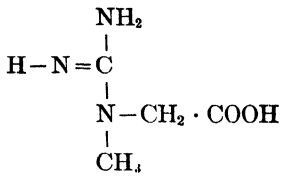
The protein metabolism with which we have been dealing has been termed *exogenous* or *intermediate metabolism* by Folin. There is another type of metabolism which is characteristic of the tissue cells and is usually constant for each individual. This is known as *tissue metabolism* or *endogenous metabolism*. One of the compounds formed in constant amounts in muscle tissue is known as *creatine*, which appears in the urine as *creatinine*, a substance easily measured by colorimetric methods. It is clear that the amount of creatinine is an index of endogenous metabolism. Creatine is a derivative of guanidine which, in turn, is related to urea.



Like other amino compounds, guanidine is capable of uniting with acids:



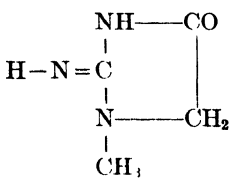
This forms creatine, which is methyl guanidine acetic acid:



Methyl guanidine acetic acid (creatine)

Fiske and Subbarrow, of Harvard University, in their study of muscle metabolism, have isolated a creatine-phosphoric acid complex which seems to be present in appreciable quantities in resting muscle but which seems to have largely disappeared in fatigued muscle. They are of the opinion that creatine may function as a buffer substance, protecting the muscle from the accumulation of lactic acid formed by excessive muscular activity.

Creatinine which is excreted in the urine is an anhydride of creatine:



creatinine

Creatinine output, therefore, is not dependent upon the diet, since it is derived from the normal muscular metabolism.

Sulfur. — Most proteins contain cystine, the sulfur-containing amino acid. When cystine metabolism takes place, the sulfur which reaches the kidney is oxidized to SO_4 and is excreted as sulfate. On the other hand, the cystine which is oxidized in the muscles and which does not reach the liver is excreted as neutral sulfur in the form of —SH(mercaptan). The urinary excretion of neutral sulfur, like creatine, is unaffected by diet and is a measure, also, of endogenous metabolism and, therefore, an index of tissue breakdown.

The diagram (after Parsons) on next page will give in a schematic way the more important changes in protein metabolism.

The Nucleo-proteins. — The nucleo-proteins are found in plant and animal tissues, particularly in the nuclei of cells. The tissues which are especially rich in these compounds are the pancreas, liver, thymus and

spleen. Chemically, the nucleo-proteins are combinations of protein with phosphorus-containing substances known as nucleic acids, of which there are a number of different kinds. Since there are many different kinds of proteins, also, it is reasonable to expect that we should find many different kinds of nucleo-proteins, and such has been found to be the case. On account of their complexity and instability it is difficult to isolate them, and many of the so-called nucleo-proteins are

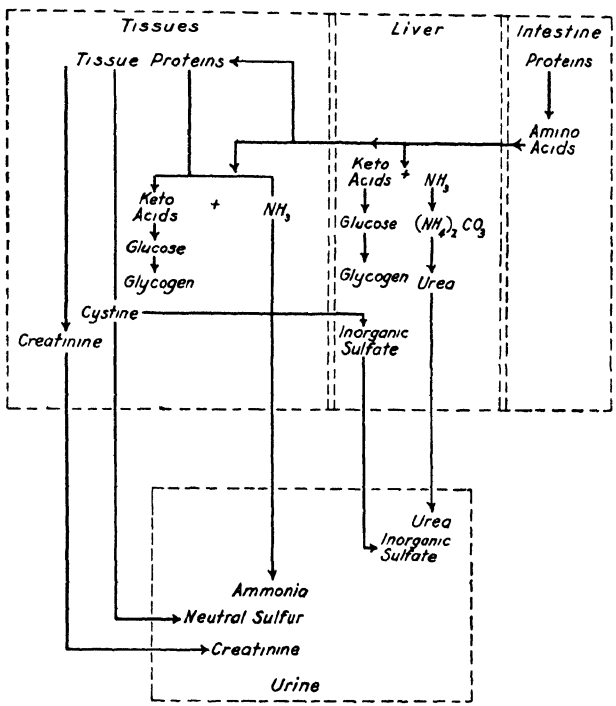


FIG 51. Showing the products of protein metabolism. (After Parsons.) (Courtesy of W. Heffer and Sons)

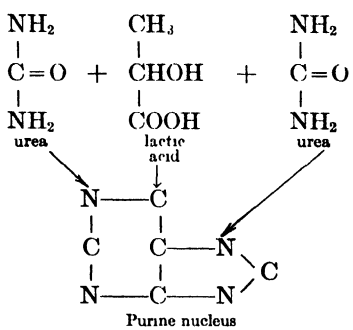
undoubtedly mixtures of protein and nucleic acids rather than definite chemical compounds. During gastric digestion, it is thought that nucleo-proteins break down to form protein and *nuclein*. The latter is thought to undergo further cleavage in pancreatic digestion, yielding protein and nucleic acid. It is for these reasons that nucleo-proteins are classified as conjugated proteins. (See Chapter V.) The plant and animal cells are capable of synthesizing these compounds,

and those found in plants are not identical with those found in animal tissues. This is true, also, of the nucleic acids.

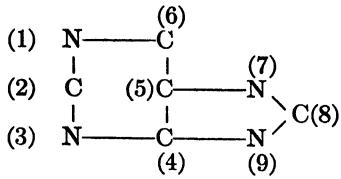
The Nucleic Acids. — Naturally, nucleic acids and their derivatives are found in those tissues which are richest in nucleated cells. The heads of spermatozoa are particularly rich in nucleic acids which are found to be combined with protamine. On hydrolysis, nucleic acids yield phosphoric acid, purines, pyrimidine bases and a carbohydrate or carbohydrate derivative. It has been thought to be characteristic of plant nucleic acids to yield a pentose sugar (*d*-ribose) whereas animal nucleic acids yield a hexose group. This observation has been questioned in recent years, because *d*-ribose has been isolated from animal tissues.

The nucleic acids are complex and variable in structure, being composed of several units called *nucleotides*, each of which contains one carbohydrate, one nitrogenous base and one phosphoric acid molecule. The types of nitrogenous bases combined in nucleic acids are also variable, some being purines and others pyrimidines. Before discussing the structure and metabolism of nucleic acids, a short description of the purines and pyrimidines should be helpful at this point.

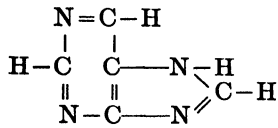
The Purine Bases. — These substances are related to uric acid (a metabolic product found in urine), and, in fact, it is from these purine bases that uric acid is formed. When two molecules of urea and one molecule of a three-carbon acid, such as lactic acid, are caused to unite in the laboratory, they form a peculiar ring-structure known as the purine nucleus.



A number of compounds contain the purine nucleus, each one differing from the others with regard to the nature and number of groups or radicals attached to the various atoms which compose the nucleus. It has become necessary to number these atoms in order that the various purine derivatives may be named. It is customary to number these atoms in the following manner:

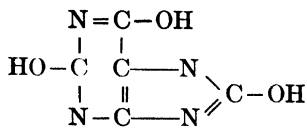


Purine, which is a definite chemical compound and the mother substance of the purine family, has the following formula:



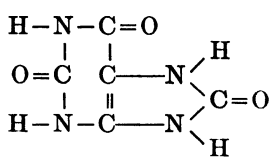
Purine

Uric acid, on the other hand, is a tri-hydroxy derivative of purine, and its structure is indicated by its other name, which is 2-6-8-trihydroxypurine:



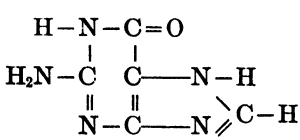
Uric acid (2-6-8-tri-hydroxypurine)

Chemists believe that hydrogen may wander or migrate from atom to atom (tautomerism) in such structures. For this reason, and also to show a more definite relationship of uric acid to urea, the formula is often written as follows:

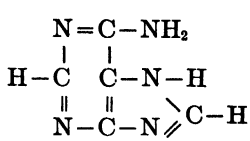


Uric acid (2-6-8-tri-oxypurine)

Guanine and adenine are purine bases which exist in nucleic acids. They are amino derivatives of purine, and their structure may be expressed as follows:

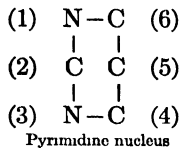


Guanine or
2-amino-6-oxypurine



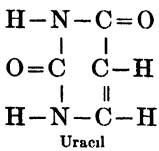
Adenine or
6-aminopurine

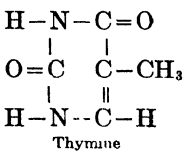
Pyrimidines. — The nucleus of these compounds differs from the purine nucleus in that it consists, if we may use the expression, of but one urea molecule and one three-carbon acid molecule. The pyrimidine nucleus is represented by the following formula:

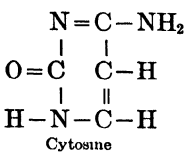


Pyrimidine nucleus

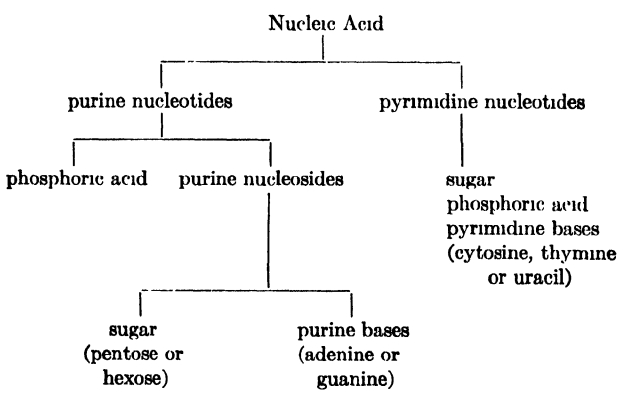
Pyrimidine bases entering into the composition of the nucleic acid molecule are *uracil, thymine* and *cytosine.*


Uracil
2-6-dioxypyrimidine


Thymine
5-methyl-
2-6-dioxypyrimidine

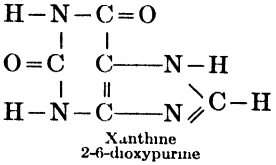

Cytosine
6-amino-
2-oxypyrimidine

Metabolic Relationships. — When nucleotides (mentioned in a previous paragraph) are broken down, they lose phosphoric acid, and nucleosides are formed. These are combinations of base and carbohydrate. The following diagram shows the outstanding features of nucleic acid breakdown:

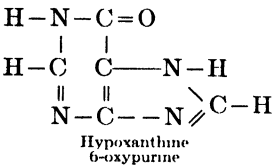


When the purine bases are metabolized, we find deaminization taking place, and this is followed by the formation of keto compounds, just

as in the case of the amino acids. These products of exogenous and en dogenous purine metabolism are xanthine and hypoxanthine.

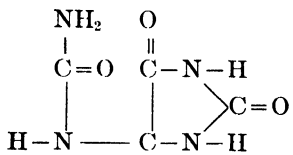


Xanthine
2-6-dioxypurine



Hypoxanthine
6-oxypurine

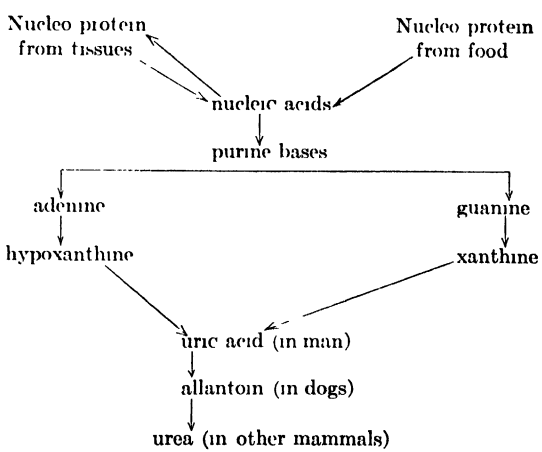
These substances are further oxidized to uric acid, 2-6-8-trioxypurine in which form they may be excreted, although it is possible for further oxidation to change some of the uric acid to a substance called allantoin



Allantoin

In poultry and birds in general, uric acid is the main nitrogenous product excreted. In man, much, if not most, of the purines are excreted as uric acid, whereas in some animals, such as dogs, allantoin is the important excretory product.

Since little is known, definitely, of the metabolism of pyrimidines we shall terminate this discussion with the following outline to show the probable steps in nucleo-protein metabolism:



CARBOHYDRATE METABOLISM

In Chapter XXII we followed the changes in carbohydrates during digestion. We learned that the digestion products (monosaccharides) were absorbed in the intestine and carried to the tissues where they were utilized or stored in the form of glycogen. It shall be our objective, in this chapter, to attempt to follow some of the more important changes through which the carbohydrates pass during oxidation in the tissues.

In normal, healthy people the glucose content of the blood is relatively constant, and so long as this amount (70 to 100 milligrams per 100 cubic centimeters of whole blood) is not exceeded, little or no glucose is found in the urine. In other words, the body mechanism, for glucose storage and combustion, is so nicely adjusted that no sugar is wasted or lost. It is evident, therefore, that the blood sugar must reach a certain *threshold* level before the kidneys allow the sugar to " spill over " into the urine.

If large amounts of glucose are ingested at one time, the liver is overloaded and, as a result of this overloading, is unable to store the sugar as rapidly as it arrives, with the result that sugar appears in the urine. This is known as dietary or alimentary glycosuria. If kidney function is impaired by disease or by a drug known as phloridzin, sugar appears in the urine owing to the disturbance of permeability relationships in the kidney cells.

When the pancreas is impaired, so that the Isles of Langerhans (see Chapter XXIII) no longer furnish insulin in sufficient quantities, the oxidation of glucose is hampered, with the result that glucose accumulates in the blood until the kidneys can no longer control its distribution. In such cases sugar appears in the urine (*glycosuria*), and the patient is said to have diabetes. When the blood sugar rises, the phenomenon is known as *hyperglycemia.*

It is through the study of natural and experimental diabetes that much of our knowledge of sugar metabolism has been derived. When insulin is not manufactured in sufficient quantities, the glucose cannot be laid down in the tissues as glycogen. The tissue glycogen already stored is changed to glucose, but it is not oxidized, and it joins the alimentary glucose arriving from the intestine, to increase the sugar in the blood.

When the urine is analyzed for sugar and nitrogen, it is found that the ratio of glucose to nitrogen eventually reaches a constant value which is known as the D/N ratio (dextrose/nitrogen ratio). This is interpreted to mean that tissue proteins are breaking down to furnish heat and other forms of energy and, since the products are being metabolized

from a constant source, the D/N ratio should be fairly constant. Inasmuch as sugar also arises from tissue breakdown, the body suffers in a two-fold manner, i.e., it not only loses the value of the glucose excreted, but the tissues are actually disappearing with no benefit to the organism.

In normal carbohydrate metabolism the glucose is stored in the liver and muscles as glycogen, which is available at all times for energy release and when occasion requires. It is known that the first step in carbohydrate metabolism is the hydrolysis of glycogen to glucose. If the glucose is not oxidized it may be used in the synthesis of fats, in which form it is stored for future use. The enzyme in the cells which influence glycogen hydrolysis is known as *glycogenase.*

When a muscle fiber contracts and shortens, work is done. Recent research work has shown that these contractions are not accompanied by combustion or carbon dioxide production, as was thought formerly. Contraction is accompanied, however, by the production of lactic acid. As the lactic acid production increases during muscle contraction, the ability of the muscle to continue contracting grows less and less. In other words, lactic acid is associated with muscle fatigue, and some authorities have attributed the "Charlie horse" troubles in muscle-bound athletes to the excessive accumulation of lactic acid in the muscles.

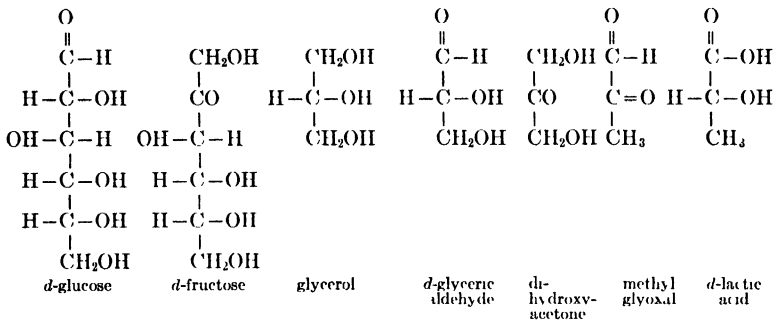
The swelling and shortening of muscle fibers during contraction is undoubtedly related to the lactic acid formed in muscle metabolism. Martin Fischer believes that the lactic acid influences the water-imbibing power of the muscle colloids, causing them to swell, which results in the shortening of the muscle fibers. This idea coincides with the fatigue-rigor mentioned above, when lactic acid becomes excessive owing to continued muscular activity It would appear that glycogen is the source of the lactic acid thus formed, and it is apparent, also, that this chemical change is a reversible one, the lactic acid disappearing to form glycogen again It is also quite clear that glucose combustion does not take place during contraction but rather following one contraction and preceding the one which follows.

Although it is difficult to say definitely just what the steps in carbohydrate metabolism may be, it is thought that glycogen may form a glucose or hexose phosphate, the phosphoric acid being liberated and re-combined in much the same way as we conceive a catalyst to act.

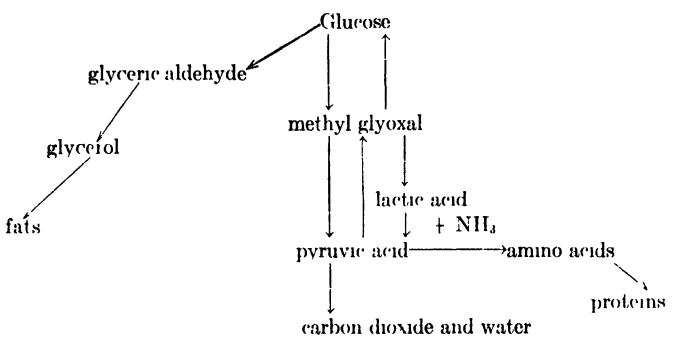
Methyl glyoxal, $CH_3 \cdot CO \cdot CHO$ (pyruvic aldehyde), has been isolated from tissues in glucose oxidation, and it is reasonable to suppose that this type of compound must be one of the intermediate products of carbohydrate metabolism. Since pyruvic acid is thought to be formed in the oxidation of fatty acids resulting from fat and protein oxidation,

this compound becomes all the more useful in explaining the metabolic interrelationships of proteins, fats and carbohydrates.

We know that glyceric aldehyde, $CH_3 \cdot CHOH \cdot CHO$; dihydroxy acetone, $CH_2OH \cdot CO \cdot CH_2OH$; pyruvic aldehyde, $CH_3 \cdot CO \cdot CHO$; pyruvic acid, $CH_3 \cdot CO \cdot COOH$; and lactic acid, $CH_3 \cdot CHOH \cdot COOH$, may be formed in the tissues, and, furthermore, it is quite evident that these compounds are closely related chemically. The following formulae show the chemical relationship existing among these compounds:



The following outline is helpful in giving the reader some idea of the possible changes through which glucose may pass during metabolism:



In brief, although many other chemical changes are possible, it is thought that glycogen is changed to glucose, which, in turn, forms methyl glyoxal, lactic acid and pyruvic acid. It is probable that those molecules of the last-mentioned acids, that are not oxidized, may form glucose and be stored as glycogen or fat. Some of the pyruvic acid may also be used in the synthesis of amino acids.

FAT METABOLISM

In Chapter XXII we followed the glycerides during the digestive process and until they were absorbed into the blood stream. We shall now consider, very briefly, some of the outstanding facts and theories regarding fat metabolism following fat deposition in the tissues. In a general way, it may be stated that the characteristic differences between fat stored in the liver, muscles or glands and that of connective and adipose tissue is the higher degree of unsaturation of the fatty acids in the former.

It was thought formerly that, in starvation, the tissue fats were completely utilized before death. Recent work, however, has shown that adipose fats may be completely lost during starvation and that muscle fat may be lost to some extent, but in the general body tissues much fat has become an indispensable part of the cell protoplasm, and analyses show that such tissues contain a normal amount of fat even after the animal has died of starvation Until recent years, the biological importance of lipids in general has not been understood or appreciated.

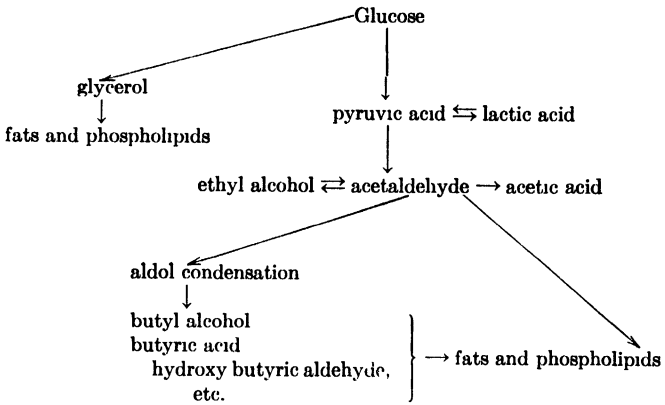
Fat Synthesis. — When animals are fed fat-free diets containing adequate supplies of proteins, carbohydrates, mineral salts and vitamins, no harmful effects are noted and the animal increases in body weight and becomes visibly fatter as the experiment progresses. It is reasonable, therefore, to conclude that animal fats may be synthesized from other carbon compounds

Quite recently, Burr has advanced the hypothesis, based on work with rats, that fats, per se, are essential for growth He contends that the body tissues are not capable of synthesizing certain fatty acids which are essential for normal cellular metabolism. This work has not been confirmed, but, if it is found to be true, it will cause us to revise our present teaching so far as functions of fats are concerned. It is entirely possible that Burr's hypothesis is correct and that the final explanation will be one of cell permeability based on emulsion changes due to the lipoidal modifications in the cell membrane, and it is not impossible to conceive that the time may come when we will find that certain fatty acids are necessary for structural purposes and, since they are incapable of synthesis in the animal body, must be considered when adequate diets are being constructed.

It is evident, to any person, that carbohydrates are capable of being synthesized into body fat. It is not possible to state the exact mechanism of fat synthesis from carbohydrates, but most authorities are of the opinion that glucose may form glycerol and a number of intermediate products which, eventually, are capable of being synthesized to fatty

acids. The following diagram summarizes the most important ideas held by workers in this field of research:

SYNTHESIS OF GLYCEROL AND FATTY ACIDS FROM GLUCOSE



It is probable that the fragments from protein metabolism form pyruvic acid, and, by this route, fat-synthesis from proteins may occur. It is also possible to show that glucose formation and glycogen storage may result from protein metabolism.

Fat Catabolism. — The liver seems to function in two ways, viz, to desaturate the fatty acids and convert neutral fats into phospholipids. It seems to be well established that the latter are much more readily metabolized than the neutral storage-fats and are universally distributed throughout the blood and body tissues. It is also evident that fats of milk and eggs are derived very largely from the phospholipids of the blood. It is thought, by Bloor, that desaturation and phospholipid formation are necessary preliminary steps in fat catabolism. The various steps are not at all clear, but it is evident that the subsequent utilization of fats involves their oxidative metabolism in the tissues.

As a result of the work of Dakin, Knoop and other investigators, it has been shown that fatty acids are not burned completely in one explosive oxidation but are oxidized gradually, losing two carbons at a time during the combustion process. This is known as the *beta-oxidation theory*. By this process, the carboxyl carbon and the alpha carbon of each fatty acid are oxidized to carbon dioxide and water, and the beta carbon is changed to a carboxyl group, forming a new fatty acid which contains two less carbons than the original acid. The steps in this type of reaction are thought to be the formation of a beta-hydroxy acid which

changes to a beta-ketonic acid, prior to the loss of two carbon atoms. Thus, butyric acid would form beta-oxybutyric acid and aceto-acetic acid. The following formulae show the relationships that may exist between caproic acid and its oxidation products:

Caproic acid	β-hydroxy caproic acid	β-keto caproic acid	Butyric acid	β-hydroxy butyric acid	Aceto-acetic acid	Acetic acid	Carbonic acid
CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CO_2
CH_2	CH_2	CH_2	CH_2	CHOH	C=O	COOH	
CH_2	CH_2	CH_2	CH_2	CH_2	CH_2		
CH_2	CHOH	C=O	COOH	COOH	COOH		
CH_2	CH_2	CH_2					
COOH	COOH	COOH					

In order that fatty acids may be completely oxidized, it is necessary that glucose-oxidation must be carried on simultaneously. If glucose oxidation is hampered, as in diabetes, fatty acid oxidation tends to stop at the aceto-acetic acid and β-hydroxy butyric acid stage. These substances tend also to form acetone, and in medicine these substances are known as *acetone bodies*.

Since fats (and proteins) tend to form acetone bodies in the absence of sugar oxidation, they are known as " ketogenic " substances. Carbohydrates, on the other hand, tend to prevent the formation of acetone bodies, and for this reason are said to have " anti-ketogenic " properties. Diabetic specialists, in planning dietaries, often calculate the various potential ketogenic and anti-ketogenic compounds in the food ingredients and derive a ratio, known as the K/A ratio, which is used in planning diabetic diets.

MINERAL METABOLISM

Although at best, the figures are but approximate, it is assumed that the human body consists of about 65 per cent of oxygen, 18 per cent of carbon, 10 per cent of hydrogen, 3 per cent of nitrogen, 1.5 per cent of calcium and 1 per cent of phosphorus, the remaining inorganic elements varying from mere traces to about 0.5 per cent. Among these last-

mentioned elements (in order of percentage composition of tissues) are K, S, Na, Cl, Mg, Fe, I, F, Si, Zn, Ni, Co, Cu, Mn and As.

We shall not devote much time or space to a detailed discussion of this important phase of animal metabolism, but there are a few fundamental facts that should be recorded.

FUNCTIONS OF SALTS

The mineral elements enter the animal body in many forms and from many sources. In general, however, we can assume that the body receives these elements in two forms, viz., as inorganic salts, and in combination with organic substances. It is evident that soluble salts are diffusible and are capable of being carried directly to the tissues. Those which are in organic combination are not available until digestive changes have made them soluble and diffusible.

When these compounds enter the blood, they may be utilized for (*a*) structural purposes, in the building of bones and supporting tissues; (*b*) for structural purposes in the building of organic molecules, which are so important in the composition of blood and the soft tissues; and (*c*) as soluble electrolytes, playing important rôles in maintaining proper chemical equilibrium, hydrogen-ion concentration, and osmotic concentration in the blood and body fluids.

Sulfur. — This element may enter the body in the form of sulfates, but by far the greater portion is ingested in organic combination. The chief sulfur-carrying ingredient in the average diet or ration is protein, in which it is combined as the amino acid cystine. There are sulfur compounds of less importance such as allyl sulfide, $(CH_2 : CH : CH_2)_2S$, found in onions and garlic, and allyl-iso-thiocyanate,

$$CH_2 : CH : CH_2 \cdot N : C : S,$$

found in mustard.

When sulfur is laid down in the tissues, it is deposited mainly in the form of cystine in tissue-protein. When these proteins catabolize, it is found that urinary nitrogen and sulfur excretion are closely correlated. The sulfur is oxidized to sulfuric acid, which unites with available inorganic bases and is excreted as inorganic sulfates. A smaller portion unites with organic radicals, such as phenol-sulfuric acid, *p*-cresol-sulfuric acid, indoxyl-sulfuric acid and skatol-sulfuric acid. These products are known as *ethereal sulfates*. A proportion is eliminated as neutral or unoxidized sulfur.

Phosphorus. — In previous chapters we have stressed the importance of the phosphorus-containing organic substances such as nucleo-proteins, nucleic acids, phospho-lipids, and phospho-proteins. These, in

fact, are the forms in which much of the body phosphorus is found. The bones and teeth, of course, contain the greatest amount of phosphorus, but the colloidal and biological properties of cells and their normal functioning are very dependent on the organic-phosphorus compounds. We should not omit reference, however, to the importance of the phosphates of potassium in the body fluids. These are not only relatively abundant, but they are most important in the regulation of osmotic and hydrogen-ion concentrations. We have learned also that phosphorus seems to be essential for proper glucose oxidation in tissues.

When the tissues are catabolized, organically combined phosphorus may be released as free phosphoric acid and excreted as inorganic phosphates in the urine. There is no evidence that organic-phosphorus compounds are essential for animal growth, for most of the evidence seems to point to the fact that the animal is capable of synthesizing its own organic-phosphorus compounds from the inorganic phosphates in the food. Most animals excrete phosphorus in the urine, in the form of soluble phosphates. In herbivora, an appreciable amount of phosphorus is excreted, in the form of insoluble phosphates, in the feces.

Calcium, Magnesium, Potassium and Sodium. — These elements are important and necessary constituents of most tissues. Blood is richer in calcium than in magnesium; the reverse is true for muscle tissues. Muscle contraction, nerve impulse, and many other phenomena depend on the presence and concentration of inorganic salts. Blood coagulation is greatly dependent upon calcium salts, and deficiencies of mineral salts lead to such diseases as rickets and osteoporosis. All these elements are excreted eventually in the urine, although in domestic animals, particularly the ruminants and herbivora, considerable mineral excretion may take place in the feces.

During the past few years nutrition workers have been much more appreciative of the value of traces of mineral elements than ever before. Although little is known regarding their function in the body, evidence is accumulating to show that the so-called accidental traces of elements in foods and tissues are not accidents, after all. The works of Bertrand, McHargue and others have shown that such elements as Ni, Co, Cu, Zn, Fe and Mn are invariably present in vital tissues. Evidence is accumulating which indicates that these substances, in traces so small that they are scarcely measurable, are capable of acting as catalysts in oxidative and metabolic processes of a most vital nature.

Iron and Copper Deficiency. — During the past few years, considerable interest has developed in medical and agricultural circles by the discovery that nutritional anemia can be produced experimentally by feeding diets consisting of corn and cow's milk. As this disease develops,

the number of red blood cells decreases, and the animal is limited in its ability to synthesize hemoglobin. When liver, lettuce or apricots are fed, there is a pronounced tendency for the red cells to increase in number, and the hemoglobin content of the blood also tends to return to the normal.

Hart and co-workers have made some very interesting and fundamental observations on the development and cure of experimental anemia

FIG. 52. Anemia in pigs. The animal on the right is afflicted with nutritional anemia induced by feeding a diet deficient in copper. The normal pig, on the left, was able to utilize the iron in the ration due to the presence of a trace of copper salts. (Courtesy of Professor E. B. Hart.)

in domestic animals. In their experiments they have used the rat, the chicken, the rabbit and the hog. These workers have found that the utilization of iron in the regeneration of hemoglobin is dependent on the presence of traces of copper. Curative foodstuffs such as lettuce and liver have been found to be relatively rich in this element. The addition of traces of copper and iron salts to the ration of anemic animals has shown a most marked effect on blood regeneration. These findings with experimental animals have been verified also in hospital cases of human anemias of certain types.

Table 61 by Elvehjem and Hart shows the copper content of some typical agricultural feeds.

These workers have also shown that the addition of copper salts to the soil influences the copper content of foodstuffs grown thereon. The evidence for the effect of copper fertilization is given in Table 62.

TABLE 61

COPPER CONTENT OF FEEDING STUFFS

Material	Copper in dry matter (100°), mg per kg	Material	Copper in dry matter (100°), mg per kg
Alfalfa hay	9 1	Linseed oil meal	21 8
Alfalfa hay, first cutting (Wisconsin)	4 5	Millet, Japanese	9 1
Alfalfa hay, second cutting (Wisconsin)	14 8	Oats	6 8
Alfalfa hay, first cutting (Colorado, Sample 1)	6 9	Oat groats	7 6
Alfalfa hay, first cutting (Colorado, Sample 2)	11 9	Oat straw	3 8
Barley	7 2	Rape	7 7
Blood meal	13 1	Red clover hay	17 6
Blue-grass	8 3	Rye	5 9
Brewer's dried grain	16 4	straw	4 4
Buckwheat feed	13 7	Sorghum fodder	8 7
Cocoanut oil meal	10 5	Soybeans	22 7
Corn, white	4 4	Soybean hay	8 2
yellow	4 7	Sugar beets	8 5
gluten meal	35 1	Sugar beet tops	6 4
oil cake meal	13 2	Sweet clover hay	11 8
silage	3 8	Tankage	23 1
stover	4 0	Timothy hay	2 2
Cottonseed meal	21 8	Tobacco	16 8
Distiller's grain	38 4	Vetch hay	9 6
Emmer	9 6	Wheat	7 8
Gluten feed	89 5	bran	16 4
Kaffir, white	8 0	germ	9 0
		gluten	17 2
		middlings, flour	12 1
		straw	2 1

Statistical records show that about 35 per cent of suckling pigs produced in this country are lost each year, and that anemia is one of the important diseases responsible for this loss. Sow milk is a poor blood builder, and it is not possible to increase the iron or the copper in the milk by feeding salts of these elements to the lactating mother. The addition of 3 6 ounces of ferric sulfate, $Fe_2(SO_4)_3$, to 5 quarts of water is recommended as a satisfactory drench for suckling pigs. As a rule, sufficient copper is present as an impurity to cause satisfactory blood regeneration.

TABLE 62

COPPER CONTENT OF CROPS GROWN ON SOILS CONTAINING DIFFERENT AMOUNTS OF COPPER

Material	Copper per kilo dry matter		Increase, per cent
	No copper addition, mg.	Copper added 50 lb. $CuSO_4 \cdot 5H_2O$ per acre, mg	
Lettuce 1	11 7	14 7	25 6
2	12 1	15 2	25 6
Tomatoes, green	9 3	10 4	11 8
ripe	11 2	12 6	12 5
Beans, navy	7 4	9 5	28 4
kidney	7 4	9 5	28 4
Carrots	8 1	8 9	9 8
Oat, grass	7 9	10 1	27 8
seed	6 5	7 5	15 4
Lettuce*	12 1	30 1	148 0

* Copper added equivalent to 500 pounds of $CuSO_4 \cdot 5H_2O$ per acre.

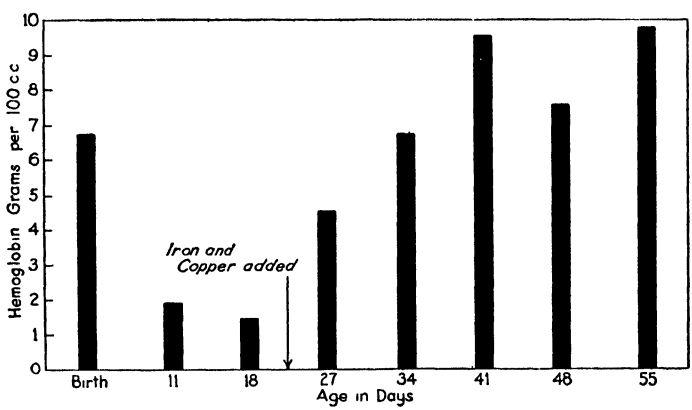


FIG. 53. Showing the loss of hemoglobin in the blood of pigs when fed on diets deficient in iron and copper and the subsequent regeneration of hemoglobin (red blood cells) when iron and copper were added to the diet. (Data of Hart and co-workers, Bull. 409, Wisconsin Agricultural Experiment Station.)

Iodine Deficiency. — Another type of mineral deficiency which has attracted a great deal of attention during the past ten or fifteen years is

that type which develops in those regions deficient in iodine. Medical authorities have shown that certain regions in this country are characterized by a large proportion of goiter, the disease being most marked in girls at the age of puberty. Authorities in Holland report 94 per cent of the girls in the city of Breda being afflicted with the disease. In this country there are large areas, particularly in the Great Lakes region and extending nearly to the Pacific Coast, where the disease has become a real problem. In Ohio, the reports indicate from 20 to 50 per cent of goiter in girls of school age, and the percentage in St. Paul, Minnesota, has been reported to be about 73.

TABLE 63

DISTRIBUTION OF IODINE IN FOODS

Expressed as milligrams of iodine per metric ton of dry foodstuff

(*Data of McClendon*)

Non-goitrous regions

Food	Iodine content	Locality
Wheat	9 3	Edgecomb, Me
Oats	175 0	Wiscasset, Me
Carrots	170 0	California Coast
Salmon	324 0	Alaska
Goat's Milk	400 0	California Coast

Goitrous regions

Wheat	6 6	Minnesota
Straight flour	3 5	Minnesota
Carrots	2 3	Oregon
Apples (peeled and cored)	3 0	Oregon
Skim Milk	12 0	Minnesota

The disease is not confined to humans, however, for hogs, sheep, colts and calves are afflicted with " big neck " in iodine-deficient regions. Smith and co-workers of the Montana Agricultural Experiment Station, and Hart and co-workers of Wisconsin, have shown that a deficiency of iodine in the ration or drinking water will cause sows to produce hairless pigs which are born dead or die soon after birth. The addition of sodium or potassium iodide to the diet of the sow during pregnancy

prevents the disease and subsequent loss of young. Fish meal and other iodine-containing food materials have been used with success in the prevention of iodine deficiency disease in livestock. Iodized salts have been used with success, but they vary so much in their iodine content that some risk is incurred in their use. Various forms of kelp and seaweed have been used by live-stock feeders in Europe, and during recent

TABLE 64

DISTRIBUTION OF IODINE IN COW'S MILK

(Data of McClendon and co-workers)

Town	County	State	Feed	Butterfat	Dry skim milk	Ratio of total iodine in butter to that in skim milk
Clemson College		S. C.	Normal	78	142	0 9
Davis	. .	Cal	Normal	26	164	0 01
Byron Rock River Farm	.	Ill	Kelp and fish	176	400	0 7
St. Paul	Ramsey	Minn.	Normal	11		
St Paul	Ramsey	Minn	Cod-liver oil	36	7,320	0 002
St. Paul	Ramsey	Minn	Iodized oil,* 100 gm daily	43	65,600	0 0006
St. Paul	Ramsey	Minn	Iodized salt containing 10 per cent iodine	378	158,000	0 0004
St. Paul	Ramsey	Minn.	4 to 6 days after discontinuing iodized salt	148	2,800	0 02

* Made by adding 20 g of ICl_3 to a gallon of linseed oil.

years kelp has been introduced with success in live-stock rations in this country. Table 64 summarizes the results of experiments by McClendon and co-workers, showing that it is possible to increase the iodine content of cow's milk by feeding iodine-containing feeds. In certain districts in the United States, it is now possible to purchase "iodized" milk and eggs produced on iodine-rich rations. Simple goiter is undoubtedly due to the absence of iodine in the diet, but there are other forms of goiter in which iodine may or may not be helpful. It is the opinion of

Kimball, McClendon and others that endemic goiter is easily controlled by iodine therapy, and that there is little excuse for the present goitrous situation in many districts.

McClendon of Minnesota and Remington and co-workers of South Carolina have made some very valuable surveys of waters, soils, and food materials with reference to their iodine content, a type of study that could be conducted by many other states with profit. Remington and co-workers have analyzed potatoes grown in Idaho, Michigan, Maine, North Dakota, Minnesota and South Carolina, and have concluded that the potato can be used as a fair index of the relative abundance of iodine in the various regions. Table 65 shows the distribution of iodine in South Carolina fruits and vegetables.

TABLE 65

IODINE CONTENT OF SOME SOUTH CAROLINA VEGETABLES AND FRUITS

(*Data of Remington*)

Values are in parts per billion on the dried sample

	Number of samples	Average iodine content		Number of samples	Average iodine content
Green Vegetables			*Fruits*		
Asparagus	26	285	Blueberries	2	206
Green beans	14	210	Dewberries	1	169
Beet tops	3	657	Peaches	3	162
Cabbage	17	263	Pears	1	65
Carrot tops	6	433	Strawberries	5	181
Chinese cabbage	1	300	Tomatoes	8	112
Collards	3	232	Watermelon	1	402
Cucumbers	2	530	*Roots*		
Egg plant	3	187	Artichokes	1	182
Kale	2	278	Beets	9	182
Lettuce	13	912	Carrots	16	213
Mustard	2	224	Irish potatoes	76	211
Okra	3	223	Rutabaga	5	200
Onions	6	222	Sweet potatoes	70	98
Pimentos	1	150	Turnips	13	271
Rape	3	300	*Seeds*		
Spinach	19	692	Cottonseed meal	1	140
Summer squash	7	625	Peas	3	136
Turnip tops	7	376	Oats in hull	1	20
			Peanuts	1	67
			Rye in hull	1	86

Phosphorus Deficiency. — Theiler, Green and Viljoen have described another disease of livestock which occurs commonly in South African cattle, known as "lamzickte." This disease is caused, primarily, by a deficiency of phosphorus in the vegetation. An abnormal appetite known as osteophagia (bone chewing) develops, in which the animals crave to eat all sorts of materials, particularly bones of dead animals, which are strewn over the large grazing areas. Death finally ensues

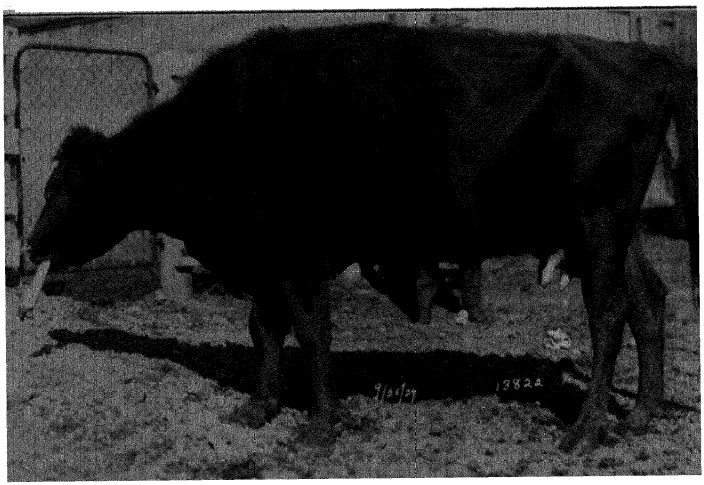


FIG. 54. A case of osteophagia or depraved appetite brought about by feeding a diet deficient in phosphorus. Such animals develop a morbid appetite and attempt to eat wood, bones and similar materials. (Courtesy of Dr. C. H. Eckles.)

owing to the toxicity of a saprophytic organism occurring on the dried bones and to general inanition.

Eckles, Becker and Palmer, of the University of Minnesota, have reported a similar dietary deficiency occurring in certain areas of Minnesota due to a lack of phosphorus in forage crops, which results in the development of a malnourished condition in dairy cattle, accompanied by a depraved appetite, or pica. This deficiency is due to a lack of phosphorus in certain Minnesota soils, which results in the production of forage crops which are likewise deficient in phosphorus. The shortage of phosphorus compounds under these conditions causes a reduction of milk flow, disturbs carbohydrate metabolism, inhibits oestrum, and produces other disturbances which result, eventually, in the mainte-

nance of uneconomical and unproductive dairy animals. The addition of phosphates such as bone meal has been found to prevent and cure the disease. The authors state that the lack of ultraviolet light and of green succulent feeds may also be contributing factors.

In concluding our brief discussion of mineral metabolism, it is only fair to state that some evidence exists to show that an excess of mineral salts in poultry rations may lead to the development of " hock disease "

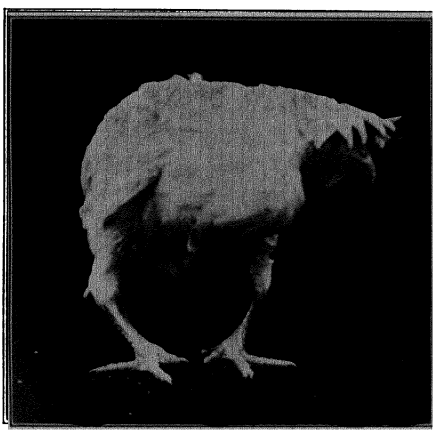


FIG. 55. Hock disease or slipped tendon disease in poultry is thought to be nutritional in origin. It can be produced experimentally by feeding normal poultry rations which have been supplemented with excessive amounts of mineral salts. The disease seems to develop best when birds are confined on wire floors, in battery brooders — although the condition is found under other types of management. (Data of Hunter, Dutcher and Knandel.)

in growing chickens. In this disease, the hock joint becomes swollen and deformed. The disease is also known as " slipped tendon disease." See Fig. 55.

In this chapter we have endeavored to point out the more important chemical phases of metabolism. In Chapter XXV we shall direct our attention to the energy transformations which result from the chemical changes just described.

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CHAPTER XXV

ENERGY METABOLISM

In the preceding chapter we discussed the chemical changes which are thought to take place in the animal body during normal and abnormal metabolism. When these chemical changes occur, energy transformations become manifest through absorption or loss of heat. Those chemical changes which are of an oxidative nature are *exothermic*, i.e., heat is given off during the reaction. Hydrolyses are characterized by no perceptible heat changes, and for this reason they are known as *isothermic* reactions. When heat is absorbed during chemical changes, as in reduction reactions, the process is said to be *endothermic*.

We have learned that fats, proteins and carbohydrates may be oxidized in normal metabolism, and that any or all of these may serve as fuel for muscular work. Owing to the fact that all these organic nutrients play a part in energy production, it has been found that energy values are most useful in making comparisons of the nutritive values of foodstuffs. To do this, it is necessary to have some unit by which everything can be measured. This unit is the large *Calorie* (spelled with a capital C) which is equivalent to 1000 small calories. For convenience in recording data involving relatively large energy changes, some writers prefer to use the *Therm* (1000 large Calories) as the unit of measurement.

Measurement of Heat of Combustion. — For the measurement of the gross energy values of foodstuffs, it is necessary to use the bomb calorimeter. There are several modifications of this instrument, but it consists, essentially, of a heavy metal bomb, lined with platinum, gold or other non-corrosive metal. The bomb contains a platinum receptacle for the weighed sample of food. A heavy metal cover is screwed tightly to the bomb and oxygen is introduced through suitable openings until the pressure reaches about 300 pounds per square inch. The bomb is immersed in a known weight of water, which is allowed to come to a constant temperature in an adiabatic container which prevents heat losses resulting from conduction or radiation. The food material is " fired " by sending a current of electricity through a very fine iron wire, which is in contact with the foodstuff inside the bomb. By means of a thermometer, which records extremely slight changes in temperature,

the temperature rise of the water may be measured. Owing to the excess of oxygen in the bomb, combustion is immediate and complete. The heat produced is conducted to the water and the temperature rise of the water is noted. From data thus obtained, it is possible to calculate the total heat released by a known weight of any food material. This is usually referred to as the *gross energy* of the feed.

When pure carbon is burned in a bomb calorimeter it is found that the heat liberated is equivalent to 8.08 Calories per gram of carbon. An equal weight of hydrogen, on the other hand, will yield 34.5 Calories under the same conditions. When hydrocarbons are burned, the amount of heat is practically equivalent to that which would have been obtained if the carbon and hydrogen equivalents had been burned separately. This is not the case, however, when carbon and hydrogen are combined with oxygen, viz., in the fats and carbohydrates. The reason for this lies in the fact that a portion of the carbon and hydrogen is already oxidized. As a result, the total heat obtained in the combustion of carbohydrates and fats is not proportional to the amount of carbon and hydrogen potentially capable of combustion. When proteins are burned, the products are carbon dioxide, water and nitrogen gas. The latter does not contribute to the heat of combustion. If sulfur is present, a slight amount of heat is produced in changing the neutral sulfur to sulfur trioxide.

Research work has shown that complete oxidation of these organic food constituents in the bomb calorimeter yield the following (average) values:

Proteins	5.65 Calories per gram
Carbohydrates	4.10 Calories per gram
Fats	9.45 Calories per gram

When these substances are metabolized in the body, however, they are not completely utilized. It is estimated that the following figures represent the average amounts of these constituents absorbed in the average diet:

Proteins	92 per cent absorbed
Carbohydrates	98 per cent absorbed
Fats	95 per cent absorbed

After absorption has taken place, it is found that the body does not burn these compounds completely. Protein, for example, is not completely oxidized, and a portion of its unburned carbon and hydrogen is excreted in the form of urea, creatinine, uric acid, etc. This loss of combustible material amounts to about 1.3 Calories per gram of protein

burned. Correcting for this loss of energy-producing fraction we now have:

From proteins (5 65 minus 1 30)	4 35 Calories per gram
From carbohydrates	4 10 Calories per gram
From fats	9 45 Calories per gram

Correcting for the loss of unabsorbed food materials, we obtain the following results:

Proteins	4 35 × 92 per cent = 4 Calories per gram
Carbohydrates	4 10 × 98 per cent = 4 Calories per gram
Fats	9 45 × 95 per cent = 9 Calories per gram

Although these figures are only approximate, they are sufficiently accurate to be used in calculating the total energy value of any feeding material. For example, the average composition of ordinary white bread is as follows:

Water	35 00 per cent
Protein	9 10 per cent
Carbohydrates	53 30 per cent
Fat	1 60 per cent

To obtain the total available Calories per 100 grams of bread we are able to make the following calculations:

	Grams per 100 grams of bread		Calories per gram		Calories per 100 grams of bread
Protein	9 10	×	4	=	36 40
Carbohydrate	53 30	×	4	=	213 20
Fat	1 60	×	9	=	14 40
Total					264 00

Since there are 453.6 grams in a pound, the total available Calories per pound of bread would be 264.00 × 4.53 = (about) 1196. Similar calculations can be made for various mixtures of food materials.

Methods of Study

One method of studying feed values has been discussed. By this method the nutrition worker endeavors to determine all the important ingredients and follow them through all the complex transformations in digestion and metabolism. By this method he may obtain, eventually, a fairly accurate picture regarding the functions and nutritive value of feeds.

A second method which has been used to a great extent in experiment station work, and which has been of considerable practical value, might be termed the " commercial production " method. By this method various feed mixtures are fed and the results obtained are expressed in live-weight-gains, slaughter tests, milk production or egg production. This type of experiment, like a metabolism test, may be controlled by chemical analyses in such a manner that it may also be considered a " balance experiment " where the materials synthesized can be measured in terms of those fed in the experimental ration. All investigations which take into account the balance between income and outgo of matter or energy may be termed " balance experiments."

Digestion trials have been used to a great extent to determine the percentage of any food or food ingredient that is actually digested and absorbed. In this type of experiment the food is carefully weighed, sampled and analyzed for those constituents which are the subjects of the investigation. The food is fed to the experimental animal for several days until all the former food residues have been removed from the digestive tract. When the animal becomes adjusted to the new feed, the experiment is started. Feces are collected by attendants (or by a special collection-harness), sampled, and analyzed. By comparing the protein, fat or carbohydrate intake with the fecal losses of these materials, it is possible to calculate the percentage digested, which is called the *digestion coefficient.*

Another method which has yielded valuable results is the type where measurements are made of the oxygen consumed and the carbon dioxide produced. Such methods are usually termed *respiration methods.* Another, known as the *calorimetrical method,* has to do with the direct measurement of heat given off by the body.

Respiration Methods. — Since heat production is due to oxidation, it is possible to measure the extent of tissue catabolism if it is possible to obtain accurate measurement of the amount of oxygen consumed and carbon dioxide produced. There are several types of respiration apparatus for this purpose, some of which measure oxygen consumption only. Most modern hospitals are now using apparatus of this type to measure the *basal metabolism* of patients. By this measurement it is possible to determine whether or not the normal (basal) metabolic rate has been impeded or hastened by disease.

Respiration studies are often termed *indirect calorimetry,* since it is possible to calculate heat-production from data obtained in such studies. The earliest form of apparatus used in respiration studies consisted of a hermetically sealed respiration chamber of known capacity in which the experimental subject was placed. Changes in the chemical composi-

tion of the air due to oxygen consumption and carbon dioxide production gave the information desired. Naturally, this type of apparatus was

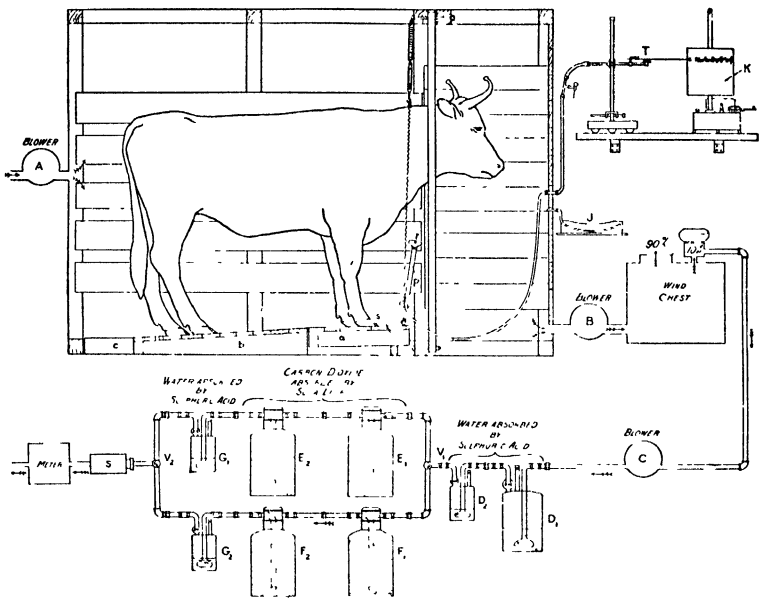

FIG. 56. Pure outdoor air introduced into chamber by blower A; is withdrawn by blower B, and forced into wind chest. Through one of two circular openings in wind chest 90 per cent of air escapes into room; 10 per cent passes into sampling can with rubber bathing-cap top. Blower C, forces air withdrawn from sampling can through two sulfuric acid bottles, D_1 and D_2. Valve V_1 deflects air through either one of two sets of soda-lime bottles, E_1 and E_2 or F_1 and F_2, and sulfuric-acid bottles G_1 or G_2. Air then passes from sodium-bicarbonate container S, through meter. Delicate petroleum manometer J, indicates pressure inside chamber. To register muscular activity of animal, floor is in two sections; at front a movable platform, a, supported by two chains attached to springs at top of chamber and by two compression springs resting on the metal floor of the chamber, only one of which, s, is shown. Rear of floor is a fixed platform, b, slanting to allow flow of urine into container, c. P, pneumograph attached at one end to one of the chains j_1, supporting movable platform, a, and at the other end to one of the stall uprights. Changes in tension of air in pneumograph transmitted through rubber tubing with safety outlet and pinchcock to tambour, T, which actuates a small pointer writing on kymograph drum, K. (Courtesy of Dr. F. G. Benedict.)

unsatisfactory, for it made no provision for a fresh supply of air, which is necessary if normal results are to be expected. As a consequence, two types of apparatus came into use, known as *closed circuit* and *open circuit*

apparatus, respectively. The former became known as the Regnault-Reiset apparatus, and the latter was called the Pettenkofer apparatus, from the names of the scientists who designed and used them.

The Closed Circuit Apparatus. — This apparatus consisted of a respiration chamber, in which the animal was placed. To overcome the objections referred to above, the chamber was equipped with a gas tank from which oxygen could be introduced as occasion required. Furthermore, a chain of absorbers was introduced, by which it was possible to absorb the carbon dioxide and water formed in respiration and, to assure circulation of air, a blower was introduced. The air remaining in the chamber was analyzed, subsequently, for methane and hydrogen. Since the nitrogen in the air could not vary, the determination of this element was unnecessary. Portable outfits are now on the market which dispense with the respiration chamber, and a special mask is substituted. With this type of apparatus it is possible to obtain the respiratory exchange of men or animals.

The Open Circuit Apparatus. — In all apparatus built on the Pettenkofer principle, fresh air is introduced and respiratory gases absorbed throughout the experimental period. The respiration chamber is equipped with a gas-meter at the point where the fresh air enters the chamber and another at the point where the respired air leaves the apparatus. Chemical analyses of the fresh and respired air give the operator the data he needs for calculating energy metabolism. This, as stated before, is also called indirect calorimetry, since it is possible to calculate heat production without actually making heat measurements.

Direct Calorimetry. — Heat may be measured in a number of different ways, but the most common is that which involves the rise in the temperature of a known volume of water. Probably the earliest type was the ice calorimeter. This type, used by Lavoisier in his studies, consisted essentially of an inner chamber totally surrounded by crushed ice. A third (outer) chamber also contained crushed ice. The experimental animal was placed in the cold inner chamber, under conditions that cannot be considered ideal for experimental work. The ice in the outer chamber functioned as a heat-buffer for the heat of the surrounding air. The inner ice chamber was constructed with an outlet pipe and stopcock. As the ice melted in the inner chamber, owing to the body temperature of the animal, the water could be collected and measured. Since it was known that 79.24 gram-calories were required to transform one gram of ice at 0° C. into water at the same temperature, it was possible to calculate the heat-output of the animal under these conditions. This type of calorimeter is known as a *latent heat calorimeter* since it involves a change in the physical state of a calorimetric substance.

Another type, known as an *emission calorimeter*, has been used to some extent. In this type of apparatus, electrical resistance is used to heat a closed chamber until the heat of radiation and heat of production come to equilibrium, the temperature of the walls of the chamber remaining constant. A second experiment is conducted in which an experimental animal is substituted as the heat-producer. When equilibrium comparable with that of the first experiment has been obtained, it is possible to calculate the heat output of the animal in terms of the heat produced in

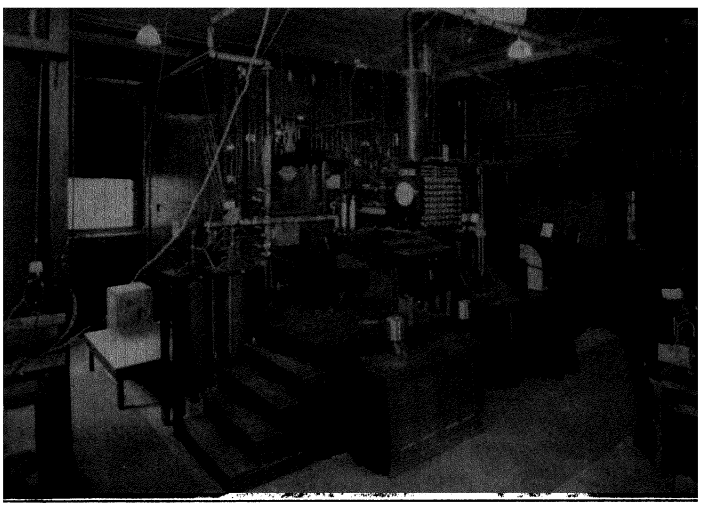

FIG. 57. Respiration calorimeter, in the Institute of Animal Nutrition at the Pennsylvania State College, used by Armsby and co-workers to determine the net energy content of feeds. (Courtesy of Dr. E. B. Forbes.)

the first experiment, since it is obvious that the total heat production in each experiment must have been the same.

The third type, which is known as a *respiration calorimeter* or *flow calorimeter*, was designed for experiments on man by Atwater and Rosa and modified by Atwater and Benedict. Armsby and Fries, of the Pennsylvania State College, have adapted this type of apparatus for experimental work with farm animals. It is impossible to describe this interesting type of apparatus in great detail, but it may be said that it consists of a chamber large enough to accommodate a mature steer. This chamber, which contains air-tight doors, is surrounded by outer walls between which dead air-spaces are maintained. In these spaces, electric resistance wires and cold water pipes are placed at regular intervals in order

that the inner walls may be maintained at a constant temperature during experimental work, thus preventing heat-losses or heat-gains so far as the inner chamber is concerned.

Air entering the chamber is passed over the pipes of a refrigeration system, which removes the excess of atmospheric moisture. The air is humidified to a constant moisture content and is measured automatically, by gas-meters, as it enters the chamber. Provisions are made for automatic sampling of the in-going air, on which the water, oxygen and carbon dioxide analyses are performed at regular intervals. Water of known volume and temperature enters the chamber and circulates through copper pipes (heat absorbers) situated immediately over the animal. The circulating water absorbs the heat produced in the chamber, and the increase in water temperature is recorded automatically as the water leaves the chamber.

Since water, carbon dioxide and methane are found in the respired air, it is necessary to determine these constituents with great accuracy. The out-going air is passed through absorbers where the water and carbon dioxide are removed and measured. The remaining gases (largely methane) are passed through tubes in an electric furnace where they are oxidized to carbon dioxide and water, which are carried to absorbers and the amount of each is determined. Provision is made, by a very clever device, whereby the feed may be introduced, as the investigation progresses, without vitiating the experimental work. Urine and feces are collected, also, and weighed, sampled and preserved for chemical analysis

It will be seen that it is possible, by means of this type of research, to make an accurate balance-record of all important ingredients which enter the animal body and to determine how they are utilized and excreted. Carbon and nitrogen balances are obtained in this manner, and it is possible to determine the amount and kind of food necessary for maintenance, fattening, milk production, etc In other words, the gross energy of the feed (income) may be studied during metabolism, and its disposition (expense) noted. By deducting this expense of operation from income received, the nutrition worker, like the banker or business man, can calculate the *net energy* remaining as physiological " profits."

The energy of the feed is necessarily *chemical* in nature, and this is true also of the form of energy excreted by the animal in the form of feces, urine, perspiration and combustible gases. If work is done by the animal during the experiment, this is expressed as *kinetic energy*, and the remaining form of energy is measured as heat energy.

Gross Energy. — When the feed mixture is analyzed chemically and burned in the bomb calorimeter the total chemical energy of the feed is

determined. Not all this energy can be utilized by the animal, however, for the reason that some of the combustible substances pass from the body in the feces as undigested material. Furthermore, we have shown in the previous chapter that a portion of the carbon in the body is excreted, unburned, in the form of urea and similar products of catabolism. In addition, fermentation in the rumen and intestinal tract produces

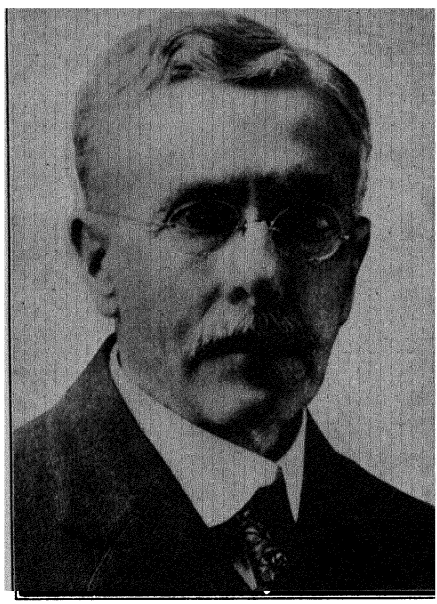

FIG. 58. Dr. Henry Prentiss Armsby (1853–1921), formerly Director of the Pennsylvania Agricultural Experiment Station and for many years Director of the Institute of Animal Nutrition at the Pennsylvania State College. He was one of the foremost authorities in the field of domestic animal nutrition, being best known for the development of the net energy conception with reference to feeding standards for livestock. (Courtesy of Dr. E. B. Forbes.)

combustible gases which are never utilized by the animal body. It is evident, therefore, that the energy content of these substances must be lost so far as the animal is concerned.

Dr. Armsby and his co-workers have been accustomed to calculate the total gross energy of the feces, urine and gases, and subtract this amount of energy from the gross energy of the feed consumed. It is evident that the difference obtained in this calculation must represent the amount of energy that was utilized by the body. Dr. Armsby desig-

nates this portion as *metabolizable energy*. The following data taken from Armsby's work will make this clear:

Energy of feed		
6,988 gm. timothy hay		27,727 Cal.
400 gm. linseed meal		1,811 Cal.
		29,538 Cal.
Energy of excreta		
16,619 gm feces	14,243 Cal.	
4,357 gm urine	1,210 Cal	
142 gm. methane	1,896 Cal.	
Total		17,349 Cal
Metabolizable energy		12,189 Cal.

The term metabolizable energy does not indicate how the energy is used; it merely indicates that it is used by the animal for some purpose. As a result some workers use the synonyms "available energy," "fuel value," and "physiological heat value" instead of "metabolizable energy."

It has been found that a certain amount of energy is necessary for the normal digestion of food, circulation of blood, etc. When this energy expense is subtracted from the metabolizable energy, the remaining portion which can be used for maintenance, fattening, growth, etc., is called *net energy*. Dr. Armsby and his co-workers have attempted, therefore, to determine the net energy of feeds for various purposes, such as body maintenance, fattening, growth, and milk production.

Conditions Affecting Energy Metabolism

Since heat is produced when work is done by the animal body, it is reasonable to expect that *activity* of the animal should be one of the most important factors to consider in measuring energy metabolism. *Age* and *size* are also important factors to consider when heat production is calculated or measured.

We have already used the term "basal metabolism." By this we mean the energy output of the body relaxed and at rest 12 to 18 hours following the last meal or intake of food. This basal metabolic rate varies somewhat for individuals and represents the amount of energy required for the maintenance of life. This, according to McLeod, is "overhead" cost that has to be charged for running the body machinery, although no external work is being done. This method has become valuable, in medical practice, to obtain information relative to unnatural

hastening or impeding of the basal metabolic rate due to disease. This method is particularly useful in detecting changes in metabolism due to disturbance of thyroid activity (goiter).

Size of Body. — Basal metabolism is more closely related to body surface than to body weight. The reason for this lies in the fact that a small body has more surface per unit of weight than a large one. This can be understood quite readily if we refer to certain calculations that are made in elementary physics.

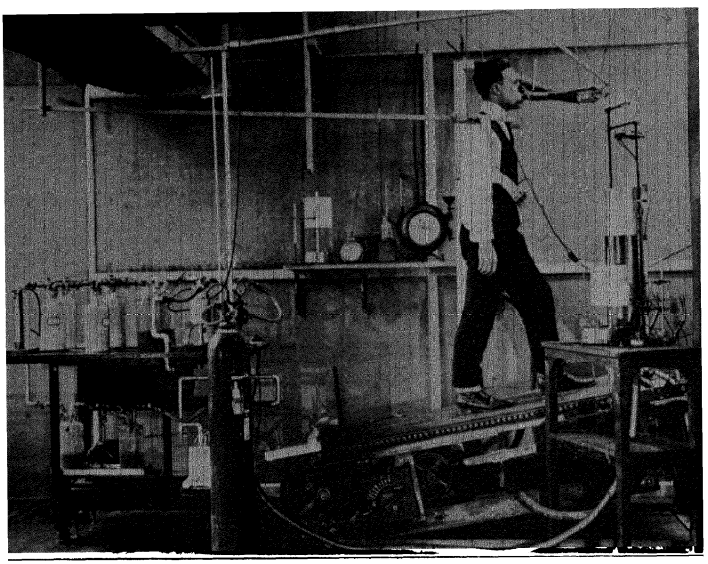

FIG. 59. Tread mill and absorption equipment to measure the heat production during walking. (Courtesy of Dr. F. G. Benedict.)

Let us assume that we have two spheres, one of them an inch in diameter and the other two inches.

Ball *A*		Ball *B*
1.00	diameter (inches)	2.00
3.14	surface area (square inches)	12.57
0.52	volume (cubic inches)	4.19
6:1	surface to volume	3:1

This means, if applied to the animal body, that small animals, on account of the larger amount of surface area per unit weight, must lose more heat and, therefore, metabolize more rapidly per unit weight than larger animals.

Armsby states that it is possible to calculate the body surface of beef cattle quite accurately by the use of the following formula, where W is the weight of the animal in grams, S the body surface in square centimeters, and K a factor or constant for all animals of the same shape.

$$S = KW^{\frac{2}{3}}$$

For beef cattle, the factor K may vary, depending upon the thinness or fatness of the animal, but, according to Moulton, it is not far from 10.34.

Shape also plays a part in the amount of surface the body may have exposed for heat radiation and heat loss. Dr. Rose has shown this graphically by the following calculations. Assuming that we have two cylinders, A wide and short and B tall and thin, but both weighing the same.

A		*B*
1 00 pound	weight	1 00 pound
2 60 inches	diameter	1 30 inches
2 60 inches	height	12 20 inches
31 80 square inches	surface area	18 30 square inches

If we wish to make a practical application at this point, we can say that a tall thin person should have more surface area, per unit weight, than a fat and "stocky" type of individual.

Other Factors Influencing Metabolic Rate. — Although it is not within the scope of this book to deal with these interesting phases in detail, it should be pointed out that *temperament, mental state, muscular tension, pathological conditions, endocrine secretions, age, sex,* and *environmental temperatures* may play important rôles in causing variations in heat production.

Specific Dynamic Action of Foods

This remarkable phenomenon deserves special mention since its effect on normal heat production may be quite marked Lavoisier was probably the first scientist to note that the consumption of food increased the oxygen absorbed by his subject Many years later Pettenkofer and Voit noted that the heat of combustion in a dog was proportional to the amount of meat ingested Fat and starch could not compare with meat in this regard, and the heat stimulation with fat was less than that of starch. In other words, it was observed that certain kinds of foods possessed different effects on the stimulation of heat production. Rubner fed bones, water and meat extract and could not obtain the metabolic stimulation that protein, fat and carbohydrate were capable of producing. He found that he obtained (in man) ten times the stimulation with proteins that he obtained with carbohydrate. He concluded that, for every

100 Calories, an extra 6 Calories must be allowed for the extra excitation of cell metabolism, whereas protein called for an increase of 40 per cent of the theoretical protein Calories. This effect of foodstuffs was called, by Rubner, *specific dynamic action.*

Later work, by Benedict and Carpenter, Du Bois, Lusk and others, has shown that this effect, especially for protein, is very marked and may amount to 30 or 40 per cent of heat in excess of that actually contained in the food. Ordinarily this represents a loss to the body. This is not true in cold winter weather, however. When the external temperature is much below that of the body, this extra heat is turned to account in keeping the body warm and, as a consequence, is not wasted.

We can see from this brief discussion that the law of conservation of energy holds here as elsewhere. The body must receive sufficient energy units to keep the body machine running and, in addition, furnish energy for all other activities of the body, no matter what these activities may be. By careful study of the body activities it is possible to calculate the exact number of energy units that must be furnished by the food to keep the body running at normal efficiency. This (energy) method of determining food requirements of the animal body has been and still is the most useful tool the scientific feeder has to work with when he desires to feed economically and efficiently.

Biological and biochemical research during recent years has shown, however, that we cannot formulate ideal rations with the mere knowledge of the facts thus far enumerated. It was discovered quite recently that animals may receive rations designed according to the best chemical and caloric feeding standards, and yet these animals will not necessarily grow or reproduce unless certain other factors are taken into consideration. These newer phases of animal nutrition will be discussed in the following chapters.

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CHAPTER XXVI

BIOLOGICAL RESPONSE TO FOODS

During the past fifteen or twenty years a type of research has come into favor which merits special emphasis at this point. This type of research involves the biological response of standardized experimental animals to purified diets, in which the various constituents are controlled by the investigator. Ordinarily the rat is used in this type of investigation, although other animals such as mice, cats, dogs, guinea-pigs and even calves and other higher animals have been used with success. Since individual animals tend to be quite variable with reference to their response to foods, it has become necessary to develop a standard type of animal which will tend to reduce this variability factor to a minimum. It is for this reason that the rat has increased in popularity as an experimental animal. The albino rat and the hooded or pied rat have been reared for generations in captivity, and by careful selection and scientific inbreeding it has been possible to develop a uniform type of animal which can be expected to grow at a reasonably uniform rate under normal conditions.

There are other reasons why the rat has become indispensable to the nutrition worker. On account of the fact that chemically purified rations are expensive to prepare in quantity, it is highly desirable to use a type of animal which will consume a minimum amount of food. On account of its size, the rat is an economical type of animal to use, since its food consumption is relatively low. The rat is omnivorous and will eat almost any kind of food, no matter how insipid and tasteless the experimental diet may be Lastly, but by no means less important, is the fact that the gestation period of the rat is short (21 days), which makes available large numbers of families in a relatively short period of time. Thus, it is possible to study the effect of diet on reproduction as well as on growth response.

Growth in animals is due, primarily, to two factors, viz., the impulse to grow, which is hereditary, and the quality and quantity of the food, which is the most important external factor. The impulse to grow is the final limiting factor, since an excess of food cannot stimulate growth beyond the natural capacity of the animal to grow. It has been shown, however, that many animals and people have a capacity to grow far in

excess of their so-called normal growth-rate when the quality and quantity of the diet are improved.

One of the first investigators to make use of the biological-response method was Lunin, who published (in 1881) the results of experiments from which he concluded that mice could not live on an artificial diet consisting of purified proteins, fats, carbohydrates and salts. This diet was improved by the addition of small amounts of cow's milk, and the animals thrived and grew normally. Lunin drew the conclusion that milk contains something, other than protein, fat, lactose or salts, which was necessary for health and growth. This work was published in an obscure journal and did not attract the attention of the scientific world for a number of years.

For centuries it had been recognized that certain diseases such as beriberi, scurvy and rickets were related in some way to the diet. It is through the study of these dietary deficiency diseases that nutrition work has received considerable impetus, and the biological-response method has been of great service in explaining the etiology of these diseases.

The older literature contains many references to the experience of ships' officers and surgeons with reference to ship beriberi and ship scurvy. Kramer, an Austrian military physician, recognized scurvy as a deficiency disease as early as 1720 and advocated the use of oranges, lemons and limes. Sailors knew that most of the crew would be afflicted with these diseases if cruises were of long duration. If ships were driven from their courses and could not touch land for months and, as a consequence, could obtain no supplies of fresh foods, sickness, incapacitation and even death resulted. This was due to the fact that the sailors were forced to subsist for long periods on hard tack, flour, beans and salt pork. When shore was reached and fresh fruits, vegetables, whole grains, milk and fresh meat were procurable the deficiency diseases were cured or became less severe.

It was natural, therefore, that medical men should ask the question, " What do fresh foods contain that gives them their healing and curative properties? " An English maritime surgeon by the name of James Lind conducted dietary experiments on shipboard and published (in 1772) his conclusions in a book entitled " Treatise on Scurvy," in which he pointed out the importance of citrus fruits and other anti-scorbutic fruits and foodstuffs.

In 1882, the Japanese Navy conducted a large-scale nutrition experiment in which different ships in the fleet, during a world cruise, were rationed in different ways with varying types of diets. On the return the " control ship," which was rationed with the regulation diet, con-

tained many cases of ship beriberi, but the sailors on the other ships, who received meat, fruit and vegetables, were practically free from the disease. As a result, the Japanese regulations regarding rations were changed immediately.

In 1897, a Dutch physician named Eijkman, working in the Dutch Colonies, made the observation that he could produce experimental beriberi (avian polyneuritis) by feeding chickens a diet consisting of white

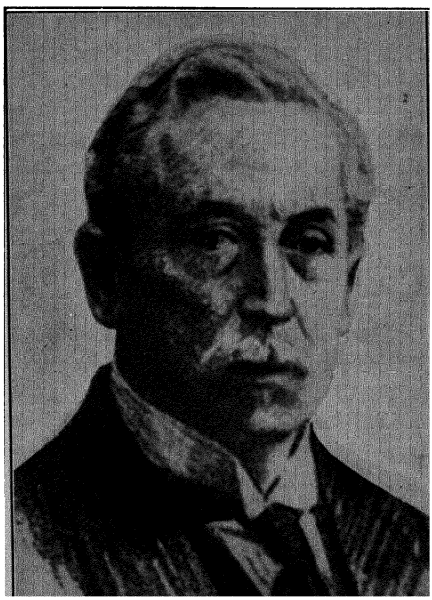

FIG. 60. Dr. C. Eijkman, whose pioneer experiments led to the production of experimental avian beriberi and which were responsible in a large measure for later work which led to the discovery of vitamins.

polished rice. The whole (red) rice did not cause the disease, and the paralytic symptoms could be prevented by oral administration or by injections of the alcohol-soluble material obtained from rice bran. Eijkman drew the erroneous conclusion that white rice contains a poison or toxin, and that the curative outer coats of the rice kernel contain an alcohol-soluble antidote for the poison. Later work showed that a curative substance (vitamin) was present in the rice bran which was absent in the polished rice.

In 1906, Hopkins, of England, described experiments in which exper-

imental animals were fed purified rations which were supposed to be nutritionally adequate, according to the best of scientific knowledge. It was found that the animals would grow for a while on this diet, after which rapid loss in body weight occurred unless milk was added to the diet. The amount of milk added (2 cc. daily) was so small that Hopkins concluded that the growth-promoting properties of milk could not be explained on the basis of protein, fat, sugars, or salts, but that the stimulatory properties were due to some hitherto unknown substance or substances. Later (1912), Hopkins suggested the name " accessory food factors " for these mysterious food constituents.

In 1911, Funk, a Polish chemist, reported that he had been able to verify Eijkman's work relative to the production, prevention and cure of avian beriberi. Funk continued the work and isolated from rice polishings a crystalline substance (containing carbon, hydrogen, oxygen and nitrogen) which was curative in avian beriberi in pigeons. Its basic chemical properties indicated that it was *amine-like,* and, since it seemed to be *vitally* necessary for his birds, he suggested that such substances be known as *vitamines.* This new word appealed to the imagination of the scientific world, and it has become an official term in nutritional and medical literature. Owing to the fact that the alkaloids terminate in the letters " ine," it was decided that the terminal letter in " vitamine " should be dropped in order to distinguish these substances from the plant alkaloids. As a result, the official term is now " vitamin," in which the first *i* is long, the second *i* is short, and the accent is on the first syllable.

In 1911, Osborne and Mendel, of the Connecticut Experiment Station and of Yale University, published their classical work in which they described biological-response studies with rats fed on purified protein, starch, agar, lard, sugar and inorganic salts. The salts were prepared from milk by removing the milk proteins and fat, after which the whey was evaporated to a powder. The agar was fed to furnish bulk or roughage.

The experimenters observed that they could obtain satisfactory growth for a considerable time, but eventually the animals ceased to grow unless the ration was modified.

In 1913, McCollum and Davis, of Wisconsin, and Osborne and Mendel, of Connecticut, announced that certain animal fats possessed growth-stimulating properties that were not possessed by the vegetable oils. Butterfat and egg fat were found to be superior in this regard.

By 1915–16 it was clear that there were at least two accessory food factors. McCollum and co-workers had established that the anti-beriberi factor in rice also possessed growth-promoting properties when

fed to rats. As a result, these investigators suggested the term *fat-soluble A* for the factor existing in butterfat and the term *water-soluble B* for the water-soluble factor found in rice bran and yeast. Since there was no authoritative evidence that the fat-soluble factor contained nitrogen, McCollum and co-workers refused to refer to it as " vitamin A." In spite of the logic of their arguments the term has persisted, so that all these food factors are referred to as vitamin A, vitamin B, vitamin C, etc.

In 1912, Andrews, working with human beriberi patients, postulated that the beriberi-curing substance could be transferred in the mother's milk to the nursing child. In 1916, McCollum and co-workers stated that the amount of water-soluble B in milk is proportional to the amount of this vitamin in the food of the lactating female. This theory was later substantiated in a quantitative way by Barnes and Hume, Dutcher, Hart and Hess and their co-workers in the United States.

During the period 1912 to 1916 much attention was given to the quality and quantity of the various ingredients in purified rations used in studies on biological response. Proteins such as casein, lactalbumin, edestin (hemp seed), gliadin (wheat), zein (corn), and gelatin were isolated, purified and studied. Of the various refined glycerides used in these studies, butterfat, egg fat, cod-liver oil, olive oil, lard and cotton-seed oil are typical. Purified carbohydrates such as starch, dextrin, sucrose and lactose were used as a source of energy in purified diets. In this early work, Osborne and Mendel furnished the salts necessary for growth by the inclusion of the powdered whey obtained from milk after removing the fat and proteins. This salt mixture, which was known as " protein-free milk," also contained the water-soluble vitamins of milk, although this was not known until later. Finally, it became necessary to make up artificial salt mixtures from inorganic salts in order that contamination might be avoided.

The following ration, which has been used, with modifications, in the laboratories of the Pennsylvania State College, is representative of the type of ration that may be used in vitamin studies:

TYPICAL BASAL RATION FOR VITAMIN STUDIES

Purified casein	18 0
Purified dextrin or starch	76 0
Agar	2 0
Salt mixture	4 0
Total	100 0

The salt mixture is known as the McCollum (No. 185) salt mixture, the composition of which is given on page 416.

COMPOSITION OF McCOLLUM'S No. 185 SALT MIXTURE

NaCl	173
$MgSO_4$ (anhydrous)	266
$NaH_2(PO_4)$ H_2O	347
K_2HPO_4 ..	954
$CaH_4(PO_4)_2$ H_2O	.540
Ferric citrate	.118
Calcium lactate	1 300

Since this ration contains no iodine, it is customary to add traces of this element to the drinking water. The vitamin-containing food to be studied may be fed separately in weighed amounts or mixed in the ration in known quantity. When a food is being examined for the presence of a certain vitamin, all other known vitamins are added to the ration and the food to be investigated is fed in graduated amounts until the presence or absence of the vitamin in question is ascertained.

In our own laboratories it is the practice to remove the young rats from the mother at 21 days of age, at which time they weigh about 40 grams. These experimental animals are placed in individual cages, with false screen bottoms, and the ration to be studied is introduced immediately. Food intake, body weight, and clinical observations are recorded at regular intervals.

It was by means of experiments of this type that Osborne and Mendel made their classical discovery that the biological value of proteins varies, depending upon the quality and quantity of the amino-acid content of the protein molecule. This work will be discussed in a subsequent chapter.

The biological-response method, using purified diets, has led not only to a better understanding of the biological value of proteins, but also to a better appreciation of the importance and function of the various inorganic elements. Lastly, our present knowledge of the importance and function of vitamins would not have been possible without this method of attack.

Returning to our discussion of vitamins, we find that the work described in previous paragraphs had laid the groundwork for the rapid development of a new field in animal nutrition.

Funk, in 1911, attempted to isolate the vitamin which was curative in beriberi. His attempts were unsuccessful, although he obtained a crystalline substance which seemed to possess pronounced curative properties. These basic nitrogenous crystals prompted Funk to coin the term "vitamine."

Quite recently Smith and Hendricks, Goldberger, Salmon, Hauge and Carrick, Axtmayer, R. R. Williams, Peters, Drummond, Chick and Ros-

coe, Reader and others have contributed experimental evidence to show that vitamin B is really a *complex* consisting of two or more factors, one of which is heat-labile, whereas the other is quite stable to heat at relatively high temperatures. British scientists have suggested the term B_1 for the former and B_2 for the latter. American investigators, on the other hand, are now restricting the letter B to designate the heat-labile (anti-neuritic) fraction, and they have coined the term " vitamin G " to refer to the heat-stable appetite-promoting and pellagra-curing fraction of the old vitamin B complex. This will be discussed in detail in a subsequent chapter.

The disease called scurvy has been known for a great many years, but it was not until recently that it has been classed among the vitamin-deficiency diseases. We have already referred to the work of Lind, who described experiments in human scurvy which showed quite definitely that it was futile to attempt to cure the disease by medication. Fresh foods, fruits and fruit juices, however, were decidedly beneficial. The classical researches of Holst and Frolich (1907–12) proved that experimental scurvy could be produced by feeding cereals and hay to guinea-pigs, and that the disease could be prevented and cured by certain fresh foods. They postulated the presence, in these fresh foods, of an anti-scurvy factor, and supported their argument by showing that the scurvy-curing properties of food could be destroyed by heat treatment, drying, etc. Later, this work was verified by other workers, and the scurvy-curing factor became known as vitamin C.

For centuries, medical men have been familiar with a disease in babies and growing children which is characterized by a failure of the bones and teeth to ossify normally. In this disease, which is known as rickets, bony structures remain soft and weak owing to failure of these tissues to calcify properly, because of the fact that calcium and phosphorus utilization is hampered.

For a time it was thought that this disease was due to a deficiency of vitamin A in the diet, since cod-liver oil was found to be a specific curative food material. Later, however, it was found that cod-liver oil contains two factors, vitamin A and a substance which was specifically curative in rickets. This substance has since been designated as vitamin D.

About 1920, Evans and his co-workers published the results of experiments in which a diet was fed which was supposedly adequate in all respects and which contained vitamins A, B, C and D. Animals grew and developed normally on this ration but, sooner or later, lost the power of reproduction. The introduction of small amounts of lettuce or wheat germ prevented the development of sterility and brought about fertility

in sterile rats. They postulated the presence of an anti-sterility factor to which they gave the tentative designation " vitamin X." Later this factor became known as " vitamin E."

At one time the same workers thought they had evidence for the existence of another factor which they named vitamin F. This work was later reinterpreted, with the result that the existence of this vitamin was denied. In the meantime, the term vitamin G had been given to the pellagra-curing, appetite-promoting, heat-stable fraction of the vitamin B complex, which has resulted in a temporary gap in the alphabetical system of vitamin terminology, as used in the United States.

With this brief account of the history of the development of this interesting phase of nutritional research, we shall now turn our attention to a more detailed discussion of the more outstanding nutritional discoveries which have been made possible through small-animal experimentation.

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CHAPTER XXVII

PROTEIN QUALITY

In previous chapters we have discussed the classification of proteins, their chemical characteristics and the chemistry of their hydrolytic products, the amino acids, and we have pointed out that all proteins contain approximately the same percentage of nitrogen (16 per cent). For this reason it was believed, for many years, that all proteins were of the same biological value and could be used interchangeably in the diet.

As early as 1872, Voit conducted experiments to determine the amount of lean meat per day required to keep a dog in nitrogenous equilibrium. In other words, he found the amount of meat which would prevent a " protein deficit " in the dog. When he substituted gelatin for meat, he discovered that he was unable to prevent a loss of body protein, and as a result of his experiments he concluded that gelatin was, in some manner, biologically inadequate when fed as the sole source of nitrogen.

Later work showed that gelatin was deficient in certain amino acids, the most important of which were tyrosine, tryptophane and cystine. It is not within the scope of this book to discuss all the steps which led to our present conception of protein quality. We shall merely attempt to point out a few of the researches which led to our present knowledge of these important nutrients.

In 1913, Abderhalden described experiments in which a dog had been kept in good health for 100 days, gaining 9.35 kilograms in body weight, on a diet containing, as its source of nitrogen, the hydrolysate of meat. In other words, the animal had lived and grown on a diet which did not contain protein. This naturally led to the conclusion that amino acids, which are the result of protein digestion, must be utilized and synthesized by the animal body. This was contrary to the prevailing conception of the time. Most scientists of that period believed that proteins were absorbed as such, and that the " circulating protein " of the blood was proof for this theory.

Kaufman, in 1905, had been able to show that gelatin could be supplemented, advantageously, by the amino acids tyrosine, tryptophane and cystine, so far as the maintenance of nitrogen equilibrium was concerned.

In 1906, Willcock and Hopkins published results of experiments with mice in which zein from corn was used as the sole source of nitrogen.

Zein was found to be an incomplete protein, and mice could not live more than two weeks when the nitrogen was furnished by zein alone. The addition of tryptophane, however, delayed death for about two weeks longer.

It remained for Folin and Denis, Van Slyke and Meyer, and Abel and co-workers to prove, experimentally, that amino acids were absorbed in the intestine; that they circulated in the blood stream as amino acids

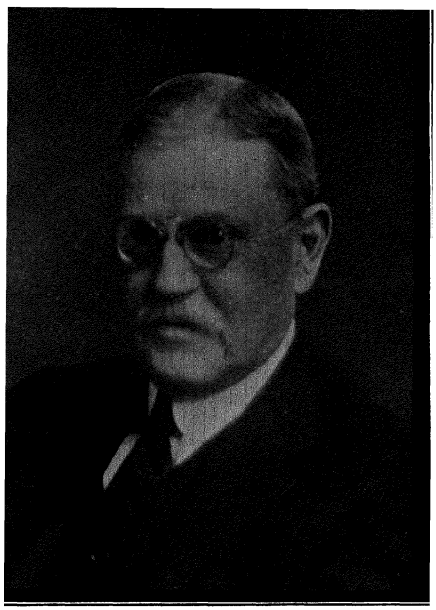

FIG. 61. Thomas Burr Osborne (1859–1929), authority in many phases of agricultural and biological chemistry, particularly in the fields of protein chemistry and animal nutrition.

and, as such, were utilized by the body in the repair and synthesis of tissue.

The classical researches of Osborne and Mendel, in spite of many handicaps due to lack of our present knowledge of vitamins, finally paved the way for a new appreciation of the biological value of proteins. These investigators used rats as their experimental animals and fed purified rations in which a single purified protein was fed as the sole source of nitrogen. Figure 62 shows one of their typical early experiments in which casein, gliadin (from wheat) and zein (from corn) are compared.

The casein was deficient in glycine but produced excellent growth, indicating that this amino acid is not essential or that it can be synthesized by the tissues. Gliadin was deficient in lysine, and growth was barely maintained, whereas zein, deficient in glycine, lysine and tryptophane, not only did not maintain growth but was so incomplete that death ensued if the ration was not changed within a reasonable time. When zein was supplemented with tryptophane the rats lived for months but did not grow. When lysine and tryptophane were added, fair growth was obtained. These, and similar experiments, led Osborne and Mendel

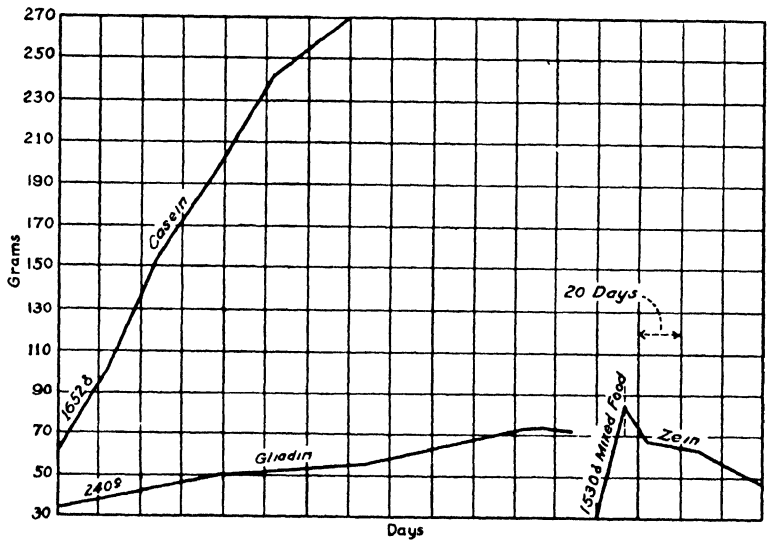

FIG. 62. Casein is superior in biological value, to gliadin (from wheat) and zein (from corn). This is due to the fact that casein contains certain amino acids which are not present in gliadin and zein. (Data of Osborne and Mendel.)

to conclude that an animal does not necessarily lose its ability to grow by being dwarfed for long periods by deficient diets. They showed that the growth impulse persisted even after the dwarfed animals had passed the age at which, under normal conditions, they would have ceased to grow.

Further work by Osborne and Mendel, McCollum and co-workers and others finally established, beyond question, that some proteins were biologically excellent. Casein and lactalbumin from milk were found to possess good biological qualities, and meat and edestin (from the hemp seed) were also satisfactory. Gelatin, gliadin, and zein were biologically inferior, and, in general, many of the cereals were found to be biologically deficient. Although it is not strictly true, it can be stated as a

rough generalization, that the animal proteins of meat, milk and eggs are biologically superior to the proteins of cereals such as wheat, corn, oats and barley. The explanation lies in the fact that the inferior proteins are deficient in certain of the amino acids necessary for growth. Considerable research is needed before this matter can be settled definitely, but it is quite evident that the animal body cannot synthesize some of the amino acids. As a result, we must search for them in plant tissues, where they are synthesized, or in animal proteins, where they have been stored following the ingestion of plant proteins by the animal. More research is necessary before we can state, with any degree of certainty, which amino acids are capable of synthesis in the animal body. At the present time, it is quite evident that lysine, tryptophane, cystine and histidine are indispensable, owing to the inability of the animal body to synthesize them.

The practical lesson that has come from this type of research is that, if we must be vegetarians, we should be certain to eat a sufficiently varied diet to insure the inclusion of all the necessary amino acids. Furthermore, it suggests the wisdom of supplementing cereal diets with animal proteins. The farmer has found by experience that this is a most profitable and economical practice. No good farmer today will feed cereal proteins, alone, to his hogs and poultry. He has found it highly advantageous to supplement his cereal rations with meat scraps, fish meal, tankage, skim milk and buttermilk This has not been done so universally in the case of cattle feeding, the problem of protein variety being solved largely through the addition of cottonseed meal, linseed meal, and other cereal concentrates. On the other hand, there has been a tendency in recent years to supplement dairy and sheep rations with animal proteins of various types, the most common of which have been fish meals and various types of meat meals

It is most difficult to express the biological value of proteins in a numerical manner so that they can be compared one with another, owing to the fact that the plane of protein intake, or percentage of protein in the feed, causes considerable variability. Likewise, the demands for growth, reproduction, lactation, egg production, etc., cause variations that cannot be controlled. Osborne and Mendel, McCollum and others have ascertained the minimum amounts of different proteins which will produce the same relative amount of growth in the rat. For example, it was found that 50 per cent more casein was required than lactalbumin and 90 per cent more edestin was needed to accomplish the same purpose.

Mitchell has made use of the Thomas method for determining the biological value of proteins, which is based on the determination of the total nitrogen fed and the nitrogen excreted, as compared with the nitro-

gen excreted on a nitrogen-free or nitrogen-poor ration. In other words, by subtracting the nitrogen wasted from the nitrogen ingested, it is possible to determine the nitrogen utilized in the animal body. By dividing the amount of nitrogen utilized by the amount ingested and multiplying by 100 he calculates the percentage utilized. This percentage is considered an index of the biological value of the protein under consideration.

The following values are given by Mitchell and Hamilton to show the relative protein values for some common food materials:

TABLE 66

PROTEIN VALUES OF FOODS FOR MAINTENANCE AND GROWTH: LEVEL OF PROTEIN FEEDING, 8 TO 10 PER CENT

(*Data of Mitchell and Hamilton*)

Food	Protein content [1]			Quality of protein		Metabolic protein in feces [2]		Protein value of food	
	Water content,[1] per cent	On fresh basis, per cent	On dry basis, per cent	Digestibility (coef.), per cent	Biological value, per cent	On fresh basis, per cent	On dry basis, per cent	On fresh basis, per cent	On dry basis, per cent
Whole egg[1]	73 2	13 2	49 3	100	94	0 4	1 4	12 0	44 9
Milk	87 0	3 3	25 4	100	85	0 2	1 4	2 6	20 2
Egg white[3]	86 2	12 3	89 1	100	83	0 2	1 4	10 0	72 6
Beef liver	71 2	20 4	70 8	90	77	0 4	1 4	14 9	51 1
Beef kidney	76 7	16 6	71 3	99	77	0 3	1 4	12 3	52 8
Beef heart	62 6	16 0	42 8	100	74	0 5	1 4	11 3	30 3
Beef round	70 0	21 3	71 0	96	69	0 4	1 4	13 7	45 7
Pork ham	60 0	25 0	62 5	100	74	0 6	1 4	17 9	44 8
Veal[4]	73 4	20 7	78 0	100	62	0 4	1 4	12 4	47 0
Rolled oats	7 7	16 7	18 1	90	65	1 3	1 4	9 8	10 6
Whole wheat	11 4	13 8	15 6	91	67	1 3	1 4	7 1	8 1
White flour	12 8	10 8	12 4	100	52	1 3	1 4	4 3	5 0
Whole corn	10 3	7 5	8 4	95	60	1 3	1 4	3 0	3 5
Potato	78 3	2 2	10 1	78	67	0 3	1 4	0 8	3 9
Navy beans[3]	12 6	22 5	25 7	76	38	1 3	1 4	4 2	6 0
Cocoa	4 6	21 6	22 6	38	37	1 4	1 4	1 6	1 8
Chocolate	5 9	12 9	13 7	38	37	1 4	1 4	0 4	0 6

[1] Average analyses taken, as far as possible, from Bulletin 28 (revised), Office of Experiment Stations, U S D A

[2] The metabolic nitrogen in the feces is assumed to equal 0 23 gram per 100 grams of dry matter of food See Bulletin of the National Research Council, 1926, xi, pt 1, no 55, p 23

[3] Cooked

[4] The cut tested was not recorded It proved to be very fibrous Analysis for shoulder cut assumed.

From the standpoint of practical feeding, therefore, it is clear that proteins differ in their biological value, and that their value for the re-

pair and synthesis of body tissues must depend upon the quality and quantity of the amino acids of which the protein molecule is constructed. The supplementary value of proteins in the diet may now be explained on

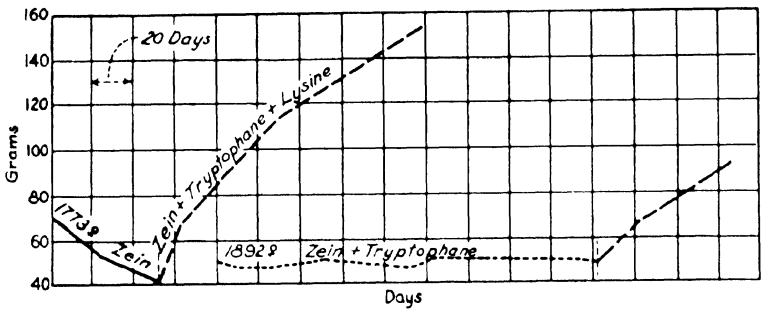

FIG. 63. Showing the biological inadequacy of the protein zein (from corn). This protein will not support growth until it is supplemented by the amino acids tryptophane and lysine. (Data of Osborne and Mendel.)

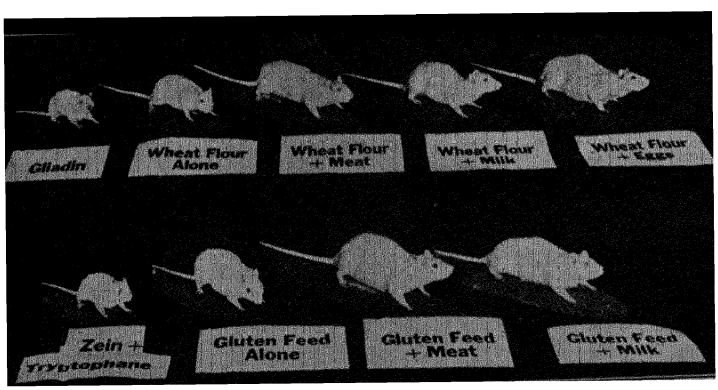

FIG. 64. These rats were all of the same age and were fed for the same length of time on diets containing the same proportion of protein. The variation in size is due to the differences in the biological value of the proteins eaten. (Data of Osborne and Mendel.)

a scientific basis, and the practice of feeding a *variety* of protein-containing foods and the wisdom of supplementing cereal diets with foods containing proteins of animal origin have been supported by experimental evidence.

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CHAPTER XXVIII

VITAMIN A

The absence of vitamin A from the diet brings about a number of abnormal changes in the body, the most noticeable of which are general debility, loss of weight and the development of an eye disease, known as xerophthalmia or keratomalacia. According to McCollum, the earliest record of a dietary ophthalmia was made by David Livingstone, who described a peculiar eye condition from which his African exploration party suffered while they were forced to subsist on a diet consisting of manioc roots, meal and coffee. This ophthalmic condition in humans had been noted by Russian physicians during religious fasts, and by Japanese physicians among Japanese children between the ages of two and five years. During the World War, a Danish physician by the name of Bloch described a large number of cases of this eye disease among Danish children. All these observers recognized that some relationship existed between the quality of the diet and the development of the disease. In fact, the Japanese had discovered that cod-liver oil, chicken livers and eel fat were efficacious in the prevention and cure of xerophthalmia.

It was not, however, until McCollum and Simmonds discovered that the disease could be produced experimentally in rats by omitting vitamin A from the diet, that a final explanation was obtained regarding the real cause of this ophthalmic condition. During the World War, another outbreak of ophthalmia occurred among children in Roumania. Owing to the lack of milk and dairy products, the children were forced to subsist on unbalanced diets consisting largely of cereal soups. The disease was soon brought under control, however, through liberal use of cod-liver oil, furnished by the American Red Cross.

The quantitative biological method for the determination of vitamin A in foods has become standardized so that it is now possible to express the vitamin content in " rat units " with a fair degree of accuracy. This is done by placing rats (at weaning time) on a diet adequate in all respects, with the exception of vitamin A. In about 30 to 40 days the rat will have used up its stored supply of this vitamin, and clinical deficiency symptoms develop which may be loss of weight, or ophthalmia, or both. At this depletion point, small weighed amounts of foods to be tested are added to the diet and the curative period is conducted for 5

to 8 weeks. The smallest amount of food necessary to maintain the animal, prevent loss in weight, cure ophthalmia, and prevent death is arbitrarily known as a "vitamin A unit." If, for example, we find that 5 milligrams of spinach fulfill the above requirements, we say that there is one vitamin A unit in 5 milligrams of spinach or 200 units of vitamin A per gram of spinach.

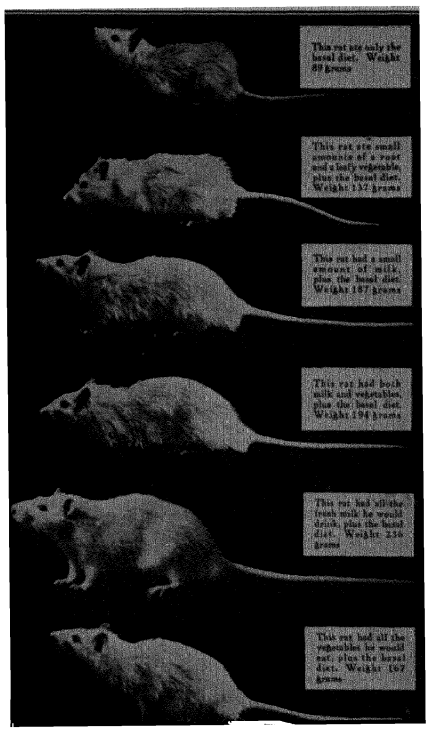

FIG. 65. Showing that the ordinary diet of bread, butter, meat and potatoes, valuable as it is, can be supplemented to advantage by additions of milk and vegetables. (Data of Bureau of Home Economics, United States Department of Agriculture.)

Just as soon as these methods were developed, it was but natural that research workers should turn their attention to the determination of the vitamin A content of foods. It was found that liver oils (particularly cod-liver oil), butterfat, cow's milk, egg yolk and similar foods were rich in this fat-soluble vitamin, but that cottonseed oil, olive oil, almond oil and similar vegetable oils were devoid of this factor.

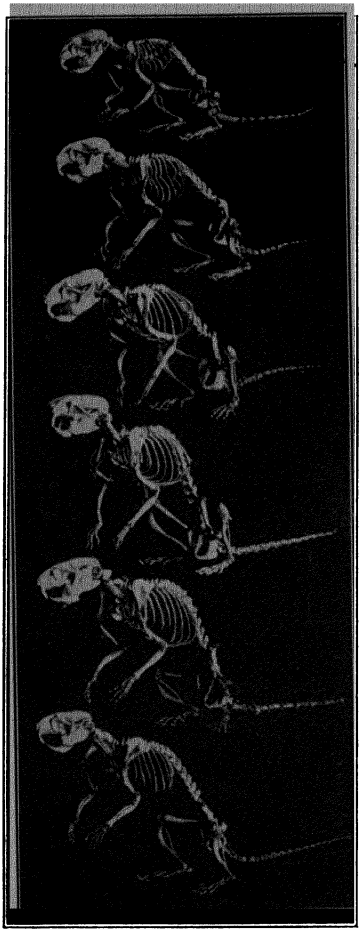

FIG. 66. Skeletons of rats which received the bread, butter, meat and potato type of diet with supplements of milk and vegetables. (See Fig. 65.) (Data of Bureau of Home Economics, United States Department of Agriculture.)

Leafy plants, particularly if highly pigmented, are usually excellent sources of vitamin A. Cereals as a group are very poor in this regard, with the possible exception of millet and yellow corn. It would appear that some relationship exists between plant metabolism and vitamin A synthesis, for the reason that the greater the pigmentation, the greater the amount of vitamin present. For example, the green outer leaves of cabbage and lettuce are relatively rich in vitamin A whereas the white inner leaves are almost devoid of this food factor.

There is some evidence that certain species of animals can synthesize other vitamins, but all experimental evidence, to date, points to the inability of the animal body to synthesize vitamin A, unless carotin is present in the diet. It is true that foods of animal origin are often rich in vitamin A, but this is due to the peculiar ability of the animal organism to store this fat-soluble factor in the liver fat and in certain other fatty tissues. This is apparently nature's method of protecting the animal organism against those times when the diet may be deficient in vitamin A. Under such conditions the animal does not develop vitamin A deficiency symptoms until this bodily store has been exhausted.

In a previous chapter it was stated that it is difficult to change the chemical composition of milk by dietary methods, but it is possible to change the vitamin content of milk by varying the vitamin content of the feed. Experiments by Kennedy and Dutcher, reported in 1922, were the first to show that the amount of vitamin A in cow's milk is dependent

on the amount of this vitamin in the dairy ration. In these experiments it was shown not only that the vitamin content of milk was dependent on the vitamin content of the ration, but that it was possible by proper winter feeding to produce a milk which compared quite favorably, in vitamin A content, with summer milk obtained when cows had access to fresh pasture grass. The same principle has been shown to apply to the enrichment of the vitamin A content of egg yolk, showing that the nutritive value of eggs can be enhanced by feeding rations rich in vitamin A. Such feeds as green sprouted grains, bright green alfalfa, yellow corn, cod-liver oil, leafy hays and green forage crops are excellent sources of vitamin A.

Although seeds are usually deficient in vitamin A, they possess the power to synthesize this vitamin during the germination and sprouting process. Seeds sprouted in the light, with the production of chlorophyll, are invariably richer in vitamin A than seeds sprouted in the dark.

Much has been written regarding the relationship between pigmentation and vitamin A content. Steenbock was the first investigator to stress the apparent interdependence of carotinoid pigments and vitamin A. He pointed out that most vegetables and foods which were rich in carotin were invariably rich in vitamin A. The question has been raised as to whether or not carotin and vitamin A were identical. Palmer and Kempster conducted experiments which showed that this could not be the case, since pig's liver was rich in vitamin A and yet was devoid of carotin. Palmer has shown also that cottonseed oil contains carotinoid pigment but contains little, if any, vitamin A. He contended that the association of carotin and vitamin A is fortuitous, and that, in general, vitamin A synthesis parallels pigmentation, but that they are not identical.

Hauge and Trost, of the Indiana Experiment Station, have shown that those varieties of yellow corn which possess colored endosperms are invariably richer in vitamin A than those which are pigmented to a less degree. These writers believe that the inheritance of vitamin A in yellow corn is according to the same genetic laws which govern color inheritance.

As this book goes to press, evidence has accumulated, through the work of Euler, Moore, Capper and others, to show quite definitely that carotin is changed in the body (probably in the liver) to vitamin A. In other words, the evidence points to the fact that carotin is the " parent-substance " or " precursor " of vitamin A. It is entirely possible that many of our so-called vitamin-A-rich foods may not contain vitamin A, per se, but their potency may be found to be due to the parent-substance, carotin.

When animals are fed fresh, pigment-rich forage crops or pasture grass, carotin pigment is deposited in the skin, body fat and in the milk. It is common practice for creamerymen to add artificial butter color to pigment-poor winter butter in order that it may have the appearance of June butter. The oleo oils, obtained from the body fat of animals on green forage, are highly pigmented and rich in vitamin A. Margarines made from such oils contain some vitamin A. In the past, one of the best "talking points" of the dairyman was that butterfat contained vitamin A whereas margarines did not. It is now possible to fortify the margarines with greater amounts of the fat-soluble vitamins A and D

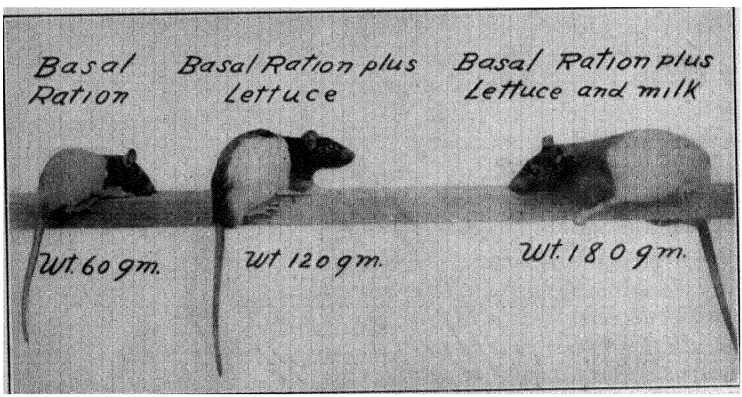

FIG. 67. These rats were of the same age and received the same basal diet with the exception of the supplements noted. This experiment emphasizes the importance of milk and leafy foods in the diet.

than are found in June butter. In fact, some of these margarines are now on the market.

When it was discovered that red palm oil was relatively rich in vitamin A, it was not long before the margarine manufacturers were importing this oil in relatively large amounts. Although it is possible to manufacture margarines fortified with vitamin-containing fats, the practice is not very general at the present time. It can be said, therefore, that butters and margarines may vary in vitamin A content, and that the former are generally superior in this regard and will probably remain so for some time to come.

Vitamin A can be extracted and these extracts can be made into concentrated preparations which possess a potency many times that of the original food material. When butterfat and cod-liver oil are saponified with alcoholic potassium hydroxide, the soaps can be separated from the

unsaponifiable material which contains practically all the fat-soluble vitamins. Attempts have been made to isolate vitamin A, but, to date, none has been successful. Takahashi describes a product called "biostearin," which he has isolated and which he believes to be identical with vitamin A. He has given it the empirical formula $C_{22}H_{44}O_2$ and believes that it is a dihydroxy alcohol, closely related to cholesterol. Takahashi reports that he has fed as little as 0.0001 per cent of biostearin in the diet of rats, with satisfactory results. He has prepared benzoates, acetates, hexabromides and ozonides of biostearin and has established the molecular weight at about 400. Drummond and co-workers, however, have attempted to repeat Takahashi's experiments and have been unable to corroborate his work.

Vitamin A is destroyed by heating at high temperatures in the presence of oxygen. In fact, this vitamin is so susceptible to oxidation that destruction occurs at all temperatures. Butter in cold storage may gradually form a bleached pigment-free outer layer owing to oxidative changes It is found that little or no vitamin A remains in this oxidized outer layer. When butterfat or cod-liver oil is mixed with feeds and stored in bags or bins, it is found that the vitamin A content disappears rapidly, because of oxidation. The presence of certain iron salts hastens this destruction, the iron acting as an oxidative catalyst. The presence of rancid fats catalyzes the destruction of vitamin A. Certain antioxidants, of which hydroquinone is representative, are capable of delaying this auto-oxidation of fats and of vitamin A.

Pasteurization of milk may or may not possess a destructive effect on vitamin A, depending upon the care of the operator and the amount of air in contact with the milk. Under proper conditions, vitamin A destruction by pasteurization is negligible, and the advantages of pasteurization often counterbalance the disadvantages of any vitamin destruction that may occur. One of the writers (Dutcher) has contended in the past that there is a place for unpasteurized grade A or certified milk in the human dietary, but that all milks of doubtful origin should be pasteurized. The discovery that undulant or Malta fever is caused by the presence of the organism which produces contagious abortion in cattle has reopened the pasteurization discussion. At the present writing, the question has not been answered, and more research must be conducted before definite recommendations can be made. Present data indicate that Malta fever is not found to an appreciable extent in children, who are the principal consumers of milk. On the other hand, there seems to be considerable evidence that contact with an infected animal seems to be the chief source of infection in humans. Until the matter is settled, it seems logical that all milks of doubtful origin should be pasteurized.

Vitamin A is not necessarily destroyed in appreciable amounts when foods or feeds are dried, evaporated or cooked, provided the heating is done in a short time in the absence of oxygen. Hart and co-workers have shown that leafy hays lose vitamin A if dried for long periods in the sun, during changeable weather. Quick drying or drying under caps

FIG. 68. A practical demonstration of modern dietetics. One of the malnourished children received at the health camp of the Philadelphia Interstate Dairy Council. After a few weeks of proper diet, rest and regulated exercise, the boy was sent home much improved in physical condition.

or covers tends to preserve vitamin A. Bright green hays are usually rich in vitamin A, whereas bleached or yellow hays are invariably poor, owing to loss of vitamin A by prolonged enzyme action and oxidative changes. Similar results have been reported by Russell and co-workers at the New Jersey Experiment Station.

Dutcher, Honeywell and Dahle have shown that ordinary commercial

evaporation methods lower the vitamin A content of milk to some extent, but that this destruction is not sufficiently great to be of nutritional importance. In fact, evaporated and condensed milks are excellent products of high nutritive value. The same statement can be made for dried or powdered milks. Modern processing has preserved most of the original nutritive value, with the result that these products are finding new markets every year and offer an excellent outlet for the excess milk produced in the periods of flush milk production.

During recent years, considerable interest has been developed in medical circles regarding the relation of vitamin A to resistance to infection. It is certain that appetite, growth and well-being are dependent on this vitamin. Evidence exists which indicates that a deficiency of vitamin A increases susceptibility to infections of the respiratory tract, sinuses, ears, kidneys and reproductive organs. Some authors believe that colds, pneumonia, otitis media, corneal ulcers, etc., can be relieved by diets rich in vitamin A. Such diets can be prepared through liberal use of cod-liver oil, butterfat, glandular tissues, cream, milk, carrots, spinach, liver, chard and other leafy plants.

The necessity of vitamin A in poultry feeding is an established fact, and Beehdel, of Pennsylvania, and Halvorsen and Sherwood, of North Carolina, have shown that dairy cattle are susceptible to a lack of this vitamin in the dairy ration. So-called "cottonseed meal poisoning" is thought to be due, at least in part, to vitamin A deficiency.

The vitamin A content of some of the more important human foods and animal feeds is appended in Table 67 at the end of Chapter XXXII. We will now consider some of the more important nutritional aspects and functions of the vitamin B complex.

For references for general reading, see end of Chapter XXXII.

CHAPTER XXIX

THE VITAMIN B COMPLEX (VITAMINS B AND G)

What was formerly called " vitamin B " is now known to consist of at least two factors: one which prevents beriberi, and one which seems to be specific in curing pellagra and which possesses appetite-stimulating properties. The former is quite easily destroyed by heat; the latter is relatively stable. Quite recently, Hunt, Williams, Waterman, Reader and others have contended that there are additional fractions of the old vitamin B. At the time these words are written, however, nutrition workers have not given definite recognition to more than the two fractions mentioned above, although the development of scientific evidence for additional members of the vitamin B family will be awaited with interest.

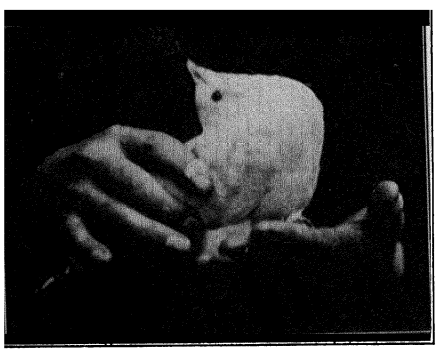

FIG. 69. A typical case of avian beriberi or polyneuritis in the pigeon. The head is retracted and the bird loses control of the muscles of the wings and legs.

The splitting of vitamin B into two fractions created a problem in nomenclature. What system of terminology could be adopted to anticipate further vitamin fractionation? Sherman suggested that the term " B " be kept for the old complex, that the anti-neuritic factor be called vitamin F, and that the term vitamin G be adopted to designate the " appetite-promoting and pellagra-curing fraction." In one sense this suggestion was a most happy one, for, as Dr. Sherman has pointed out, it would not be difficult to remember these vitamins, since Funk was

primarily responsible for the recognition of vitamin F, and Goldberger was responsible for the discovery of vitamin G.

British workers, in the meantime, had suggested that the term "B" be retained to refer to the complex and that the anti-neuritic factor be called "B_1," the anti-pellagrous factor be known as "B_2," and additional fractions be designated "B_3," "B_4," etc., as soon as their identity could be definitely established.

An American committee on vitamin B nomenclature appointed by the American Society of Biological Chemists objected to the use of the letter "B" to designate a complex containing more than one factor. Furthermore, these workers contended that the British system of terminology led to the inference that B_1 and B_2 were related. This inference, they contended, had no scientific evidence to support it, and, as a result, they were unwilling to adopt the British system of terminology.

As a result the American committee recommended that the term "B" be restricted to refer to the anti-beriberi fraction, since this vitamin was the first one recognized by scientific workers. In the meantime, Evans and co-workers had submitted evidence to show that they were dealing with a hitherto unrecognized factor which they called vitamin F.

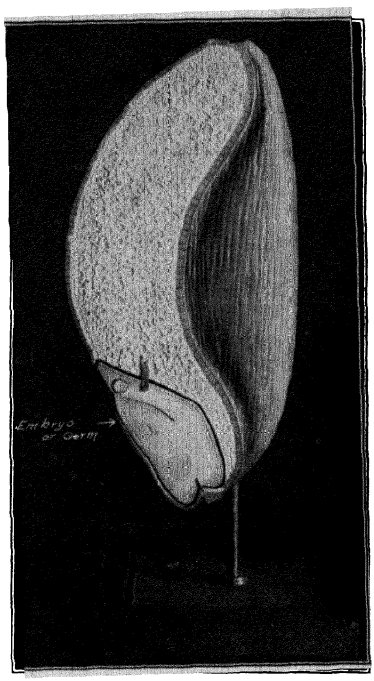

FIG. 70. Model of a kernel of wheat showing the embryo or germ which is removed when wheat is milled into flour. The germ contains relatively large amounts of oil and enzymes as well as appreciable amounts of the vitamin B complex. The keeping qualities of wheat flour are dependent upon complete removal of the germ.

The American committee recommended, therefore, that the anti-pellagrous, appetite-promoting fraction of the vitamin B complex be known as vitamin G. Subsequently, Evans obtained evidence which forced him to doubt the existence of vitamin F, which now leaves a gap in the alphabetical list of food factors. As a result, we find ourselves in a most unfortunate situation so far as vitamin terminology is concerned,

the anti-neuritic factor being called vitamin B_1 by European writers and vitamin B by many American workers. The anti-pellagrous factor is referred to in the literature as vitamin B_2 and vitamin G. Although the terms B and G have not been adopted officially in this country, their usage has become quite universal in American publications.

Since new technique must, of necessity, be developed to study these new vitamins and since much of the old work will have to be re-interpreted when the new studies are made, it will be necessary to discuss the vitamin content of foods in terms of the complex rather than in terms of individual fractions.

When the anti-neuritic factor, vitamin B, is omitted from the diet, birds and rats eventually become afflicted with a form of paralysis known as experimental beriberi. Pigeons, for example, may become afflicted with the disease after subsisting no longer than 15 to 20 days on a vitamin-B-free diet. The addition of small quantities of yeast or other foods which contain vitamin B will bring about almost miraculous relief from the paralytic symptoms. The apparent cure can be brought about in less than 3 hours by feeding or injecting vitamin-rich concentrates.

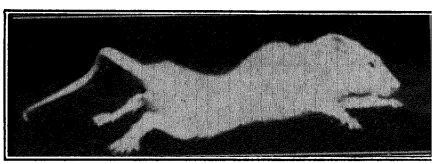

FIG. 71. Typical paralysis in the rat induced by feeding a diet deficient in the anti-neuritic factor, vitamin B.

Human beriberi has been brought under control in the Orient through the applications of present knowledge regarding vitamin therapy and proper dietary treatment. When experimental beriberi is studied by the pathologist, it is found that there are degenerative changes in the nervous system, enlargement of the heart and suprarenal glands, degeneration of lymphoid tissue and atrophy of many other organs.

Vitamin B is soluble in water, alcohol, glacial acetic acid and dilute acid solutions. It can be adsorbed from acid solutions by fuller's earth, Lloyd's reagent and similar hydrous aluminum silicates. It is quite stable in acid solutions but is readily destroyed in alkaline solutions. It can be precipitated from solution by the addition of salts of the heavy metals, by silico-tungstic acid and similar reagents. It will form picrates, which can be purified to a fair degree of purity. All evidence points to the fact that it is a basic nitrogenous compound. Vitamin B differs from its associated factor, vitamin G, in that it is much less stable to heat and

to oxidation. Much remains to be done, now that the fractions of the old complex have been identified, before definite statements can be made regarding the stability of the anti-neuritic factor during the various stages of food preparation, such as storage, drying and cooking.

When yeast is autoclaved, the anti-neuritic or anti-beriberi factor is destroyed, but it is found that the yeast still possesses a water-soluble

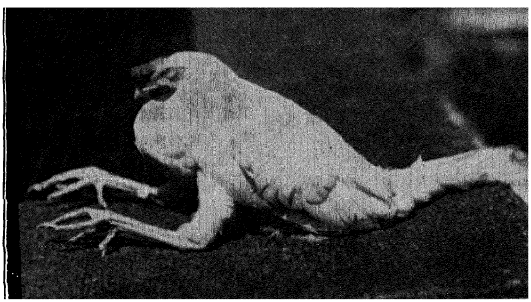

FIG. 72. Typical case of avian beriberi in the chicken induced by feeding a diet deficient in vitamin B.

factor (vitamin G) which stimulates appetite, thereby causing animals to grow more readily, and which also possesses the property of preventing the appearance of pellagrous-like skin lesions. Pellagra is a dietary deficiency disease which has occurred to an appreciable extent in the

FIG. 73. Pellagra-like skin lesions produced by feeding a diet deficient in vitamin G. (Courtesy of Dr. N. B. Guerrant.)

southern states and has been studied successfully by Goldberger and co-workers. Ordinarily the disease occurs most often among the poor who are forced to subsist on a diet consisting largely of corn and pork. The disease has been studied to some extent in penal institutions of the South.

The feeding of diets containing milk, vegetables and meat was found to prevent the disease, and, in late years, yeast has been fed with success.

The writers are informed that pellagra became a real problem during the recent Mississippi floods, and that the American Red Cross distributed large quantities of yeast and yeast concentrates among the refugees. The heat-stable factor which possesses anti-pellagrous properties is now known as vitamin G or vitamin B_2 (Brit.). According to the latest governmental records, pellagra has been increasing in the United States during the past few years, and at the time this book is being written (1931) the number of pellagrous cases is about 5.5 in every 100,000 of population.

A pellagra-like disease can be produced in experimental animals by feeding diets deficient in vitamin G. This disease is characterized by

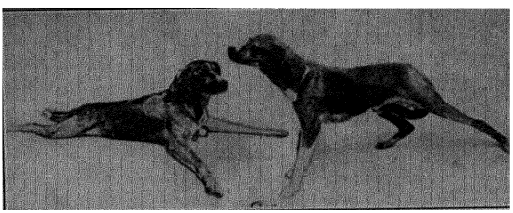

FIG. 74. Deficiency of the anti-neuritic factor, vitamin B. The dog on the left is paralyzed due to the absence of vitamin B in the diet. The photograph on the right shows the same dog 18 hours after receiving tomato juice. (Courtesy of Dr. G. R. Cowgill.)

loss of hair, hyperemia and ulcerations accompanied by hemorrhages. Some authors believe that the experimental dietary disease in dogs known as " black tongue " is identical with pellagra in man. This theory has been disputed.

Cowgill and co-workers have conducted interesting experiments with dogs, relative to the appetite-promoting properties of the vitamin B complex. These investigators describe experiments in which diets deficient in vitamin G were fed. In the course of two or three weeks appetite failed, followed by the loss of body weight. Upon the introduction of vitamin G preparations, appetite was stimulated and growth was resumed. One dog ate this artificial ration for a period of three years without the loss of the urge to eat, when the diet contained sufficient quantities of a yeast vitamin concentrate. Cowgill believes that vitamin B plays the dominant rôle in the stimulation of appetite, although the entire complex seems to be necessary.

Sure has shown that nearly four times as much vitamin B complex is necessary for successful lactation as is required for growth. He found that lactating rats dissipated about 60 per cent of the vitamin B complex

in the metabolism of the transfer to the milk. In other words, it would appear that vitamins B and G are secreted into the milk sparingly. As a result of his findings, he is of the opinion that vitamin B concentrates can be fed to advantage to nursing children. Sure has found that nursing rats afflicted with uncomplicated vitamin B (complex) deficiency, are characterized by possessing subnormal blood sugar and liver glycogen.

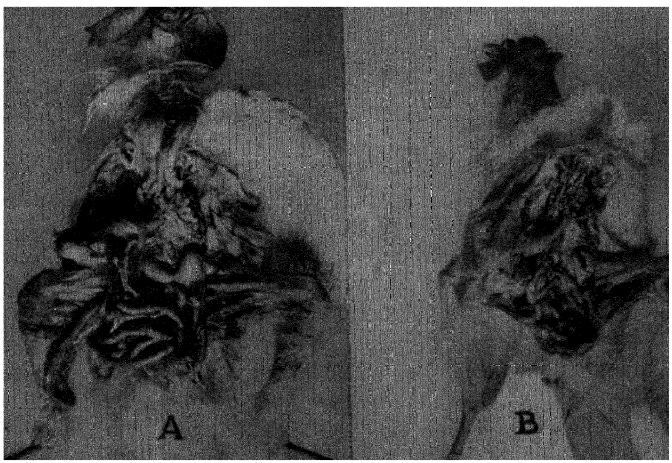

FIG. 75. Photograph showing the atrophy of organs as affected by the absence of the vitamin B complex. Chickens (*A*) and (*B*) received the same diet with the exception that (*A*) received adequate amounts of the vitamin B complex in the form of yeast, while yeast was omitted in the ration of chicken (*B*). Note the poorly developed organs of chicken (*B*) as compared with the normal bird (*A*). Particular attention is called to the size of the testes (bean shaped organs in center of photograph). Testicular development was almost completely absent in chicken (*B*). (Data of Souba.)

In a previous chapter we stated that Carlson and his associates have accumulated considerable evidence to show that contractions of the empty stomach are the physiological cause of hunger. Cowgill and coworkers have shown that gastric motility is enhanced by administration of the vitamin B complex. Birds afflicted with vitamin deficiency of this type are found to show some evidence of crop paralysis. Vitamin administration usually alleviates this condition. Some evidence exists also which leads us to believe that the vitamin B complex tends to promote intestinal motility. Attempts have been made, but without pro-

nounced success, to show that the vitamin B complex promotes secretory activity of the digestive glands. Some evidence has been advanced to show that biological oxidations are dependent upon vitamin B, but these findings have been questioned by some workers who believe that the lowered body temperatures in polyneuritis can be explained on the basis of inanition or starvation.

In conclusion, it can be said that vitamins B and G are necessary for optimal growth and development. Young growing animals should be

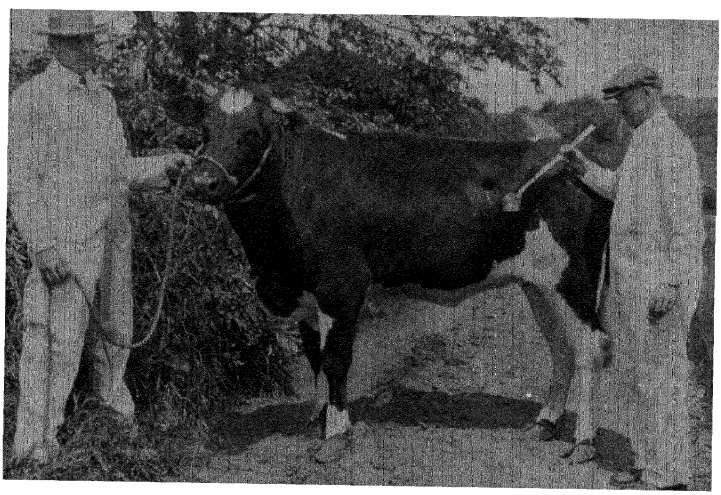

FIG. 76. Pennstate Homestead Jessie, showing permanent fistula in the rumen from which samples of the rumen contents were removed and studied. It was found that the rumen harbored an organism (Flavobacterium vitarumen) which possessed the power to synthesize the vitamin B complex. (See Fig. 77.) (Data of Bechdel, Dutcher, Honeywell and Knutsen.)

fed liberal amounts of these vitamins to insure good health and maximal growth. It would appear, in the light of our rather meager knowledge of the distribution of these vitamins, that vitamin B predominates over vitamin G in the cereal grains, and this seems to be true also for the tomato. Milk, on the other hand, is just the reverse, containing larger amounts of vitamin G. Fresh meat also contains the latter vitamin, but it is relatively poor in the anti-neuritic factor.

The vitamin B complex is stored in the liver and other tissues and in milk and eggs. The experimental work of Dutcher and co-workers did not show that the vitamin B complex in milk could be affected appreci-

ably by the feeding of rations deficient in the B complex. This was explained later by the researches of Bechdel, Dutcher, Honeywell and Knutsen. The experiments of Bechdel had shown that dairy cattle could be made to grow and reproduce normally on a ration deficient in

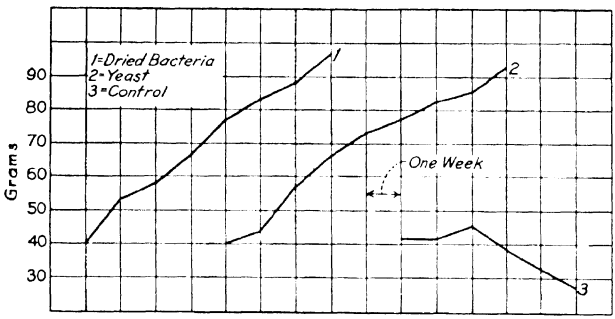

FIG. 77. Showing how rats grew when dried bacteria served as the source of the vitamin B complex. Group 3 received no vitamin B complex. Group 2 received yeast which is rich in these vitamins, while group 1 received bacteria (dried) obtained from the rumen of a cow, which received a ration deficient in the vitamin B complex. (Data of Bechdel, Dutcher, Honeywell and Knutsen.) (Courtesy of the Journal of Biological Chemistry.)

the vitamin B complex. Biological tests on the milk of these cows showed that the vitamin content was comparable with that of the milk from the regular college dairy herd. It was evident that the vitamin B

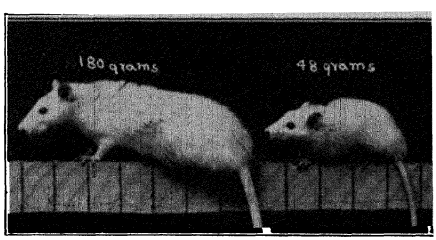

FIG. 78. These rats were from the same litter and at the beginning of the feeding experiment were practically equal in size and weight. Both received the same basal ration with the exception that the larger rat received daily allotments of the vitamin B complex while the smaller received none. (Data of Dutcher and Honeywell.)

complex was being synthesized in the animal body. To solve this problem, it became necessary to produce a permanent fistula (opening) in the rumen of one of the experimental animals.

Fermented food, removed from the rumen through this opening, was

extracted with alcohol and the extract was found to be potent in the vitamin B complex, showing that vitamin synthesis had taken place in the rumen (paunch). Subsequent studies revealed the fact that the vitamin was synthesized by a pigmented organism, of the genus Flavobacterium, which was named *Flavo bacterium vitarumen.* Damon and others have shown that other microorganisms are capable of synthesizing the vitamin B complex.

Although pasteurization, evaporation and sterilization undoubtedly destroy a portion of the vitamin B content of milk, the amount originally present is not large and destruction by heat treatment is not of much nutritional significance. There is little evidence that the vitamin G content of milk is affected appreciably by heat treatment

In the following chapter we shall consider the anti-scorbutic factor, known as vitamin C. References for general reading may be found at the end of Chapter XXXII For the distribution of the vitamin B complex in foods, see Table 67.

CHAPTER XXX

VITAMIN C

Vitamin C is known as the anti-scorbutic or " anti-scurvy " vitamin, since its presence in the diet prevents the development of the dietary-deficiency disease known as scurvy. In a previous chapter we have referred to the classical experiments of Holst and Frolich, of the University of Christiania (Norway), which showed that experimental scurvy could be produced in guinea-pigs by feeding certain types of diets. With the establishment of this technique, it has been possible to study the anti-scorbutic properties of foods and the factors influencing the quantity of vitamin C in feeding materials.

The researches of Holst and his colleagues established the fact that scurvy could be prevented or cured, at will, by supplementing a scurvy-producing diet with fresh vegetables, fruits and fruit juices. It was found that small amounts of cranberries, cabbage and dandelion leaves possess anti-scorbutic properties, and that drying and heating reduced the potency of these food materials to a marked degree Similar observations were made when fruit juices were stored for various lengths of time. They noted also that acid fruit juices, such as lemon juice and sorrel juice, tended to remain stable during storage. Subsequent researches led to the recognition of vitamin C as a specific factor in the prevention and cure of scurvy.

When guinea-pigs are fed scurvy-producing diets consisting of oats, skim milk powder (heated), butterfat and salt, or oats, bran and water, the animals grow for a time and subsequently develop symptoms of scurvy. Such symptoms are cessation of growth, tender and swollen joints, tender jaws, loose teeth and a tendency to lose control of the hind legs. Post mortem examination of scorbutic animals reveals a type of pathology very similar to that found in human beings who have died from scurvy, viz., fragility of bones and hemorrhagic areas in muscles and intestines.

Although the guinea-pig has become the classical experimental animal for the study of scurvy, the monkey has also been used quite successfully. It would appear that the rat, the chicken and pigeon are immune to this deficiency disease, for they can be reared successfully for long periods on diets which are devoid of vitamin C. Parsons was the first to show

that rats could be made to grow for many months on diets so deficient in vitamin C that they would cause the onset of scurvy in guinea-pigs within 10 to 25 days. After many months these rats were killed and their livers were fed to guinea-pigs which had become afflicted with scurvy. The number of guinea-pigs which recovered from scurvy was

FIG. 79. Feeding a guinea pig with a medicine dropper. This procedure is often necessary when it is desired that quantitative intake of certain vitamin-containing foods be assured.

sufficient to prove quite clearly that the rats must have been able to synthesize and store vitamin C in their body tissues. This finding has been substantiated by Parsons and Hutton and by Lepkovsky and Nelson. Parsons and Reynolds were able to cure scurvy in guinea-pigs by feeding the livers of normal guinea-pigs which had had access to ample quantities of the anti-scorbutic vitamin. On the other hand, the livers of

scorbutic pigs possessed no curative effect when fed to other animals afflicted with the disease.

Oranges, lemons and tomatoes are excellent sources of vitamin C, and for this reason most of the chemical studies have been made on the juices from these fruits. Acidity tends to preserve and alkalinity tends to destroy vitamin C. Dutcher, Harshaw and Hall showed that the anti-scorbutic properties of orange juice can be destroyed by hydrogen peroxide and oxygen at room temperature, and that the speed of destruction is hastened by heating. Hess is authority for the statement that copper in solution may act as a catalyst, and aid in the destruction of vitamin C during the heating of milk

Dutcher and co-workers have shown that milk which has been pasteurized in closed bottles at 145° F. for 30 minutes has lost little, if any, vitamin C. When the same raw milk was pasteurized in open vessels at the same temperature, with air bubbled through the milk, vitamin C destruction was complete. " Flash " pasteurization at a higher temperature was less destructive than ordinary pasteurization (when milk was agitated in contact with air), and boiling for ten minutes was not appreciably destructive

Vitamin C is soluble in water and in all concentrations of ethyl alcohol, and it can be dialyzed through membranes. Zilva and co-workers have studied the chemistry of this vitamin more thoroughly than have other workers. These workers have de-citrified lemon juice and have been able to remove the sugars by fermentation without appreciable destruction of the vitamin. By further chemical treatment and fractionation, Zilva has been able to concentrate practically all the vitamin C of lemon juice in 0.03 to 0 07 per cent of the total solids of the original juice Chemical analysis of this potent fraction showed very little nitrogen and only traces of phosphorus It also possessed a reducing action on ammoniacal silver nitrate and decolorized potassium permanganate.

La Mer, Campbell and Sherman studied the effect of heating and hydrogen-ion concentration on the stability of vitamin C in tomato juice. They concluded that time and temperature must be considered in controlling the destruction of this vitamin, the amount destroyed being proportional to these two factors. They also confirmed previous work which emphasized the importance of acidity in preventing vitamin destruction.

It is evident that vitamin C is gradually lost during the storage of fruits and vegetables. This is due, in all probability, to respiratory oxidation, which goes on continuously in the living plant tissues Kohman and Eddy have been able to prevent the loss of vitamin C during the commercial canning of apples, by immersing the freshly cut apples

in salt water, thereby preventing the activity of oxidative enzymes and shutting off the supply of atmospheric oxygen. They have succeeded in modifying manufacturing methods to the point where canned fruits and vegetables have been shown to contain much of the original vitamin content of the fresh food materials. (See Figs. 80 and 81.)

FIG. 80. A mother and her 28-day old litter, representing third and fourth generations of rats whose food supply consisted entirely of canned foods, the first generation having been put on such a diet at weaning time, i.e. 28 days of age. Growth was good and reproduction and lactation were all that could be expected on any diet consisting of ordinary foods. (Data of Kohman, Eddy and Gurin.)

It is difficult to generalize regarding the vitamin content of cooked, dried and stored foods, owing to the large number of variables that must be taken into account. As a general rule, the vitamin content of growing plants is greatest during the rapidly growing stage when metabolic

FIG. 81. A mother and her 90-day old litter, representing first and second generation guinea pigs whose supply consisted entirely of canned foods. Guinea pigs as well as rats, thrive on such a diet. (Data of Kohman, Eddy and Gurin.)

processes are at their maximum. It is quite evident that we obtain the maximum amount of vitamins when vegetables are picked at the tender, rapidly growing period. Young carrots, for example, are richer in vitamin C than mature carrots. Seeds are devoid of vitamin C, but this vitamin becomes abundant during seed germination. During the World War, the British army combated scurvy by feeding the soldiers germinated pulse. Although pigmented leaves are usually rich in vitamin C, there is no evidence that pigmentation is a necessary environmental factor for vitamin C production, since white cabbage and the white heart of head lettuce are potent sources of this vitamin.

Cabbage, which is an excellent source of vitamin C, loses its potency if the cabbage is dried slowly. If drying is conducted rapidly, at higher temperatures, less destruction of vitamin C is observed. If oxidation is prevented, considerable vitamin C remains in sauerkraut after the completion of the fermentation process.

Tomatoes, which are comparable with the citrus fruits in vitamin C content, have been studied more than many fruits Ethylene ripening does not seem to hinder or enhance vitamin C synthesis in the tomato, and greenhouse tomatoes are not considered as good sources of vitamin C as those ripened in the field.

Reference has been made to the storage of vitamin C in the liver. The same principle seems to apply to the vitamin C potency of milk. In 1919, Barnes and Hume and Dutcher, Pierson and Biester announced that they had noted seasonal variations in the nutritive and anti-scorbutic properties of cow's milk. Later work by Dutcher and co-workers showed that vitamin C could be increased or decreased in cow's milk by feeding rations rich or poor in vitamin C. It was concluded that the cow doles out her own supply of vitamin C slowly, since the anti-scorbutic properties of the milk become poor slowly. On the other hand, when the cows were fed a vitamin-rich diet, the anti-scorbutic properties of the milk increased rapidly, showing how nature seems to be attempting to protect the nursing calf. The influence of diet on the vitamin C content of milk has been shown by Hart and co-workers at Wisconsin and Hess and co-workers in New York.

It is possible to manufacture milk powders, evaporated and condensed milks in such a manner that vitamin C is not completely destroyed. Since milk is never a rich source of this vitamin and since the amount of vitamin C fluctuates with the vitamin content of the dairy ration, physicians have learned to regard cow's milk and milk products as an undependable source of the anti-scorbutic vitamin. As a result, orange juice and tomato juice are usually prescribed for bottle-fed babies

Zilva and Wells, in England, and Howe, of the Forsyth Dental Infirmary of Boston, have shown, in recent years, that the pulp of the teeth deteriorates when diets are deficient in the anti-scorbutic vitamin. Hanke, of Chicago, has shown, also, that dental caries and pyorrhea can be prevented and cured by dietary measures. Vitamin C seems to be quite specific in this regard, and orange juice, lemon juice and lettuce are recommended to correct these dental diseases. Research and clinical experience are now emphasizing the importance of dietary measures in the prevention of dental troubles, and the old saying, " a clean tooth is always a sound tooth," is now known to be incorrect. Oral hygiene may

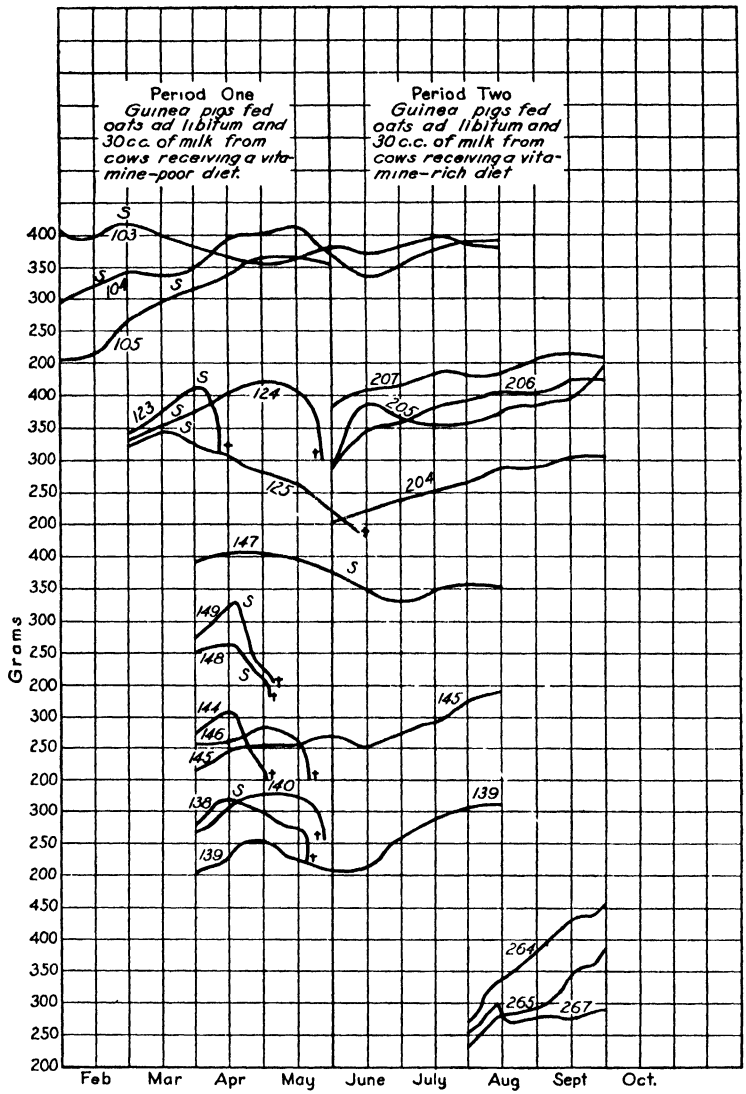

FIG. 82. Showing the influence of the diet of the cow on the amount of the antiscorbutic factor, vitamin C, found in cow's milk. When cows are fed a vitamin-poor ration, there is a tendency for the milk to become vitamin-deficient rather slowly. When vitamin-rich rations are fed, there is a tendency for the milk to become vitamin-rich rather rapidly. (Data of Dutcher and co-workers.)

be perfect, but teeth will deteriorate and decay if physiological conditions are not right for the manufacture of healthy dental tissues.

Infantile scurvy is not uncommon in this country, but there are few authentic records of scurvy in adults. This has been brought about by the fact that most people obtain sufficient vitamin C in fruits and vegetables, although it is probable that many people do not eat enough of these foods to prevent dental disturbances. There is little experimental evidence to show that vitamin C is of much importance, so far as the feeding of farm animals is concerned. The vitamin C content of some of the more common foods and feeds may be found in Table 67.

Vitamin D and ultraviolet light will be discussed in the following chapter. References for general reading will be found at the end of Chapter XXXII.

CHAPTER XXXI

VITAMIN D AND ULTRAVIOLET LIGHT

The deficiency disease which is prevented by vitamin D is known as *rickets*. This disease has been known for centuries and is found most commonly in young, growing children. The disease is characterized by a disturbance in calcium and phosphorus utilization, with the result

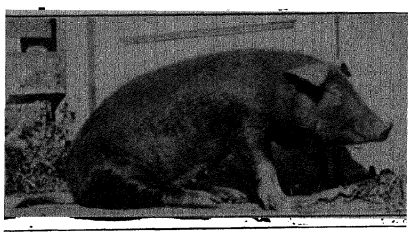

FIG. 83. Posterior paralysis in the hog induced by feeding a ration deficient in vitamin D. This hog was raised in the dark. (Courtesy of Dr. L. A. Maynard.)

that normal ossification or bone-formation does not take place. The bones become soft, muscles become weak and flabby, and, although the disease is seldom fatal, pathological changes may develop to the point

FIG. 84. Photograph of a hog raised on the same ration that induced paralysis shown in Fig. 83. This animal was not afflicted with posterior paralysis because it was fed in an open yard on a cement floor where it received the beneficial action of sunlight. (Courtesy of Dr. L. A. Maynard.)

where the child's health and strength are permanently jeopardized. It is not uncommon to see children and adults with misshapen heads, sunken chests, crooked arms, bowed legs and twisted pelvic bones as the result

of rickets in early childhood. Science has found that the disease can be prevented and cured by dietary treatment and by sunlight. When the disease occurs in adults, it is known as *osteomalacia*. Osteomalacia is also quite common in mature domestic animals, particularly those animals raised almost wholly on dry pasture grass, which possesses an unsatisfactory content of calcium and phosphorus.

FIG. 85. Typical case of nutritional posterior paralysis in the hog, induced by an improper ratio of calcium to phosphorus and an inadequate supply of vitamin D. (Courtesy of Dr. L. A. Maynard.)

Rickets is not peculiar to human beings, for it has been observed in animals during captivity, particularly the carnivora, such as dogs, lions and tigers. The disease in hogs, known as *nutritional posterior paralysis* is thought to be identical with rickets, and a form of leg weakness in poultry is believed to be a similar disease.

Credit for the development of feeding technique for the production of experimental rickets is due to Mellanby, of England, who described (1918–19) over three hundred feeding experiments with puppies. Mellanby found that he could produce soft bones and bowed legs, in dogs, which seemed not unlike rachitic bones in children, and these observations were substantiated by X-ray and histological examinations, as well as by chemical examinations of the bones.

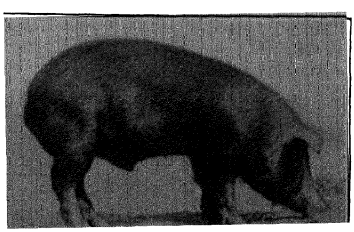

FIG. 86. Photograph of the paralyzed hog shown in Fig. 85, taken two months after the administration of cod-liver oil. (Courtesy of Dr. L. A. Maynard.)

He found that he could prevent or cure the disease by feeding cod-liver oil and, to a less satisfactory degree, by feeding butterfat and suet. He was unable to prevent rachitic symptoms by feeding lard or vegetable oils. Mellanby concluded that rickets is a dietary-deficiency disease, and that foods which contained appreciable quantities of vitamin A possessed rickets-curing properties.

McCollum and co-workers, knowing that vitamin A is easily destroyed by oxidation, heated cod-liver oil and aerated the hot solution with oxygen. The cod-liver oil lost its typical ophthalmia-preventing properties but still retained its rickets-curing potency. As the result of these and

other studies, it became evident that cod-liver oil contains two vitamins, viz., vitamin A and an anti-rachitic factor which is now known as vitamin D.

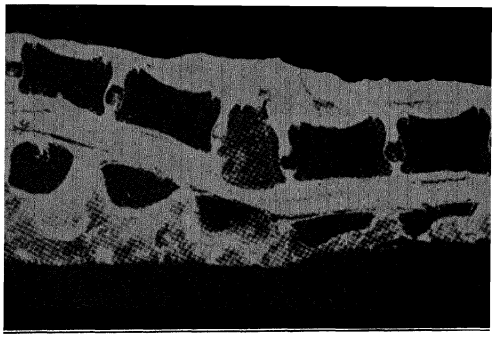

FIG. 87. Longitudinal section of the spinal column of a hog afflicted with nutritional posterior paralysis. Note that one of the lumbar vertebrae has slipped out of position and is pressing on the spinal cord, thereby causing paralysis of the hind quarters of the hog. This abnormality is due to improper calcium and phosphorus utilization. (Courtesy of the Ohio Agricultural Experiment Station.)

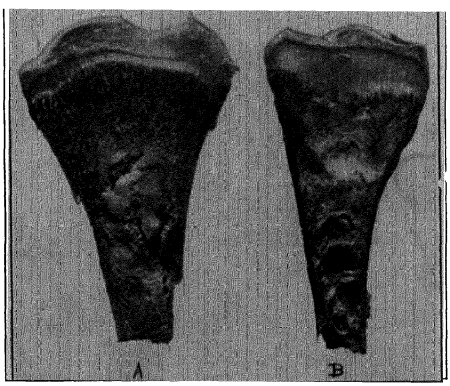

FIG. 88. Showing the effect of diet on the efficiency of bone calcification in the chick. Bone *A* is normal and shows satisfactory deposition of calcium and phosphorus which is indicated by the narrow epiphyseal line. Bone *B* came from a bird afflicted with leg-weakness induced by feeding a ration deficient in phosphorus and vitamin D. Note the width of the uncalcified area (epiphyseal line) and the diminution of mineral deposition in the shaft. (Data of Hunter.)

Rickets may be studied by means of the X-ray. By this method the observer can follow the ossification process without disturbing the rachi-

tic person or animal. It is possible, also, to draw blood samples which can be analyzed for calcium and inorganic phosphorus. Normal blood tends to remain fairly constant in its content of these ions. In rickets, the concentration of these ions falls quite markedly, which is considered an index of the development of the disease.

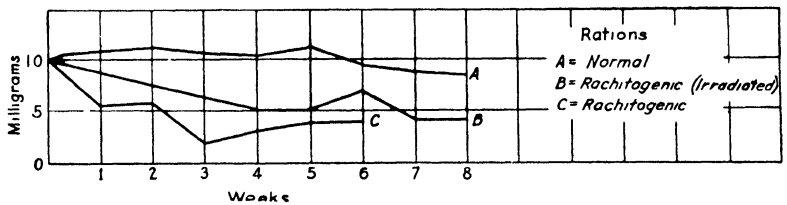

FIG. 89. Showing the amount of inorganic blood phosphorus in the blood of rats (expressed in milligrams per 100 cc) when fed for several weeks on (*A*) normal, (*B*) rachitogenic and (*C*) irradiated rachitogenic rations. Inorganic phosphorus tends to fall during the development of rickets (Data of Dutcher and co-workers.) (Courtesy of the Journal of Biological Chemistry)

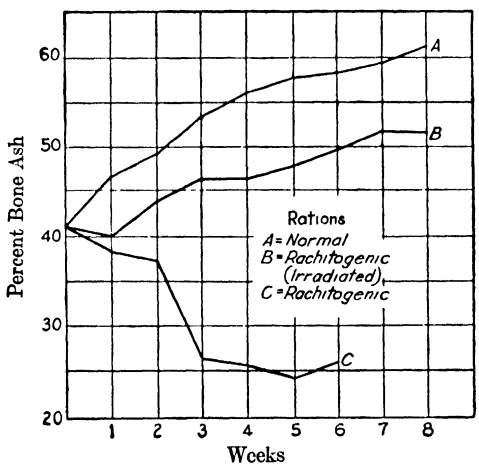

FIG 90. Showing the amount of bone ash in rats described in Fig 89. The deposition of mineral salts is hindered and bone salts are actually lost from the bones when animals are fed rachitogenic diets. (Data of Dutcher and co-workers) (Courtesy of the Journal of Biological Chemistry)

When normal and rachitic bones are split lengthwise, immersed in silver nitrate, subjected to light and examined under a low-power microscope, it is possible to note definite differences in the respective bones. Normal bones show a well-calcified metaphysis and a smooth, narrow cartilaginous line known as the epiphyseal line. In rachitic or poorly

calcified bones this line is wide and irregular, owing to cessation and failure of calcium and phosphorus deposition. This test is known as the "line test" and is used by most laboratories. It is also possible to obtain information regarding the efficiency of calcification by making ash, calcium or phosphorus determinations on moisture-free and fat-free bones. A good bone will be dense and rich in ash, whereas a rachitic bone will tend to contain much less mineral matter.

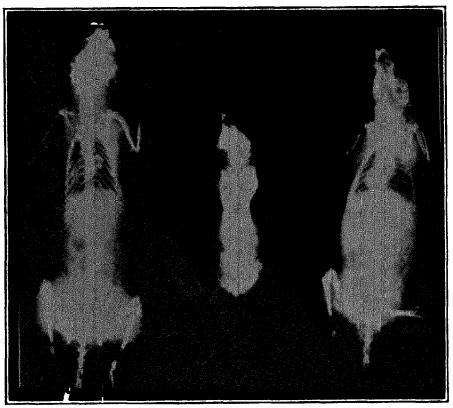

FIG. 91. X-ray photograph showing good and poor ossification in rats. Note the denseness of the leg bones of the two larger rats as compared with the small rat in the center. The bones of the latter were so poorly calcified that they do not show in the photograph. (Courtesy of Dr. Helen Mitchell.)

The ossification process depends on the amounts and ratio of calcium to phosphorus in the diet and, eventually, to the presence of vitamin D. If the calcium-phosphorus supply is physiologically adequate, the need for vitamin D in the diet becomes of secondary importance, since most animals can obtain the equivalent by means of sunlight.

When dietary calcium is low and phosphorus is high, rickets develops rapidly. This type of rickets may be accompanied by tetany, owing to the inadequate supply of calcium. The usual type of rickets is produced experimentally by feeding diets low in phosphorus but rich in calcium. The following ration, designed by Steenbock and co-workers, has been used successfully in the production of experimental rickets in rats:

Yellow corn	76
Wheat gluten	20
$CaCO_3$	3
NaCl	1
	100

When young rats are placed on this diet they will develop moderate rickets in about 18 to 20 days. The least (daily) amount of anti-rachitic food which will bring about definite healing of these rachitic

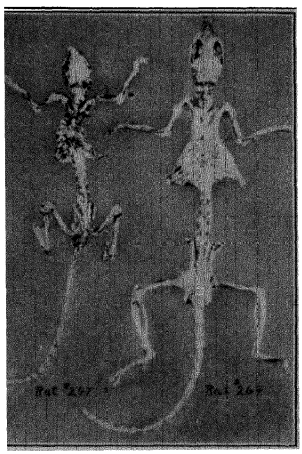

FIG. 92. Skeletons of the rats shown in Fig. 91. Compare the soft mis-shapen bones of the smaller rat with the heavy, well-formed bones of the normal rat. (Courtesy of Dr. Helen Mitchell.)

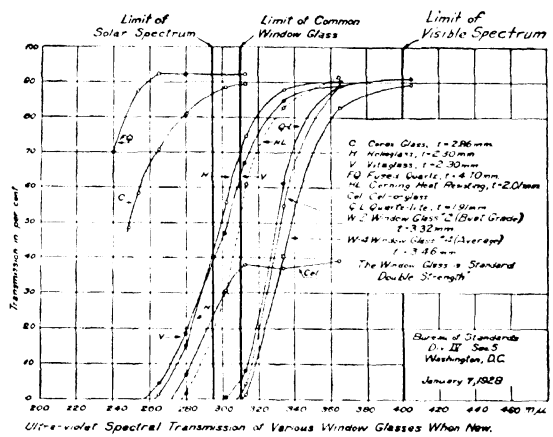

FIG. 93. Showing the percentage of ultraviolet light transmitted by various types of window glass substitutes. Note the small amount of the light of short wavelength that is able to pass through ordinary window glass.

lesions in a period of 10 days is known as a " vitamin D unit." For example, if 2 milligrams of cod-liver oil are necessary to accomplish this

result, it is clear that the cod-liver oil contains 500 vitamin D units per gram of oil. This is the type of unit used by the American Drug Manufacturers Associations.

Bills is of the opinion that certain fish may have the power to synthesize vitamin D and store it in the liver oil. Others believe that this vitamin cannot be synthesized by the animal, but that the animal must obtain it from the plant. Regardless of what the mechanism may be, it is evident that fish and other animals are able to store large quantities of vitamin D in their livers. Since the vitamin is fat-soluble, it can be obtained in the liver oils in high concentration, the discovery of which has had a very stimulating effect on the cod-liver-oil industry. When cod-liver oil is saponified, vitamin D is found to be concentrated in the sterol or unsaponifiable fraction, which constitutes, roughly, 1 per cent of the original oil. The vitamin is relatively stable to heat and oxidation, although it can be destroyed by these agencies.

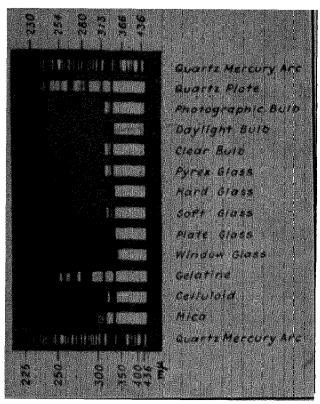

FIG. 94. Showing how glass absorbs ultra violet, while quartz and gelatin transmit considerable ultraviolet light.

It has been known for many years that rickets in humans is susceptible to seasonal variation, more cases being observed during the winter than

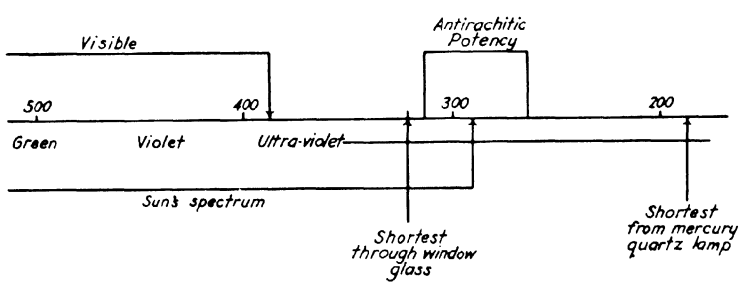

FIG. 95. Diagram showing portions of the spectrum ranging from 500 millimicrons to 200 millimicrons.

the summer months. This was finally correlated with the amount and intensity of sunlight. When sunlight is passed through a quartz prism, the light is separated into bands or zones varying in wave-length. The

visible portion of the spectrum ranges from the short-wave-length rays in the violet portion to long-wave-length rays in the red end. These wave-lengths are measured in terms of millimeters. A micron (m) is 1/1000 of a millimeter, and a millimicron ($m\mu$) is 1/1000 of a micron. The Ångström unit is 1/10 of a millimicron or one ten-millionth of a millimeter. Most light studies are expressed in Ångström (A) units.

Light waves just shorter than 3900 Å are known as ultraviolet light. These are invisible to the eye. Likewise, waves longer than 8000 Å in the infra-red are also invisible. That portion of the sun's spectrum which falls in the range 1800 Å to 3000 Å possesses definite physiological effects so far as calcium deposition in bones and teeth is concerned. This is known as the true ultraviolet zone. These rays are very readily absorbed by glass, and for this reason ordinary window glass prevents the transmission of ultraviolet light.

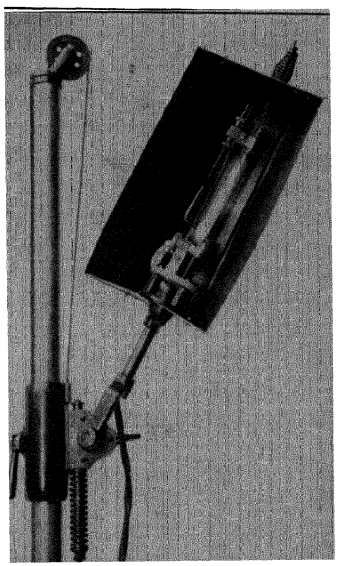

FIG. 96. Quartz mercury vapor lamp for production of ultraviolet light, used to irradiate animals and foods for the synthesis of the antirachitic factor and in preventing and curing rickets.

Intensity is also a matter of prime importance and must be considered in all heliotherapy problems. Light intensity is affected by altitude, being greater at high altitudes. Intensity is greater in tropical countries than in those of the temperate zones. In summer, when the rays are most direct, the intensity is greater than during the winter months, when the sun is farther from the earth. Studies have shown that smoke, fog and suspended dust particles cut down light intensity, with the result that rural districts receive light of greater intensity than can be obtained in large cities.

Substitutes for window glass, which transmit ultraviolet light, are now available for use in hospitals, sun parlors, factories and farm buildings. Special glasses have been used successfully. Another product has found wide use in the glazing of poultry houses. This product is not a glass, but consists of cellulose acetate incorporated in a thin layer into ordinary wire mesh. Another product, is a loosely woven cloth

which has been made semi-transparent by treatment with a paraffin-like preparation.

For experimental and therapeutic purposes, various types of lamps have been placed on the market. These, as a rule, are capable of producing more intense ultraviolet light, without the fluctuations that must be expected when it is necessary to depend on sunlight. Up to the present time, two types of lamps have been used for therapeutic purposes, viz., the quartz mercury vapor lamp and the carbon arc lamp. With the latter type it is possible to obtain a more varied distribution of the various portions of the spectrum by using carbon electrodes which are " cored " by various types of inorganic elements and salts. Quite recently special types of electric bulbs which are efficient and economical have been constructed of special glass and equipped with special filaments, and are being placed on the market. One type contains a quantity of mercury in the bulb which eventually vaporizes, owing to the heat, giving appreciable amounts of ultraviolet light. These lamps are designed, primarily, for use in the home. Similar bulbs, equipped with special filaments and constructed of special glass, are being used to a limited extent in commercial poultry plants.

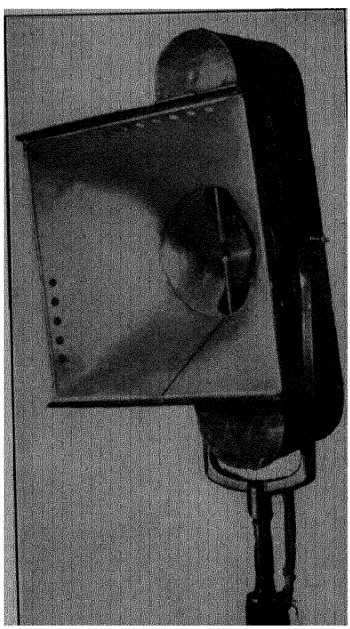

FIG. 97. Carbon arc lamp used as a source of ultraviolet light. Different types of flames of varying intensity and quality can be obtained by varying the type of "core" in the carbon electrode.

The question of the comparative values of light and cod-liver oil in poultry feeding is still under discussion. Some poultry raisers feel that they can raise healthy and productive birds in the absence of sunlight when a good grade of cod-liver oil is used as the source of vitamin D. Other commercial poultrymen believe that light, and particularly sunlight, is preferable. These poultrymen usually use cod-liver oil during the winter months, when sunlight is not available. Experimental work at Wisconsin and at Pennsylvania has led to the conclusion that sunlight is superior to

artificial ultraviolet light, so far as appearance and physical condition of the birds are concerned. Research work now in progress at the Pennsylvania State College indicates that wave-lengths longer than the ultra violet possess definite physiological value and play a part in promoting normal calcium and phosphorus retention.

Hart and co-workers of Wisconsin, Martin and Insko of Kentucky, and Hughes of Kansas have shown quite conclusively that light affects hatchability of eggs. When birds are fed in the dark without access to vitamin D or ultraviolet light, the hatchability eventually falls to zero. (See Fig. 98.) When ultraviolet light, sunlight or cod-liver oil were

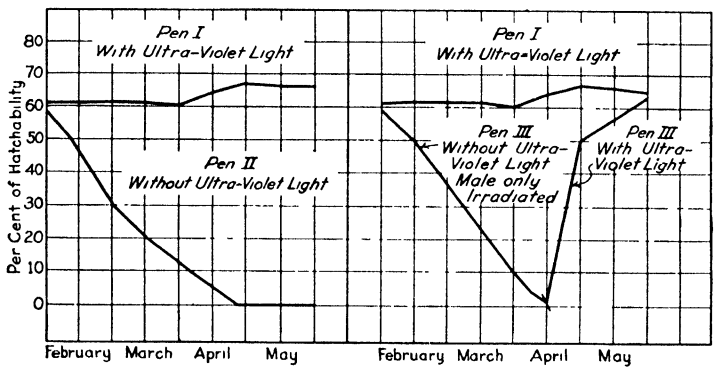

FIG. 98 Showing the effect of ultraviolet light on the hatchability of eggs (Courtesy of Dr Harry Steenbock and Journal of Biological Chemistry)

used, hatchability was stimulated. Most work, to date, indicates that hatchability is usually higher when vitamin D is furnished by sunlight than when it is furnished by cod-liver oil.

Although sunlight and ultraviolet light have been used to irradiate high-producing cows, it has not yet been shown that the amount of vitamin D in the milk can be increased appreciably by this method. Experimental work at Wisconsin indicates that light treatment tends to increase calcium retention in milk-producing goats, but to a less extent in dairy cattle. The amount of vitamin D in goat's milk was increased by irradiation of the animals, but no appreciable increase could be noted in cow's milk. Steenbock and co-workers, Krauss and Bethke and Thomas and McLeod have shown that it is possible to increase the vitamin D potency of cow's milk by feeding activated ergosterol or irradiated yeast. The last-mentioned investigators claim to have increased the amount of vitamin D, in milk, 16 times through feeding these highly potent materials.

In 1924, Hess and Steenbock announced that it is possible to irradiate foods with ultraviolet light and endow them with anti-rachitic potency. Hess showed that cottonseed and linseed oil did not contain vitamin D until they had been subjected to ultraviolet light. Steenbock drew similar conclusions after irradiating certain mixtures of dry food materials used in his experimental work. When oils were irradiated and endowed with the anti-rachitic factor, it was found that this factor remained in the unsaponifiable fraction after saponification. It was found that cholesterol, which is present in animal oils and animal tissues, could be highly activated with ultraviolet light. Subsequently, it was discovered by Windaus that the activatable substance was not cholesterol, but an alcohol known as ergosterol, which was present in the cholesterol preparations as an impurity.

Ergosterol is an unsaturated alcohol containing three sets of double bonds; it has been given the empirical formula $C_{27}H_{42}O$. Inactive ergosterol may be activated by treatment with ultraviolet light. The latter (activated ergosterol) possesses physical properties not possessed by the former, indicating that intermolecular changes have been induced by irradiation. Preparations of ergosterol have been made that are 200,000 times more potent than cod-liver oil It is evident that ergosterol is the parent-substance of vitamin D. It is prepared commercially from yeast and is sold on the market in standardized "units" under the trade name "Viosterol." It is found that, when Viosterol is fed in excessive quantities, calcification occurs in the soft tissues, blood vessel walls, etc. Although the dosage must be quite high to bring about pathological conditions and death, it is much safer to use it only on the advice of a physician At this point is should be stated that ergosterol preparations, since they contain no vitamin A, are not complete substitutes for cod-liver oil.

Food materials are now being treated with ultraviolet light on a commercial scale under patents taken out by Steenbock and administered by the Wisconsin Alumni Research Foundation, the income of which goes to the University of Wisconsin for research work Cereal foods, milk, milk powders, etc., have been shown to contain appreciable amounts of vitamin D, after irradiation, and some of these are now sold on the market. It is too early to predict what the future of irradiated foods and feeds may be, but indications point to a successful future for certain types of irradiated food materials. Irradiated yeast is now being manufactured for use in poultry feeding.

Mellanby and Holst postulate the presence of an "anti-calcifying substance," called "toxamin," in certain cereals. It is their belief that this substance actually discourages the deposition of calcium and phosphorus in the bones.

Vitamin D is relatively stable to heat and oxidation and is not easily destroyed by storage, cooking and drying. Since vitamin D is the most limited, in distribution, of all the vitamins, it behooves the practical feeder to pay some attention to the supply of this factor in the rations of farm animals during the winter months when sunlight is less available and less intense.

In the following and final chapter we shall consider the so-called anti-sterility or fertility vitamin, known as vitamin E. References for general reading will be found at the end of Chapter XXXII.

CHAPTER XXXII

VITAMIN E

In 1922, Evans and Bishop announced that they had been able to obtain sterility in rats by feeding purified diets, which were presumably adequate with respect to all the known constituents which were regarded as essential for normal growth. Rats grew well on these diets and possessed every appearance of health. Some of the females showed transitory periods of fertility, but, sooner or later, sterility developed. Evans and Bishop reasoned that, since the diet appeared to be adequate for all other physiological functions of the body, it must be lacking in some unknown type of dietary factor which is necessary for the proper functioning of the reproductive processes

When certain natural foods were added to these " anti-fertility diets," it was impossible to produce sterility. Similarly, animals which had been made sterile by the dietary treatment could be made fertile by the addition of certain natural foods to the diet. As a result of a great many experiments of this type, Evans and Bishop postulated that this type of nutritional sterility could be regarded as a dietary-deficiency disease, and that the dietary factor necessary for its prevention and cure was fat-soluble. In the early stages of their investigations, these workers used the term " vitamin X " to designate the new dietary factor which is now known as " vitamin E." This vitamin has been referred to as the " vitamin of reproduction," the " fertility vitamin " and the " anti-sterility vitamin."

Some nutrition workers have contended that other dietary factors are also necessary for normal reproduction and that the term " vitamin of reproduction " cannot be reserved for any particular factor. For example, it may be pointed out that diets which are deficient in vitamin A bring about a type of sterility which is reproducible and typical. It seems to be established that the absence of vitamin A interferes with ovulation, whereas the absence of vitamin E permits of normal ovulation, but reproductive failure appears to be associated with the placental functions of vitamin E.

Evans and associates have developed interesting physiological and microscopical technique in which the whole reproductive cycle can be studied. It is possible, with their experience and technique, to deter-

mine whether or not oestrus is occurring, whether or not breeding has been accomplished, and whether or not fertilization and implantation have occurred. In other words, it is possible, by this technique, to prophesy whether or not young will be born and, if sterility has developed, the type of sterility which is responsible for reproductive failure.

When sterility is produced in male rats by this method, it is found that the loss of fertility is due to destruction of the germ cells and, eventually, the entire seminiferous epithelium. In the female, on the other hand, ovulation and fertilization are not impaired. In fact, implantation occurs on the wall of the uterus but the foetus is resorbed, resulting in reproductive failure. According to Evans and Bishop, other types of sterility may be due to failure of oestrus, ovulation, fertilization or implantation, but vitamin E is the only anti-sterility factor which is seemingly specific in preventing resorption of the embryos after implantation has occurred.

These authors have determined that foetal death occurs, usually, on the twelfth or thirteenth day of the gestation period. It has been their practice to use (for vitamin E tests) only those females which have exhibited one typical resorption. When this has occurred, it is evident that the animal is ready for a second gestation period. Prior to and during the second gestation period the food is fed which is supposed to contain vitamin E. If vitamin E is present in adequate amounts, resorption will not take place, and normal reproduction occurs. If vitamin E is absent, sterility again results, owing to resorption of the embryos. The necessity of producing one resorption, prior to food testing, is evident, since it is necessary to prove that the normal storage of vitamin E in the body has been depleted before it is possible to assay a food for this vitamin.

Vitamin E is low in visceral tissues such as pancreas, liver, spleen and heart. It is present in musculature in larger amounts than in liver. It is present, but extremely low, in butterfat. When the diet contains one-third of its weight in the form of dried milk, sterility results. When the entire diet consists of dried whole milk, fertility occurs. Milk obtained from cows which have had access to alfalfa pasture seems to be richer in vitamin E. Cod-liver oil is devoid of this factor at all levels that are usually fed in farm practice.

Seeds and leaves furnish vitamin E in relatively large amounts, and the leaves of alfalfa, lettuce, pea and tea plants are potent sources of this factor, even after drying, which is also an indication of the relative stability of the vitamin. It is concentrated in the embryo of wheat, oats and corn, the most potent preparations having been prepared from wheat-germ oil. Cottonseed oil contains vitamin E, and "Crisco," which is

made by hydrogenating cottonseed oil, also contains the vitamin in appreciable quantity.

Potent preparations of vitamin E have been made which are soluble in such solvents as methyl alcohol, ethyl alcohol, ether, pentane, benzene, acetone and carbon bisulfide. It is classified, therefore, as a fat-soluble vitamin because of its solubility in the fats and fat solvents. Vitamin E is stable to heat, light and air, and wheat-germ preparations have been heated at 170° C. until charring has occurred, without destruction of vitamin E potency. Similarly, preparations have been heated in superheated steam at 180° C. and distilled *in vacuo* as high as 233° C. without impairing the action of this vitamin. Ordinary saponification does not seem to destroy vitamin E, although prolonged treatment with boiling solutions of alkali (KOH 20 per cent) seems to cause some destruction.

Evans and Burr state that 73 per cent of the unsaponifiable fraction is a sterol known as sitosterol. After many chemical manipulations and subsequent distillations a yellow oil is obtained from which it is not possible to derive crystalline material. This oil is so potent that a single injection of 5 milligrams has proved efficacious. Chemical examinations show a trace of ash, but no nitrogen, sulfur, phosphorus or halogen could be identified.

Considerable other work has been done with reference to vitamin E and sterility, by workers such as Sure, McCollum and co-workers. This brief discussion is designed to give the student some idea of the functions and properties of vitamin E. It should be borne in mind that all experimental evidence to date indicates that vitamin E is distributed so universally that there is little reason to believe that nutritional sterility of this type will ever be of clinical importance. In domestic animal feeding, vitamin E deficiency should be of rare occurrence, owing to the large proportion of cereals and leafy plants in the average farm ration.

Experience leads to the belief that vitamin E is of little clinical importance in human reproduction, since reproduction is almost excessively efficient among those classes of people where most of the nutritional rules are violated. Among such people it is possible to find most of the pathological conditions which accompany poor dietary habits, but, certainly, sterility does not seem to be one of them.

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TABLE 67

Distribution of Vitamins A, B (complex) and C

Note The relative distribution of vitamins A, B (complex) and C, given in the following table, will give the reader some idea of the vitamin potency of some of the more common foodstuffs The data have been compiled from many sources, but more particularly from Circular No 84 of the United States Department of Agriculture, prepared by Miss Sybil Smith, Senior Chemist in the Office of Experiment Stations

Vitamins B (B_1), G (B_2), D and E have not been included for reasons that are evident from the discussions in the preceding chapters

The following symbols are used in expressing the relative vitamin potency of the various food materials

− The vitamin is not present
* The vitamin is not present or the experimental data are insufficient
+ The vitamin is present
++ The vitamin is present in appreciable quantity
+++ The vitamin is present in relatively large quantity

Fruits

Type of food	A	B	C
Apples, canned commercially	*	*	+ to ++
Apples, raw, fresh	+	+	++
Bananas, raw	+ to ++	+	++
Grapefruit (or juice), fresh	+	++	+++
Grapes (or juice), fresh	+	+ to ++	+
Lemons (or juice), fresh	+	++	+++
Orange juice, fresh	++	++	+++
Peaches, raw	+ to ++	+	++
Pears, raw, fresh	*	+ to ++	+
Pineapples, raw	++	++	++
Prunes, dried	++	++	−
Raisins	−	+	−
Raspberries, raw	*	*	++

Vegetables

Asparagus, green, raw	++	+++	*
Beans, string, raw.	++	++	++
Beets, root .	− to +	+	+
Cabbage, head, raw	+	++	+++
Cabbage, leaves, green, fresh	++	++	+++
Cabbage, leaves, white, fresh	− to +	++	+++
Carrots, raw, old	+++	++	+
Carrots, raw, young	+++	++	++
Cauliflower, raw	+	++	+
Celery, stalks, bleached	− to +	++	*
Chard, Swiss, raw	++	+ to ++	*

TABLE 67 — *Continued*

DISTRIBUTION OF VITAMINS A, B (COMPLEX) AND C

Vegetables — *Continued*

Type of food	A	B	C
Dandelion greens	++	++	+
Lettuce, head	+ to ++	++	+++
Lettuce, leaves, green	+++	++	+++
Mushrooms, raw	− to +	*	−
Onions, raw	− to +	+	++
Peas, green, raw	++	++	+++
Potatoes, raw	+	++	++
Rhubarb	*	*	+
Rutabagas, raw (Swedes)	+	++	+++
Sauerkraut	+	+	+ to ++
Spinach, raw	+++	++	+++
Sweet potatoes, raw	+ to ++	++	++
Tomatoes, raw, vine-ripened	++	++	+++
Turnip greens, raw	+++	++	++
Turnips, white	− to +	++	++

Food from Animal Sources

Beef muscle, lean	+	+	− to +
Buttermilk	+	++	− to +
Cheese, Swiss	+ to ++	*	*
Egg yolk	+++	++	*
Eggs, hen, fresh	++	+ to ++	*
Fish, fat (such as herring, salmon)	+	+	*
Ham	− to +	++	−
Kidney, pig	++	++	+
Liver, calf, ox, sheep	++ to +++	++	*
Milk, cow's, whole, condensed	+++	++	+
Milk, cow's, whole, dried	+++	++	− to +
Milk, cow's, whole, evaporated	+++	++	− to +
Milk, cow's, whole, raw	+++	++	+
Milk, goat's	+++	++	+
Milk, human, raw	++	+	− to +
Oysters, raw	++	++	+
Pork	− to +	++	*
Poultry	− to +	+	*

TABLE 67 — *Continued*

DISTRIBUTION OF VITAMINS A, B (COMPLEX) AND C

Cereals and Cereal Products

Type of food	A	B	C
Bread, white, milk	+	+	− to +
Bread, white, water	*	+	−
Bread, whole-wheat, milk	++	++	− to +
Bread, whole-wheat, water	+	++	−
Corn (maize), white, whole	+	++	−
Corn (maize), yellow, whole	++	++	−
Oatmeal	− to +	++	−
Oats, whole	− to +	++	−
Rice, polished	−	−	−
Rice, unpolished, brown	+	++	−
Rye, whole	+	++	−
Wheat, bran	+	++	−
Wheat flour, white, patent	−	− to +	−
Wheat, whole	+	++	−

Fat-rich Foods

Bacon	− to +	+	*
Beef fat.....	++	−	*
Butter ..	+ ++	−	*
Cod-liver oil	+++	−	−
Corn oil, yellow, refined	−	−	−
Cottonseed oil	− to +	−	−
Cream ...	+++	++	− to +
Lard	− to +	−	−
Mutton fat	+	−	−
Oleo oil .	+ to ++	−	−
Olive oil	− to +	−	−
Peanut oil	+	−	−
Salmon oil .	++	*	*
Soybean oil...	+	*	*
Yeast.....	− to +	++ to +++	*

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