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NEW CONCEPTIONS IN BIOCHEMISTRY

BY

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PREFACE.

Since the days of Lavoisier, animal metabolism has been a fascinating subject of research for chemists and physiologists alike. Despite the attempts of numerous distinguished workers, the enigma of animal metabolism still remains unsolved.

In this book, which comprises a course of lectures delivered by the author in the Patna University, an effort has been made to discuss the general physico-chemical principles underlying animal metabolism. The book deals with the chemical aspects of biochemistry which have any bearing on metabolism. It is therefore intended in no way to be a book of reference. No attempt has been made to describe systematically all diseased conditions occuring in cases of improper metabolism. The dominant idea in the book is that several diseases are due to lack of a proper and balanced oxidation of the three classes of food materials, the carbohydrates, fats, and proteins. The exaggerated oxidation of one of the three classes of food materials in preference to the other two may lead to the incidence of several diseases. The author has ventured to include in the list of metabolism diseases even such ailments as rickets, pellagra, beri-beri, scurvy, special cases of diarrhœa, and cancer. All factors, which lead to increased metabolism, should therefore prove beneficial in the treatment of such diseases. A case has been made out in support of the theme that iron preparations, mild alkalis, phosphates, vitamins, internal secretions, and light act as accelerators in the oxidation of food materials; and as such they should have good curative effect in the treatment of metabolism diseases.

Moreover, the experiments carried out in the author's laboratory have shown that all food materials can be oxidized at ordinary temperatures by air in the presence of sunlight; and this fact has, for the first time, given a rational explanation of actino-therapy.

The author has interpreted, to the best of his ability, all the wealth of observations in this particular branch of science. He is conscious that what we imagine an absolute fact today may be disproved by our successors; and that the future may prove his interpretations wrong.

He must therefore be satisfied with the consciousness that all those engaged in unravelling the mystery of life are engaged in a glorious service.

In conclusion the author wishes to express his thanks to the authorities of the Patna University, and to the members of the Physics and Chemistry Departments of the Patna College, for their help in making the lectures a success. The author also takes this opportunity of expressing his great indebtedness to Mr. G. Gopala Rao, M.Sc, research Fellow of the Andhra University, now working at Allahabad, for his help in the preparation of the book.

In writing this book especially the first Chapter, the author is greatly indebted to Graham Lusk's *Science of Nutrition* and L. B. Mendel's *Nutrition*. The author also acknowledges his indebtedness to the *Annual Reports of the Progress of Chemistry* and von Fürth's *Chemistry of Metabolism*. He thanks them for their kind permission to utilize their books.

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Allahabad, April 1932.

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NEW CONCEPTIONS IN BIOCHEMISTRY

CHAPTER I

An Introduction to Views on Food Principles and Chemical Aspects of Metabolism.

In recent times physical chemistry has found increasing application in the domain of biochemistry. Graham Lusk in his *Science of Nutrition*[1] states: "In another decade the development of scientific knowledge will probably permit the formulation of the subject from the standpoint of physical chemistry". Prof. Otto Meyerhof writes in his *Chemical Dynamics of Life Phenomena*[2]: — "The more closely we study the machinery of life in the isolated cell, the clearer it becomes that the chemical and physico-chemical processes predominate in it. Physiology in its classical period was studied chiefly by physicists, at least in Germany, among whom may be mentioned Helmholtz, Dubois-Reymond, Ludwig, Fick. In the field of cellular physiology, however, physiological and physical chemistry have to assume the leadership — not, however, by analyzing dead material in regard to its constituents, but by studying the chemical dynamics of the processes of life. Almost a hundred years ago, Pasteur initiated this path. With extraordinary success, Jacques Loeb, whom many of our younger generation call their teacher, has continued along these lines."

Already physical chemistry has done much in the elucidation of many problems of biochemistry. Organic chemistry, important as it is, can help only in the analysis and synthesis of body materials. But when we come to the dynamics and energy relations of throbbing life, a knowledge of physico-chemical principles is indispensable.

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(1) 3rd edition, Philadelphia and London (1919).

(2) pp. 23—24 (1924).

In the following pages an attempt is made to discuss some aspects of biochemistry from the standpoint of physical chemistry. First, however, the development of our views about food principles will be traced.

VIEWS ON FOOD PRINCIPLES.

As early as the time of Hippocrates (460—370 B.C.), there existed a belief in the occurrence of some specific universal nutrient substance present in all food materials, which was abstracted for the body through the alimentary functions. This idea persisted in many quarters till the early part of the last century. It remained for the rapid development of chemistry, the beginning of biochemistry in its present significance, finally to dispel this belief in a single universal food principle. The change came about a century ago with the better realization of the existence of different types of substances in the products termed foods. Thus Dr. William Prout (1785—1850), who was the originator of the hypothesis that all elements are condensations of hydrogen, advanced the view that organized bodies are constituted of three great staminal principles — Saccharina, Oleosa, and Albuminosa, all of which are found in milk, the prototype of a perfect food. One may recognise here the earliest development of the classification of foodstuffs in vogue today. To Prout it seemed logical to assume that because "all the more perfect organized beings feed upon other organized beings, their food must necessarily consist of one or more of the above staminal principles". Thus it seemed that the principles — apparently identical in the various substances serving as food, — became transferred as such into the organism which consumed them; in other words, the body was constructed directly from foodstuffs ingested. The doctrine of Hippocrates of a single specific aliment thus gave way to the view that there are several nutrient principles. "A diet to be complete", Prout remarks, "must contain more or less all the three staminal principles". There is no indication in Prout's theory of nutrition of any special differences in the nutritive values of his three staminal principles.

It was the great merit of the French physiologist François Magendie (1783—1855) to have demonstrated the different nutritive values of the three foremost groups of food materials. To him is due the clear distinction between the nitrogenous and non-nitrogenous groups. He conducted experiments in which animals were fed on diets consisting essentially of non-nitrogenous food materials—sugar, gum, olive oil, butter, etc., and observed failure of nutrition in every case. From

his researches he concluded that the nitrogen of the tissues is derived from food nitrogen, and that non-nitrogenous foods are not converted into nitrogenous components in the organism. Thus Magendie may be looked upon as the founder of the modern experimental investigations in the science of nutrition.

The recognition of the relative importance of nitrogenous foods for nutrition ushered in a new era of progress; indeed, before long it brought about a glorification of the albuminous substances, a creed which has persisted in its extreme form almost until the present time. The Dutch physiological chemist, J. G. Mulder (1802—1880), who in 1839 coined the name "protein" (from the Greek word *proteios* = primary), early recognized the resemblance between the albuminous substances that can be extracted from animal and plant tissues respectively. More than half a century later the belief that nitrogenous food plays the prominent rôle in nutrition was still voiced in the statement that "the life processes consist in the metabolism of the proteins" (M. Verworn, *General Physiology* (1899)). J. B. Boussingault (1802—1887), in his *Économie rurale* published in 1844, was so highly impressed by the importance of the nitrogenous constituents of fodder for domestic animals that he rated fodder largely by its contents of nitrogen, although he realized that the non-nitrogenous constituents were not without value.

To the teachings of Baron von Liebig (1803—1873) above all others is due the prominence which, before the middle of the last century, began to be attached to the nutritive rôle of the proteins. He regarded blood — the immediate source of those organic products which are involved in the transformation of matter and energy in the organism — as the prototype of real food. In his *Familiar Letters on Chemistry*, the dominant importance of proteins is expressed as follows: — "Everywhere throughout organized nature where animal life is developed we find the phenomenon of life depending on the presence of albumin. The continuance of life is indissolubly connected with its presence in the blood, that is, in the nutrient fluid . . . Only those substances are in the strict sense nutritious articles of food which contain either albumin or a substance capable of being converted into albumin[1]." For a clear understanding of Liebig's conception of food values it is necessary, however, to refer to his theory of metabolism. To him the various physiological functions, such as muscular contraction and glandular activity, appeared to proceed at the expense of

[1] J. Liebig, *Familiar Letters on Chemistry*, 3rd edition, London (1851).

the albuminous tissue structures. Thus, the origin of the energy liberated when work is performed was referred by Liebig to the muscle protein. The function of nutrition from his standpoint was to replace the destroyed tissue protein. According to Liebig, the nitrogenous food stuffs could be converted into blood, whilst non-nitrogenous substances lacked this property. The sulphurized and nitrogenous constituents of foods determine the continuance of manifestations of force. They are the builders of organs and organized structures and the producers of force. Starch, sugar, and fat, according to Liebig, serve to protect the organized tissues, and, in consequence of the combination of their elements with oxygen, to keep up the temperature of the body, and thus support the respiratory process.

Starch, sugar, and fat were held by Liebig to be materials necessary for respiration. Even today the belief is still widespread that the production of flesh and blood requires a liberal consumption of protein, which in turn is commonly interpreted to mean meat. Liebig's teachings abound in much that has meant progress in scientific knowledge, but owing to the force of his great reputation, many wrong conceptions were fostered unduly long. The doctrine that protein, and particularly the muscle protein itself, is the only source of the energy liberated has long been disproved. The artificial distinction between nitrogenous and non-nitrogenous food is untenable. It was fallacious to teach that starch or fat does not serve nutrition, but merely facilitates the respiratory process. The momentous consequence of Liebig's conception was that, at the time of its formulation and long after, attention was centred on protein, which was regarded as the chief and foremost food material. Indeed, protein was considered to be the sole food principle, because it alone was supposed to replace the losses entailed in metabolism, and nutrition was supposed to be identical with the reconstruction of tissue destroyed by work. Thus, the one and unchangeable aliment of Hippocrates was replaced by protein, which became endowed with exclusive nutritive powers, and into which every nutritious substance had to be converted.

It is now well established that proteins can form glucose, and that under special circumstances fats are generated from carbohydrates. It appears, therefore, that inside the animal body, proteins, fats, and carbohydrates are interconvertible to a limited extent, and protein may be looked upon as a universal nutrient substance. Consequently, the view of Hippocrates regarding the existence of a universal nutrient substance, appears to be partially true in the light of modern researches on metabolism.

Meanwhile, much experimental work was being conducted during the last third of the 19th century, notably by the Munich School of Physiology under the leadership of Carl von Voit (1831—1908). The energy aspects of nutrition were becoming recognized, and the interchangeability of the organic foodstuffs was demonstrated. In many respects Voit's definition of food given in 1881 remains unchallenged today. He wrote "the foodstuffs are those substances which bring about the deposition of a substance essential to the composition of the body, or diminish and avert the loss therefrom".

In explaining this dual function of food in replacing losses or preventing them, Voit referred to the older view that the only use of the food components is to restore what has been destroyed in the organism. This he says is true when protein and fat are replaced after starvation, but ingestion of these foods serves, primarily, he adds, to avert loss because of their being themselves consumed. The constituents of diets for the most part avert the necessity of the destruction of the body tissues, and thus protect the latter from disintegration. It matters not whether muscular activity or some other transformation of energy results; the question as to what forces are set into play is not concerned in the definition of a food.

Atwater, a pupil of Voit, emphasized in 1895 the energy yielding function of food materials as follows: — "Food may be defined as a material which, when taken into the body, serves either to form tissue or yield energy or both." This definition includes all the ordinary food materials, since they yield both tissue and energy. It includes sugar and starch because they yield energy and form fatty tissue. It includes alcohol because the latter is burnt to yield energy, though it does not build tissue. It excludes creatinin and other so-called nitrogenous extracts of meat, and likewise thein or caffein of tea and coffee, because these neither build tissue nor yield energy, although at times they may be useful aids to nutrition. The ideal food is a palatable mixture of foodstuffs which is capable of maintaining the body in an equilibrium of substance, or capable of bringing it to a desired condition of substance, and arranged together in such proportion as to burden the organism with the minimum of labour.

The development of our ideas on the chemical aspects of metabolism will now be considered.

CHEMICAL ASPECTS OF METABOLISM.

Though John Mayow, writing in London in 1668, stated that the atmosphere contained a constituent which supported combustion

as well as animal life, the modern era of the science of nutrition was opened by Lavoisier in 1780. He was the first to apply the balance and the thermometer to the investigation of the phenomena of life, and declared "La vie est une fonction chimique". The work of today is but the continuation of that done more than a century ago. Lavoisier and Laplace made experiments on animal heat and respiration; and Liebig, owing to his residence in Paris in 1822, became acquainted with their work. Liebig's conception of the process of nutrition fired the genius of his countryman, Carl von Voit, to undertake the painstaking researches which laid the foundation of his Munich school. These have been repeated and extended by his pupils, notably by Rubner, and others the world over. Thus the knowledge transmitted personally from the master to the pupil, to be in turn extended and elaborated, had its seed in the intellect of Lavoisier. It was he who first discovered the true importance of oxygen gas, to which he gave its present name. He declared that life processes were those of oxidation, with the resulting evolution of heat. He believed that oxygen was the cause of decomposition of a fluid brought to the lungs, and that hydrogen and carbon were produced in this fluid and then united with oxygen to form water and carbon dioxide. He reported that perspiration regulated the quantity of heat lost from the body, and that digestion replenished the blood with the materials eliminated through respiration and perspiration. It was he who first made respiration experiments on man, the results of which were briefly described in a letter to M. Terray written in Paris and dated November, 1790. There is no existing record of the apparatus with which Lavoisier obtained those important results, which are in strict accordance with the knowledge of our own day. We know more details, but the fundamental fact that the quantity of oxygen absorbed and of carbon dioxide excreted depends principally on (1) food, (2) work, and (3) temperature was established by Lavoisier within a few years of his discovery that oxygen supported combustion. Writing in 1849 Regnault and Reiset say "Les recherches modernes ont confirmé ces vues profondes de l'illustre savant".

It was, however, quickly noted that if carbon and hydrogen burned in the lungs, the greatest heat would be developed there, a result not in accordance with observation. It was then suggested that the blood dissolved oxygen, and that the production of carbon dioxide and water took place through oxidation within the blood. In 1837, Magnus discovered that the blood did hold large quantities of oxygen and carbon dioxide, which gave support to this theory. Ludwig, in his later years,

believed that oxidation took place in the blood. Through the critical studies of Liebig, which were published in 1842, it was seen that it was not carbon and hydrogen which burned in the body, but protein, carbohydrates, and fat. Liebig's original theory was that while oxygen caused the combustion of fat and carbohydrates, the breaking down of protein was caused by muscle work. It will be shown later that oxygen is not the cause of oxidation of materials in the body, but that this change proceeds from causes, the nature of which is not yet clear, and that the products involved unite with oxygen. The sum of these chemical changes of materials under the influence of living cells is known as metabolism. Liebig was also the father of the modern methods of organic analysis, and he began to apply to the problems of biology the mental wealth of the newer chemistry which he himself was creating. He knew that protein contained nitrogen, and in 1842 he suggested that the nitrogen in the urine might be made a measure of the protein destruction in the body. Bidder and Schmidt(1) were the first to make systematic experiments upon this subject. They gave meat to dogs and cats and found that almost all the nitrogen contained in the meat was excreted in the urine and fæces. They make the following striking statement concerning protein metabolism which sounds acceptable to modern thought:—"Almost all the nitrogen of protein and collagen is split from its combination, and carries with it enough carbon, hydrogen, and oxygen to form urea; the remaining part, containing 5/6th of the total heat value of the protein, undergoes oxidation to carbon dioxide and water which are eliminated in respiration, the calorific function having been fulfilled." The results obtained by Bidder and Schmidt were opposed, and were not finally accepted until proof was afforded by Voit, who established the fact that an animal could be brought into what he called nitrogenous equilibrium. In this condition the nitrogen of the protein eaten was equal to the nitrogen eliminated from the body in urine and fæces. Urea, the principal nitrogenous end-product derived from protein, was therefore shown to be not an adventitious product, but one normally proportional to the protein destruction. It is evident that if protein nitrogen is retained in the body a new construction of body-tissue is indicated, whereas if more nitrogen is eliminated than is ingested with the food a waste of the body tissue must take place. The discovery of the method of calculating the protein metabolism led Voit to suggest to Pettenkofer that he should construct an apparatus with which the total

(1) *Verdauungssäfte und Stoffwechsel*, p. 353, 387 (1852).

carbon excretion might be measured, including that of the respiration as well as that of the urine and fæces. Voit saw that with these data it would be possible to determine just how much of each food stuff was actually burnt in the animal body. He has described the delight which he and Pettenkofer experienced when their wonderful machine began to tell its tale of the life processes.

Before giving a detailed account of the experiments of Pettenkofer and Voit, it is necessary to survey briefly the earlier work done in this line by Lavoisier, Regnault, and Reiset.

The form of Lavoisier's respiration apparatus is unknown. In 1850, Regnault and Reiset published an account of respiration experiments in which small animals were placed under a bell-jar containing a known quantity of oxygen. The air was kept free from carbon dioxide by pumping it through potassium hydroxide, and oxygen was added from time to time. The gaseous exchange between the animal and its environment could be readily ascertained by determining the amount of carbon dioxide given off and the amount of oxygen absorbed. No attempt was made to determine from what materials the carbon dioxide arose. According to the method of Regnault and Reiset, the animals were placed in a confined space, where poisonous exhalations other than carbon dioxide could collect, and where the atmosphere was saturated with water vapour. However, these factors were without influence on the health of their animals. They planned to work in one of the large hospitals of Paris, but unfortunately the project proved too costly and had to be abandoned. They write, "L'étude de la respiration de l'homme dans ses divers états pathologiques nous paraît un des sujets les plus dignes d'occuper les hommes qui se vouent à l'art de guérir, elle peut donner un diagnostic précieux pour un grand nombre de maladies et rendre plus évidentes les revolutions qui surviennent dans l'économie". (The investigation of the respiration of human beings under different pathological conditions is a subject of great importance for those who profess the art of healing human diseases, because of its value in the diagnosis of a large number of maladies, enabling one to visualize the changes which take place in the animal economy during disease.)

The problem to be solved by Pettenkofer included the maintenance of a man in normal surroundings. A small room was constructed which was well ventilated by a current of air, 500,000 litres passing per day. Knowing the quantity of carbon dioxide and water entering and leaving the room, it was easy to calculate how much was derived from the man living in it during the period of experimentation. As an illustration

of the practical working of the respiration apparatus used by Pettenkofer and Voit (*Zeit. f. Biol.* ii, 478 (1866)), the following data are interesting:—

A man on entering the living room of the apparatus, weighed 71·09 kgs., and drank during the day 1·0548 litres of water, making a total body weight of 72·1448 kgs. Twenty-four hours later he weighed 70·16 kgs.; and his excreta amounted to 0·7383 kg. of carbon dioxide, 0·8289 kg. water from the lungs and skin and 1·1975 kgs. of urine. The final body weight plus all the excreta amounted to 72·9247 kgs. A total body weight of 72·1448 kgs. was converted into a body plus excreta amounting to 72·9247 kgs. The difference of 0·7799 kg. represents the amount of oxygen absorbed, in order to convert the body substance lost into the excretory products obtained. The tabular statement reads thus:—

Man in starvation.

Weight at start	71·09 kgs.	Weight at the end. . .	70·16 kgs.
		Excreta:	
Water drunk	1·0548 ,,	Carbon dioxide	0·7383 ,,
		Water	0·8289 ,,
Oxygen absorbed . . .	0·7799 ,,	Urine	1·1975 ,,
	72·9247 kgs.		72·9247 kgs.

Pettenkofer and Voit proved that the quantity of oxygen needed in metabolism depends upon the chemical composition of the materials that burn in the organism, and also that the relation between the amount of oxygen absorbed and carbon dioxide excreted depends on the same factors. The ratio of the volume of carbon dioxide expired to the volume of oxygen inspired during the same time is called the *respiratory quotient.* When carbohydrates burn the respiratory quotient is unity. When proteins burn the quotient is 0·781, and when fats burn it is 0·71. Pettenkofer and Voit found the respiratory quotient in a fasting man to be 0·69. This indicates a combustion of fat in the organism. If fat and meat were ingested, and the carbon and nitrogen excreta collected, then it could be determined from these data how much of each food stuff was oxidized, and whether there was a storage or loss of either in the body. If a mixed diet which included carbohydrate were given, the carbon dioxide elimination increased, and the oxygen absorption was such as indicated the combustion of carbohydrate. It was assumed that after deducting the protein carbon from the total carbon eliminated, the balance of the extra carbon was derived from the destruction of the carbohydrates in so far as these were ingested; any carbon in excess of this was attributed to the combustion

of fat. Thus the law of the conservation of mass was definitely established in the phenomenon of animal metabolism.

Voit in his note on Pettenkofer's work writes "Imagine our sensations as the picture of the remarkable processes of metabolism unrolled before our eyes and a mass of new facts became known to us. We found that in starvation protein and fat alone were burnt; that during work more, and during rest, especially during sleep, less fat was consumed; that the carnivorous dog could maintain himself on an exclusively protein diet, and that if to such a protein diet fat were added, the fat was entirely deposited in the body; that carbohydrates, on the contrary, were burnt, no matter how much was given, and that they, like the fat of the food, protected the body from fat loss, although more carbohydrates than fat had to be given to effect this purpose; that the metabolism in the body was not proportional to the combustibility of the substances outside the body, but that protein, which burns with difficulty outside, metabolized most easily, and then carbohydrates; while fat, which readily burns outside, is the most difficultly combustible in the organism."

Among the important conclusions reached by Voit was that concerning the manner of metabolism. It has been stated that Liebig believed fat and carbohydrates to be destroyed by oxygen, while protein metabolism took place on account of muscular work. Voit (*Zeit. f. Biol.* ii, 525 (1866)) showed that muscle work did not increase protein metabolism, and that the metabolism was not proportional to oxygen supply.

The absorption of oxygen does not cause metabolism, but rather the amount of metabolism determines the amount of oxygen to be absorbed. The metabolism of the tissues through its oxygen requirements and its carbon dioxide production changes the condition of the blood, and thereby regulates the respiration. Phenomena of life are phenomena of motion due to liberation of energy caused by the breaking down of molecules. The physical activities noted in life are the results of chemical changes in the body. Metabolism vivifies the energy potential in the chemical compounds.

Lavoisier (*Académie des Sciences*, p. 379 (1780)) was the first to recognise that animal heat was derived from oxidation of the substance of the body, and to compare animal heat with that produced by a candle. To prove this he burnt a known quantity of carbon in an ice chamber and noted the amount of ice melted. He then calculated the amount of heat produced from a unit of carbon. He and Laplace put a guinea pig in an ice chamber, and noted the amount of ice which melted

during ten hours, and then calculated the heat given out by the animal. They then determined how much carbon dioxide the animal gave off. The animal yielded 31·82 calories to the ice chamber, while a calculation from the respiratory analysis showed that 25·408 calories could have been derived by the burning of enough carbon to yield the same amount of carbon dioxide as was eliminated by the animal. Lavoisier realized several errors in his work and knew that cold would raise the carbon dioxide output, also that cold reduced the heat in the animal itself, and further that the water of respiration was added to that of the melting ice, but he concluded that the source of the heat lay in the oxidation of the body. This was a discovery of far-reaching importance.

About 1842 James P. Joule supplied the chief experimental data which established the mechanical equivalent of heat. In 1845 J. R. Mayer laid down the law of conservation of energy, and in 1847 Helmholtz independently made the same discovery. It is interesting to observe that both these great contributions were rejected by the leading German journals of the day.

Rubner (*Zeit. f. Biol.* XXI, 250, 337 (1885)), a pupil of Voit, established the isodynamic law which showed that food stuffs may under given conditions replace each other in accordance with their heat producing value. Rubner gives the following as the quantities of the different food stuffs which are isodynamic:—

100	grms.	fat.
232	,,	cane sugar
234	,,	dried meat.

The heat value to the body of burning starch and fat were obviously the same as that determined in the calorimeter, because in both cases the end products were carbon dioxide and water. The heat value of protein in the calorimeter was different from its fuel value in the body, since the end products were different in the two cases. When protein is oxidized in the body the products of its metabolism are lost in three different ways — through respiration, in urine, and in fæces. The last two contain the heat lost to the body, which must be deducted from the protein value determined calorimetrically.

Rubner saw that the heat value of the urinary constituents themselves, which had to be subtracted from the heat value of the protein to the body, needed to be determined; hence he burnt the dry urine. He also determined the amount of heat produced from one gram of fæces after meat ingestion; he thus had all the data necessary for the determination of the heat value under different physiological conditions,

and was in a position to establish the law of conservation of energy in the metabolism of the animal body.

Rubner also discovered that the heat value of the metabolism of the resting individual is proportional to the area of the surface of his body; for example a man in starvation or on a medium diet, an infant at the breast, and a starving dog were shown to give off similar quantities of heat per sq. cm. of surface. This law of surface area has been extended so that it applies to all warmblooded animals.

From his work on protein, Rubner concluded that the heat value of one gram of protein in an average mixed diet might be placed at 4·1 calories, for fat at 9·3, and for carbohydrates at 4·1 calories. He also showed that if the diet were increased from a medium to an abundant amount, the metabolism as indicated from the heat production rose. This dynamic action resulting from the excessive ingestion of food stuff was greatest with protein. Finally, Rubner evolved an animal calorimeter which could accurately measure the amount of heat a dog produced in 24 hours; and the results obtained were a triumphant demonstration of the truth of the law of conservation of energy. Thus the amount of heat calculated (*Zeit. f. Biol.* XXX, 73 (1894)) as the quantity that should have been derived from the metabolism of the dog during the day spent in the calorimeter, was found to be the amount actually given off by the dog to the calorimeter. Metabolism, the source of the energy of bodily motions, was also the source of heat lost by the body. The result achieved constituted a final verification of the method of calculating the total metabolism originated by Pettenkofer and Voit. The precise and elaborate experiments of Atwater and Rosa confirm Rubner's results, and have shown that the energy spent by a man in doing any work such as bicycle-riding is exactly equal to the energy set free by the metabolism in the body.

Thus we have to look upon the living organism as a machine like a moving locomotive. We burn more if we have to attain a faster speed; or again we burn more to keep all parts warm in the winter's cold. In both cases motion and heat are derived from the power in the fuel. The physiologist busies himself with answering the how and why regarding the mechanism of living things.

This brief historical survey of the chemical aspects of nutrition may appropriately be closed with the following well-considered statement of Carl von Voit (*Münch. med. Woch.* **49**, 233 (1902)), perhaps the greatest authority on the subject of metabolism:—

"The unknown causes of metabolism are found in the cells of the organisms, the mass of these cells and their power to decompose mate-

rials determine the metabolism. It is absolutely proved that protein fed to the cells is the easiest of all the food stuffs to be destroyed, next come carbohydrates, and lastly fats. Metabolism continues in the cells until their power to metabolize is exhausted. All kinds of influences may act upon the cells to modify their ability to metabolize, some increasing or others decreasing it. To the former category belong muscular work, cold of the environment in warm blooded animals, abundant food, and warming the cells; to the latter, cooling the cells, certain poisons, etc.

The metabolism of the different food stuffs varies with the quantity and quality of the food. Protein may burn alone, or little protein, much carbohydrate, and fat may metabolize.

All the phases of the metabolism originate from the processes in the cell. In a given condition of the cells, available protein may be used exclusively if enough be furnished to them. If the power of the cell to metabolize is not exhausted by the protein furnished, then carbohydrates and fats are destroyed up to the limit of the ability of the cells to destroy them.

From this use of materials arise physical results such as work, heat, and electricity, which can be expressed in heat units. This is the energy derived from metabolism.

It is possible to approach the subject in the reverse order, i.e. to study the energy production and then to draw conclusions regarding the metabolism. It is perfectly possible to say that the energy requirement of the body, or the production of the heat necessary to cover heat loss or energy to do work, are controlling factors of the metabolism, since on cooling the body, or on working, correspondingly more material is destroyed; but one must not conclude that the loss of body heat and muscular work are the immediate causes of this increase in metabolism. The causes lie in the peculiar condition of the organism, and muscle work and loss of heat are merely factors acting favourably upon those causes, raising the power of cells to metabolize. In virtue of this more is destroyed, and secondarily the power to work and increased heat-production are determined. The requirement of energy cannot possibly be the cause of metabolism any more than the requirement of gold will put it into one's pocket. Hence the production of energy has a very definite upper limit, which is afforded by the ability of the cells to metabolize. If the cells will metabolize no more, then further increase of work ceases even in the presence of direst necessity, and this is also the case with heat production, even though it were very necessary, and we were likely to freeze."

CHAPTER II

Biological Oxidations.

The chemistry of the vital changes taking place in the animal body may fairly be said to date from the publication in 1780 of Lavoisier's "Expériences sur la respiration des animaux et sur les changements qui arrivent à l'air en passant par leur poumon". This savant clearly recognized from his experiments on animal heat and respiration that life processes are those of oxidation, resulting in the liberation of heat. It was shown by Lavoisier that oxygen is taken up by the lungs and in part converted into carbon dioxide. He was convinced that the whole process is one of combustion, and that it constitutes the natural mechanism for the supply of animal heat. With a clarity of vision, which is the unique characteristic of this investigator, Lavoisier clearly saw the necessity of furnishing the body with combustible material in the form of food in order to avoid the loss of body substance, and also the increased output of carbon dioxide following muscular activity.

Oxidation then is the central life process supporting the entire complicated machinery of the living being. The substances undergoing metabolism in the animal body, comprising proteins, carbohydrates, and fats, are entirely resistant to oxidation by molecular oxygen under ordinary conditions. Yet in the animal body they are oxidized with the greatest ease into their end-products. One can at once realize the great riddle of the vital combustion process by reflecting on the intense heat requisite to burn completely a fragment of protein on platinum foil, although it is very easy for the body to break down large quantities of protein completely. The problem of how this is accomplished in the body has occupied students of science ever since they began to delve into the enigma of life from the chemical point of view. It is generally conceded that the oxygen must in some way be activated inside the animal body. Many theories have been put forward from time to time to explain the mechanism of this activation; only the most important ones will be discussed here.

The idea of ozonization of oxygen emanating from Schönbein has not survived criticism, although it created a deep impression on his

contemporaries. Van't Hoff and others had suggested that probably ionization of molecular oxygen occurs. This theory has no adequate experimental basis, and appears to be speculative. Hoppe-Seyler, and later Baumann, were inclined to ascribe the activation of oxygen to the rupture of its molecule by nascent hydrogen, or some other reducing agent present in the cells, with the formation of water and an atom of active oxygen. This view, too, lacks experimental foundation.

PEROXIDASES.

A theory which deserves our attention was put forward originally by Traube. This author seems to have been the first to adopt the idea of an oxidizing ferment, and to give expression to the theory that there occur in the body readily oxidizable substances which have the power of transferring oxygen in an active form to substances which can be oxidized with difficulty. Later, this conception was developed by Engler, Bach, and Chodat into the now well-known peroxide theory. According to these workers living cells contain thermo-labile substances (the oxygenases), which are capable of taking up oxygen to yield peroxides. These peroxides are in themselves inactive, or only slightly active, but acquire powerful oxidizing properties by contact with certain ferments called peroxidases. Engler and Herzog postulate the following scheme: $A + O_2 \rightarrow AO_2$. A second substance, B ("acceptor"), not an autoxidizable one, can be oxidized by AO_2 in the sense: $AO_2 + B \rightarrow AO + BO$. AO is capable of oxidizing another molecule of B, $AO + B \rightarrow A + BO$, and the autoxidizer A is thereby regenerated; so that the final effect of the whole process seems to be $2B + O_2 \rightarrow 2\,BO$. In the above case A simply acts as a catalyzer, and conveys two atoms of oxygen to two molecules of B.

These views have acquired increased physiological interest from certain findings of Kastle and Loevenhart (*U.S. Hygienic Lab. Bull.* **50**, 24 (1909)) indicating that inorganic and organic peroxides are capable of producing a blue color with tincture of guaiacum, just as vegetable tissues do. Battelli and Miss Lina Stern have collected the literature on oxidases up to 1912 in a little monograph (*Ergebn. d. Physiol.* **12**, 96—268 (1912)).

The name given by Bach and Chodat to the autoxidizable substances, namely oxygenases, suggests the ferment character of these substances. But the recent work of Mrs. Onslow indicates that the substance which takes up molecular oxygen is not an enzyme but a cell-unit — she favours the idea that it may be related to catechol —

which forms a peroxide under the influence of an enzyme. However, Gallagher has presented evidence that the autoxidizable cell-unit may be of the nature of a lipin which does not require any enzyme to accelerate the formation of the peroxide, and that the only enzyme required is the peroxidase to decompose this compound. Similar views have been expressed recently by Thurlow. Pugh and Raper (*Biochem. J.* **21**, 1370 (1927)) have suggested a convenient classification of oxidases. The recent work of Keilin (*Proc. Roy. Soc. B.* **104**, 206 (1929)) deals with the close association of the respiratory pigment cytochrome with the oxidase system of the cell.

An extensive literature has accumulated on the oxidizing capacity of peroxidases. However important the part played by these oxidases and peroxidases may be in effecting the oxidation of formic acid to carbonic acid, of leuco-malachite green to the colour dye, of aldehydes to acids, of purin bases to uric acid, of cyclic protein cleavage products to melanins, or generally of easily attackable hydroxyl compounds, yet it is not likely that the peroxidases and oxidases take any important part in the vital combustion processes. That these play a prominent rôle in all sorts of detoxifying processes in the animal body cannot be denied. But nobody has ever been able to break down a single milligram of sugar with their aid(1). Von Fürth remarks in his *Chemistry of Metabolism*, p. 555 (Lippincot Company, 1916 edition): — "The study of oxidases has not, however, brought us appreciably nearer to the final secrets of life". Battelli and Miss Stern express a similar opinion.

CRITICISM OF PEROXIDE THEORY.

The supposed similarity between the oxidations effected in the body and those brought about by hydrogen peroxide *in vitro* (Dakin and Jolles) is believed to lend great strength to the peroxide theory. Thus Dakin writes (*Oxidations and Reductions in the Animal Body*, p. 8, 1912 edition), "An extraordinarily close similarity as regards the types of reactions exists between the two sets of phenomena. Thus normal saturated fatty acids in the body undergo oxidation in the β-position, butyric acid yielding aceto-acetic acid, acetone, and other products; hydrogen peroxide alone of all the various chemical oxidizing agents brings about precisely the same reaction ... Amino-acids and ketonic acids, such as leucine and phenyl pyruvic acid,

(1) Compare, however, Lyon (*Amer. J. Bot.* **14**, 274 (1927)).

are oxidized to lower fatty acids with the liberation of carbon dioxide and either ammonia or water . . . Glucose may be oxidized in the body to glycuronic acid, while hydrogen peroxide is the only reagent capable of effecting this change outside the body. A great many other biochemical reactions of less strikingly characteristic types such as the oxidation of simple alcohols, aldehydes, etc. may be reproduced *in vitro* by means of hydrogen peroxide".

If we consider this statement, it will be at once clear that the similarity between oxidations in the animal body and those *in vitro* effected by hydrogen peroxide, is only superficial. The intermediate products selected here for bringing the similarity into relief, namely glycuronic acid in the case of glucose, aceto-acetic acid, acetone, and other products in the case of butyric and other fatty acids, and carbon dioxide and ammonia in the case of amino acids, are not products of normal metabolism, but are only obtained in certain pathological conditions. The end-products of normal metabolism of glucose and fatty acids in a healthy individual are carbon dioxide and water. The introduction of camphor, chloral, etc. into a normal as well as a diabetic person, leads to the excretion of combined glycuronic acids. This fact unfortunately has led to the misleading supposition that glycuronic acid is an intermediate product of sugar metabolism. Existing evidence strongly contradicts such a view: gluconic, glycuronic, and saccharic acids undergo ready catabolism in the diabetic (Baumgarten, *Zeitschr. f. exper. Pathol.*, **2**, 53 (1905)). Hence it might be thought that the diabetic has merely lost the ability to manage the first step in sugar combustion—that leading to glycuronic acid. But that the diabetic has the power to carry on sugar oxidation as far as glycuronic acid is shown by the fact that he reacts to the introduction of chloral, camphor, etc. just as a normal individual does, by excretion of combined glycuronic acids (Baumgarten, *Zeitschr. f. exper. Pathol.* **8**, 206 (1910)). One might conclude, therefore, with von Fürth (*Chemistry of Metabolism*, 1916 edition, p. 258) that "normal physiological combustion of sugar does not follow the path through gluconic and glycuronic acids", and that "glycuronic acid is only produced exceptionally, if occasion demands detoxification of some foreign substance". The dangers of drawing conclusions as to normal metabolism from the results obtained by the introduction of some foreign substance are very clearly indicated here. We have seen that the introduction of phenol, chloral, etc. has definitely changed the normal course of sugar catabolism. Great caution needs, therefore, to be exercised in interpreting such results.

Similarly it can be shown that aceto-acetic acid and other acetone bodies are not the products of normal metabolism. The excretion of acetone bodies in the urine only occurs in conditions of carbohydrate starvation, and is independent of the method by which starvation is brought about. It may be due to disease such as diabetes, to the influence of drugs like phlorhizin, as a result of continued vomiting or the voluntary abstention from carbohydrate food. When acetonuria has made itself manifest as a result of simple carbohydrate starvation, the administration of carbohydrate causes its prompt disappearance. Fats are oxidized completely only when carbohydrates are being metabolized at the same time. Deprivation of carbohydrate increases the body's need of fat, and this flooding of the metabolic mill with fat results in its rapid and incomplete oxidation, leading to the production of acetone bodies. It is evident therefore that aceto-acetic acid is not a final product of normal metabolism.

Thus the similarity drawn by Dakin between biological oxidations and oxidations *in vitro* effected by hydrogen peroxide, holds only in the case of the products of abnormal metabolism.

SURFACE OXIDATION THEORY.

During recent years Prof. Otto Warburg has brought forward some indirect evidence to show that oxidations taking place in the living cell are catalytic reactions in heterogeneous systems (*Pflüger's Arch. ges. Physiol.* **145**, 277 (1912); *Z. physiol. Chem.* **92**, 231 (1914)). The hypothesis that the high velocity of oxidation of food stuffs in the cell is based on catalysis at surfaces containing iron, has been shown to be probable by Warburg from his work on Merck's blood charcoal suspended in aqueous solutions (*Pflüger's Arch. ges. Physiol.* **155**, 547 (1914); *Bioch. Zeit.* **119**, 34 (1921); *ibid.* **113**, 257 (1921)). It is well known that amino-acids in aqueous solution are perfectly stable when exposed to air. If, however, animal charcoal is added, the amino-acid is burnt to carbon dioxide and ammonia. This oxidation, taking place on the surface of the charcoal, is inhibited by different narcotics in exactly the same way as respiration in living cells. Warburg conceives with Langmuir that unimolecular adsorption layers of the substance are formed at the surface of the charcoal or the cell as a preliminary to oxidation, and that the effect of narcotics consists in the displacement of amino-acid molecules from the surface of the carbon. In order to explain the function of iron, Warburg assumes that activation of oxygen occurs at the iron-rich centres on

the surface of the charcoal. On this theory, Warburg successfully explains the powerful inhibiting action of cyanides, as being due to the destruction of active centres containing iron by the formation of a complex cyanide.

Attractive and interesting as may seem this hypothesis of Warburg, it does not yield satisfactory results when we come to sugars and fats. Meyerhof (*Bioch. Zeit.* **135**, 558 (1923)) has investigated the behaviour of carbohydrates on charcoal. Glucose is perfectly stable. It is interesting to note that hexose-phosphoric acid is oxidized to a noticeable amount, although much less than amino-acids are. Fructose is oxidized still less, and lactic acid almost imperceptibly. This peculiar stability of carbohydrates at the surface of the charcoal model makes it doubtful whether such surface catalytic actions can alone account for most biological oxidations; suffice it to say that such surface actions do take place to a certain, though limited extent, the animal body being a veritable net of colloidal systems.

THE DEHYDROGENATION THEORY OF WIELAND.

Only a brief reference need be made to the ideas of Wieland (*Ber.* **45**, 484 (1912); **47**, 2085 (1914)). This investigator is of the opinion that most oxidations are dehydrogenations:

$$R-CH_2-OH \rightarrow R-C\begin{matrix}O\\ \diagdown\\ H\end{matrix} + H_2$$

$$X + H_2 \rightarrow XH_2$$

where X is a substance, termed the hydrogen acceptor, having great affinity for hydrogen. Hence in the complete absence of oxygen, glucose is partly oxidized by palladium black at the ordinary temperature to carbon dioxide, and the palladium is found to be charged with hydrogen. If p-benzoquinone, methylene blue, or oxygen be present as "hydrogen acceptor", the oxidation proceeds further. Palladium may be replaced by *bacterium aceti*. In an interesting paper, Thunberg (*Skand. Archiv. physiol.* **40**, 1 (1920)) has adopted Wieland's views, and studied the action of a thermolabile agent present in muscle tissues, and capable of causing the reduction of methylene blue under anaerobic conditions, in the presence of hydrogen donators like succinic acid, etc.

According to Wieland, oxygen is the most common acceptor of hydrogen in the living tissues. How far such dehydrogenation pro-

cesses play an important rôle in the metabolism of nutrient substances cannot yet be judged with certainty in the light of the scanty experimental evidence available. Suffice it to say that there was a bitter controversy between Warburg and Wieland regarding the latter's dehydrogenation hypothesis. In recent years attempts are being made to introduce electrochemical considerations into biochemical oxidation — reduction systems, notably by Quastel (*J. Hygiene* **28**, 139 (1928)), Cannan, Cohen, and Clark (*United States Health Service Pub. Supp.* **55** (1926)).

INDUCED OXIDATION OF CARBOHYDRATES, FATS, etc.

Some interesting observations made in the author's laboratory seem to point to far reaching conclusions with regard to the metabolism of carbohydrates and fats in the normal healthy animal. Before proceeding further a few words need be said about induced oxidation. An example will make the subject clear. It has long been known that sodium sulphite in solution is very readily oxidized by atmospheric oxygen; whereas sodium arsenite alone in solution is not oxidized. If, however, sodium arsenite and sodium sulphite are mixed together in solution, both will be readily oxidized by atmospheric oxygen at ordinary temperatures. That is, the oxidation of sodium sulphite, which proceeds spontaneously, induces the oxidation of sodium arsenite, which is normally resistant to the action of atmospheric oxygen. In general, whenever the oxidation of an auto-oxidizable substance *A* brings about the oxidation of another substance *B*, which under normal conditions does not proceed, then the phenomenon is called induced oxidation. The substance *A* is termed the inductor and *B* the acceptor. Much has been said and written about the mechanism of induced oxidations in general, but our knowledge about most of these reactions still remains obscure.

In several publications[1] from the author's laboratory, it has been shown that substances like glucose, starch, cane sugar, and fatty acids like palmitic and stearic acids, which are not directly oxidized by atmospheric oxygen at the ordinary temperatures, can be oxidized when they are mixed with substances like sodium sulphite, and freshly precipitated ferrous, cerous, manganous, and other hydroxides, all of which readily undergo oxidation; and it is important to note

[1] Dhar. *Proc. K. Akad. Wetensch. Amsterdam* **33**, 1074 (1921); Mittra and Dhar. *Z. anorg. Chem.* **122**, 146 (1922); *J. Phys. Chem.* **29**, 376 (1925); Palit and Dhar. *ibid.* **29**, 799 (1925); **30**, 939 (1926); **32**, 1663 (1928); **34**, 710 (1930).

that in each case the substance is oxidized to carbon dioxide and water, without the formation of any intermediate compound. A molecule of acetic acid containing two carbon atoms, and a molecule of stearic acid which contains eighteen carbon atoms, are equally well oxidized to carbon dioxide and water; the same is the case with alcohol containing two carbon atoms and glucose containing six carbon atoms.

One cannot but be struck by the close analogy between these slow and induced oxidations and biological oxidations in a normal individual. The normal metabolism of carbohydrates and fats gives rise to carbon dioxide and water only, and to no intermediate products. Such products, as glycuronic acid from glucose and diacetic acid from fatty acids, make their appearance only in abnormal metabolism, as in intoxication with phenol, chloral, etc. or in diabetes, or in fasting as a result of continued vomiting or in poisoning with drugs like phlorhizin, etc. It is also interesting to note that exactly similar intermediate products are obtained in the case of rapid oxidation with hydrogen peroxide and ferric salt (Dakin, Jolles, Chakravarti and Dhar etc.). But the products of the slow and induced oxidation of carbohydrates are exactly identical with those of the normal metabolism of these substances in the animal body.

The behaviour of some of the polybasic acids is also instructive. Oxalic acid, the simplest member of the group, although very rapidly oxidized by many laboratory reagents, is oxidized with great difficulty in the animal body. Malonic, succinic, glutaric, tartaric, and citric acids, however, are readily oxidized in the body, although they are more stable than oxalic acid towards many oxidizing agents *in vitro*.

A systematic investigation of induced oxidations of the above type has been carried out in the writer's laboratory, a detailed account of which will be given in the succeeding chapters. Suffice it to say here that oxidation of a wide variety of compounds has been effected by atmospheric oxygen in the presence of inductors like sodium sulphite, colloidal phosphorus, freshly precipitated ferrous, cerous, manganous, uranous, and other hydroxides, at the ordinary temperature (about 20° C). It is highly significant that many of these substances yield carbon dioxide and water, products of normal metabolism. Knowing what one does of the products which are formed by the action of other oxidizing agents, one cannot but be impressed by the parallel which this suggests. Not only this. The oxidation of these substances to carbon dioxide and water is quantitative. It seems probable, therefore, from the analogy of these experiments *in vitro*,

that biological oxidations can be classed under induced reactions; and that there exist in the animal body readily oxidizable substances, the oxidation of which induces the oxidation of food materials.

Within recent years some evidence has accumulated in favour of this hypothesis.

GLUTATHIONE AND THE WORK OF MEYERHOF AND HOPKINS.

Paul Ehrlich was the first to recognise that tissue respiration is in some way connected with the reducing power of tissues, and the presence of auto-oxidizable tissue components. He injected methylene blue into animals and found that this is changed by reduction into the leuco-base, which by oxidation may be converted back again into the original dye. A number of other examples of reducing action of tissues have been met with from time to time. Such reductions have been long looked upon by many workers as indicating the existence of reducing ferments. All these observations have appeared in a new light since Heffter (*Hofmeister's Beitr.* **5**, 213 (1904); *Arch. f. exper. Pathol. Schmiedberg Festschr.* 253 (1908), and B. Strassner, (*Biochem.* 2, **29**, 295 (1910)) subjected them to a systematic study. It was shown that the phenomena which are connected with the auto-oxidizability and reducing power of tissues are occasioned by the presence of hydrosulphide groups (SH), shown by the characteristic purple colour given with sodium nitroprusside and ammonia. Heffter clearly realized that a substance containing (SH) groups can function in the tissues in oxidation-reduction reactions.

Meyerhof found that respiration in yeast cells and muscle can be stopped by extracting with water. It is remarkable that the respiration can again be induced in the washed muscle or yeast by the addition of the aqueous extract. He further showed that a compound causing the hydrosulphide reaction always passes into the aqueous extract, while the residue becomes free of (SH). So there is no doubt that the respiration in muscle tissues and yeast cells is connected with an auto-oxidizable hydrosulphide compound. Meyerhof further showed that the addition of artificially prepared substances containing hydrosulphide groups, like thioglycollic acid $\begin{matrix} CH_2SH \\ | \\ COOH \end{matrix}$, also renews the activity of washed muscle tissues.

The isolation in a pure condition by Sir Gowland Hopkins in 1921 of a simple substance present in tissues and containing the

sulphydril group marked a very great advance. This substance he termed glutathione, and it was supposed to be a dipeptide of glutamic acid and cystein. Hopkins demonstrated that the addition of this substance to washed yeast or muscle tissues restores the power of the cells to effect oxidations, as represented by oxygen utilization, carbon dioxide liberation, or the decolorization of added methylene blue. By oxidation glutathione is converted into the disulphide form

$$2G-SH+O \rightleftarrows G-S-S-G+H_2O.$$

This oxidation of glutathione into the disulphide form induces the oxidation of the cell constituents, as is shown by the fact that more oxygen is taken in than is required for the formation of the disulphide compound. It is revealed by the investigations of Meyerhof and Hopkins that the tissues are also capable of effecting the reduction of the disulphide compound into glutathione

$$G-S-S-G+XH_2 \rightarrow 2G-SH+X.$$

The nature of the compound XH_2 in muscle tissues is not known. The compound XH_2, glutathione, and its disulphide are all thermo-stable, so that the possibility of thermolabile enzymes is excluded.

In a recent communication from Hopkins' laboratory, Harrison (*Bioch. J.* **18**, 1009 (1924)), has shown that traces of iron cause a marked acceleration in the auto-oxidation of glutathione; and hence the oxidation of tissue components induced by glutathione will also be accelerated by iron. H-ion concentration appears to influence the system, muscle preparation — glutathione. At p_H 3·0—4·0 fat alone is oxidized, at p_H 6 both fat and protein are oxidized, whereas at p_H 7·6 the oxidation of fat is suppressed and that of protein is predominant.

Recent work shows that glutathione is not a dipeptide of glutamic acid and cystein, but a tripeptide of glutamic acid, cystein, and glycine (E. C. Kendall and his associates, *J. Biol. chem.* **84**, 656 (1929); Hopkins, *ibid.* **84**, 1269 (1929); *Nature* **124**, 445 (1929)). In the meanwhile, Dixon and Meldrum (*Nature* **124**, 512 (1929)) have stated that the tripeptide is physiologically inert; the undoubted activity of the older impure glutathione preparations in this respect, therefore, still remains an unsolved mystery, and is probably due to some impurity acting as a promoter.

These new findings do not in any way affect the view here taken that there exist in the tissues auto-oxidizable substances, the oxidation of which by atmospheric oxygen induces the oxidation of nutrient

materials. It is interesting to note that experiments done in the author's laboratory have shown that insulin can also act as an inductor in the oxidation of glucose.

CONCLUSION

This much can be said in summarizing our views about the oxidation of food principles. The studies of Otto Warburg have made it probable that oxidations in the cell are to a limited extent examples of catalysis in heterogeneous systems. The results of Warburg on the oxidation of amino-acids at the surface of a blood charcoal model, when taken in conjunction with the fact that the proteins are oxidized most readily in the animal body, suggest that possibly protein metabolism takes place through catalytic reactions at surfaces. That this mechanism cannot satisfactorily explain the metabolism of carbohydrates and fats is evident from the failure of Meyerhof to get perceivable oxidation of glucose, lactose, etc. at a charcoal surface. The results of the work carried out in the author's laboratory, in which the normal biological oxidations have been successfully imitated, make it probable that there exist in the animal tissues readily oxidizable substances, the oxidation of which induces the oxidation of sugars and fats. That such thermo-stable, auto-oxidizable substances exist in the tissues is made evident from the work of Meyerhof and Hopkins on glutathione. This fact, then, emerges from the author's investigations: that in a normal healthy individual carbohydrates and fats are completely oxidized to water and carbon dioxide, without the formation of intermediate products, and that the latter make their appearance only in abnormal metabolism.

CHAPTER III

Carbohydrate Metabolism.

It is well known that carbohydrates are not easily oxidized by air at the ordinary temperature; but in the animal body they are oxidized readily to carbon dioxide and water, and this oxidation is the main source of animal heat. Generally speaking two-thirds of the energy produced by the animal organism is derived from the oxidation of carbohydrates. Not without reason is bread considered the staff of life.

The gastro-intestinal tract converts starch into glucose, inverts sucrose into glucose and fructose, and lactose into glucose and galactose, so that these soluble monosaccharides become fuels transported by the blood for the nourishment of the body cells. The enzymes maltase, invertin, and lactase, which respectively convert maltose, sucrose, and lactose into monosaccharides, are present in the intestinal mucosa of the new-born infant. Of these various monosaccharides, glucose deserves our attention most as it is the most widely-occurring sugar in the animal body. Glucose is in a large measure stored as glycogen in the muscles and the liver, and on utilization glycogen yields glucose once more. It follows, therefore, that the problems of carbohydrate metabolism are principally centred around the chemical changes that glucose may undergo.

BLOOD SUGAR.

It has been frequently asked whether blood sugar is present in the body in the free or combined condition. Thanks, however, to the researches of Schenk and Asher (*Bioch. Zeit.* **8**, 351 (1907)) and Michaelis and Rona (*ibid.* **14**, 476 (1908)), we now know that most of the sugar exists free in the blood, or at least in a condition capable of becoming free spontaneously.

Recently Robison and co-workers (*Bioch. J.* **18**, 1161 (1924); *ibid.* 740, and 755) have produced evidence to show that some of the glucose exists in combination as phosphoric esters of hexose-mono-

phosphoric and hexose-diphosphoric acids. It will be remembered that for a long time Embden and collaborators (*Z. physiol. Chem.* **93**, 1, 94, 124 (1914)) have advanced the opinion that phosphorus compounds are concerned in the early stages of the metabolism of hexoses.

More recently Winter and Smith (*Chem. Soc. Ann. Report* **19**, 195 (1922); *Proc. Roy. Soc.* (*B*) **97**, 20 (1924)) have stated that the sugar present in normal blood is an unstable form of glucose of low initial rotatory power, and possibly the so-called γ-glucose or ethylene oxide form of dextrose. This view is based on their observation that the protein-free filtrates of normal blood at first show disagreement between their copper-reducing and polarimetric values, but that after two to three days the latter gradually approach the former values until agreement is reached. In the case of diabetic bloods, however, the polarimetric readings gave higher values than the copper method. It is suggested that the blood-sugar in cases of diabetes is α, β-dextrose, and that the failure of the organism to utilize the sugar in this disease is due to the absence or inactivity of an enzyme, which in the normal subject converts the α, β-sugars into the active γ-form.

This work of Winter and Smith has been severely criticized by several investigators. Recently Lundsgaard and Holbøll (*Compt. rend. soc. Biol.* **91**, 1108 (1924); **92**, 387, 395, 398, 525 (1925); also *J. Biol. Chem.* **68**, 457, 485; **70**, 71, 79, 83, 89 (1926)) have shown that in presence of muscle tissue, insulin brings about a marked reduction of the rotation of glucose solutions. These results can, however, be explained by a change in the equilibrium between α- and β-glucose without assuming the formation of hypothetical γ-glucose. The experimental methods and the interpretation of the results of the above workers have been criticized by Paul (*J. Biol. Chem.* **68**, 425 (1926)), and by Anderson and Carruthers (*Bioch. J.* **20**, 556 (1926)). The whole question must be regarded as an open one.

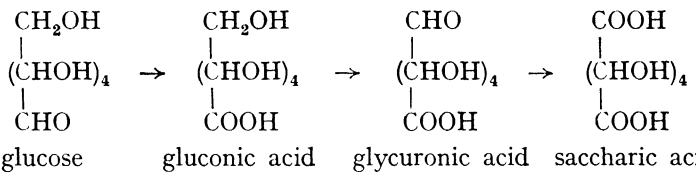
CATABOLISM OF GLUCOSE.

The mechanism by which dextrose is broken down in the animal body into its final products of oxidation, carbon dioxide and water, has been and is likely to be a subject of widespread interest. A large mass of literature has accumulated as regards the possible intermediate products of oxidation of glucose.

The fact that it is apparently possible to isolate small quantities of ethyl alcohol by the distillation of aqueous extracts of tissues,

had led at times to the supposition that sugar might undergo alcoholic fermentation in the animal body. This view was found to be incorrect long ago. In the same way it had been advanced that oxalic acid is a product of sugar catabolism, on the basis that oxaluria is at times found associated with diabetes. But the well known observation that oxalic acid is attacked very little in the animal body, and is excreted as such in the urine of a normal healthy individual, strongly points against the acceptance of such a view.

Glucose can be made to pass through the following stages by careful oxidation *in vitro*:—



glucose gluconic acid glycuronic acid saccharic acid

The administration of chloral, camphor, etc. leads to the elimination of combined glycuronic acid in the urine; this observation has led to the unlikely suggestion that a process such as that indicated in the above scheme takes place in the animal body. That saccharic acid cannot be regarded as a product of normal metabolism of glucose is shown by the experiments of Wirth (*Bioch. Zeit.* **33**, 49 (1911)), which indicate that saccharic, gluconic, and mucic acids, when added in sufficient quantity to the blood supplying perfused dogs' livers, may lead to a marked aceto-acetic acid formation.

IS GLYCURONIC ACID A PRODUCT OF NORMAL METABOLISM?

It has been shown by Baumgarten (*Zeit. f. exper. Pathol.* **2**, **53** (1905)) that glycuronic acid undergoes ready combustion in the diabetic organism. It might, therefore, be thought that the diabetic has merely lost the ability to manage the first step in sugar combustion. If that were the case, however, it would be difficult to interpret the fact that the diabetic reacts to the introduction of chloral, camphor, etc. just as the normal individual does, by the excretion of combined glycuronic acids. This shows clearly that the diabetic has the power to carry sugar oxidation as far as glycuronic acid. One might conclude, therefore, that the normal, physiological combustion of sugar does not follow the path through gluconic and

glycuronic acids, and that glycuronic acid is only produced under exceptional circumstances, if occasion demands detoxification of some foreign substance.

Most physiologists regard it as a settled fact that lactic acid is an intermediate product in sugar metabolism. Lactic acid can be obtained *in vitro* by means of lactic fermentation of glucose, or by the action of alkalies on glucose, the yield being more than 50 per cent. The investigations of Hopkins and Fletcher (*J. Physiol.* **35**, 247 (1907)) have shown that muscle accumulates lactic acid anaerobically on electric stimulation. On admitting oxygen lactic acid disappears, and the intake of oxygen above the resting value only lasts as long as lactic acid exists in the muscle. But the total amount of this excess of oxygen is only sufficient to account for the oxidation of about one-fourth of the lactic acid which disappears; the remaining three-quarters of the lactic acid is assumed to be reconverted quantitatively into glucose. It has not yet been decided experimentally, however, whether it is the lactic acid that undergoes oxidation on admitting oxygen, or an equivalent amount of glucose. The respiration coefficient with both lactic acid and glucose would be unity. It seems probable that all the lactic acid is converted back into glucose, and that a quantity of glucose equivalent to one-fourth of the lactic acid lost undergoes oxidation. The rôle of lactic acid in muscular exercise has been the subject of a valuable series of papers by Hill, Long, and Lupton (*Proc. Roy. Soc.* (*B*), **96**, 438; **97**, 84 (1924)).

Embden has always postulated that there is a precursor of lactic acid; this he called lactacidogen. Recently Embden and Zimmermann (*Z. physiol. Chem.* **167**, 114 (1927)) have isolated a hexose-monophosphoric acid from rabbit's muscle; this observation has been confirmed by Pryde and Waters (*J. Soc. Chem. Ind.* **46**, 1182 (1927)). Embden and Zimmermann state that the new hexose-monophosphoric acid differs from the two known natural hexose-monophosphoric acids (Robison's and Neuberg's). They have further shown that the new muscle monophosphoric acid is converted by muscle press-juice into lactic acid. They are, therefore, disposed to identify lactacidogen with the new mono-phosphate.

An important advance in this line is the isolation by Meyerhof (*Bioch. Zeit.* **178**, 395, 462 (1926)) of an active lactic-acid-forming enzyme from muscle. He has shown that it is possible to separate completely the lactic acid ferment from frog or rabbit muscle, and to obtain it in aqueous solution free from carbohydrates of the muscle. A co-enzyme, which is dialyzable and thermostable, can be separated

from the enzyme mixture. It has been shown that this water-soluble lactic acid ferment splits hexoses only under special conditions. On the other hand, in the presence of inorganic phosphate, it readily acts upon starch, glycogen, the starch components amylose and amylopectin, and the simpler compounds derived from them, such as tri- and di-hexosans, splitting them all to lactic acid with about the same velocity. During hydrolysis of the polysaccharides a phosphoric ester accumulates, at first quickly and then more slowly, and this ester can be completely decomposed into lactic acid and phosphate by warming to 37°. It would appear, however, that different enzymes are concerned in these reactions (Compare *Annual Reports on the Progress of Chemistry*, **24**, pp. 258—261 (1927)).

Embden (*Bioch. Zeit.* **45**, 186 (1912)) considers that acetaldehyde is an intermediate product in the oxidation of lactic acid to carbon dioxide and water. Simon and Piaux (*Bull. Soc. Chim. biol.* **6**, 412 (1924)) have shown that lactic esters are oxidized spontaneously with the formation of pyruvates, acetaldehyde, and carbon dioxide. Neuberg and Gottschalk (*Bioch. Zeit.* **146**, 164, 185, 582 (1924)) have shown that acetaldehyde is produced from glycogen in guinea-pig-liver pulp, and that the amount is increased by the addition of sugars. These investigators observed that the addition of insulin increased the production of acetaldehyde considerably. Toenniessen (*Z. physiol. Chem.* **133**, 158 (1924)) assumes that insulin is necessary for the production of acetaldehyde from lactic acid by chopped muscle. Indeed, this writer maintains that insulin acts on lactic acid rather than directly on carbohydrates.

Lambie (*J. Soc. Chem. Ind.* **46**, 300 (1927)) suggests that possibly dihydroxyacetone is an intermediate product in sugar metabolism; he further adds that the diabetic primarily lacks the power of transforming glucose into dihydroxyacetone. These suggestions are contradicted by Markowitz and Campbell (*Amer. J. Physiol.* **80**, 548 (1927)), who have shown that when dihydroxyacetone is given to depancreatized dogs, it is quantitatively excreted as glucose. Moreover, dihydroxyacetone is not oxidized in the body and has no antiketogenetic action.

SLOW AND INDUCED OXIDATION OF CARBOHYDRATES.

Notwithstanding all that has been said about the probable intermediate products in carbohydrate metabolism, the problem has not advanced much beyond the stage of hypothesis; and the question

why the carbohydrates, which are resistant to atmospheric oxygen outside the body, are metabolized with the greatest ease into carbon dioxide and water inside the body, remains still unanswered.

In the previous chapter the author has ventured to put forward the view that possibly glucose is burned in the animal body directly into carbon dioxide and water without the formation of intermediate products. This view has been advanced on the basis of results obtained on the slow and induced oxidation of carbohydrates.

In these experiments a slow current of air was passed through a series of bottles, containing solutions of carbohydrates and cerous hydroxide or some other inductor. The amount of carbohydrate oxidized was found both by direct estimation of carbon dioxide obtained, and by estimation of the residual sugar after removal of the peptized ceric hydroxide by coagulation with potassium sulphate. The precipitate of ceric hydroxide thus obtained was always analyzed and shown to be nothing but ceric hydroxide. It therefore becomes evident that a molecule of cerous hydroxide $Ce(OH)_3$ becomes oxidized to one of ceric hydroxide $Ce(OH)_4$, and that this oxidation induces the oxidation of the carbohydrates to carbon dioxide and water. The amount of carbon dioxide liberated quantitatively accounted for the amount of sugar that disappeared. This result, taken in conjunction with the failure to detect any intermediate compound, effectively eliminates the possibility of intermediate compound formation. Whilst an appreciable amount of oxidation in presence of inductors has been noticed in neutral solution, alkalinity has always been found to increase the amount of carbohydrate oxidized. Indeed the greater the amount of alkali, the greater is the quantity of sugar oxidized. Experiments showed that even in the complete absence of any inductor, oxidation still takes place if alkali is present.

The various carbohydrates fall into the following order according to the readiness with which they are oxidized:—

Starch > maltose > arabinose > laevulose > galactose > glucose > lactose > cane sugar.

In other words, starch is the most readily, and cane sugar the least readily oxidized of the carbohydrates investigated.

Besides $Ce(OH)_3$, a number of other inductors, like freshly precipitated ferrous, manganous, uranous, and other metallic hydroxides, sodium sulphite, and colloidal phosphorus, have been employed with similar results.

The following are the experimental results.

Inductor $Ce(OH)_3$; Volume of air passed was 36·5 litres in 5·5 hours.

Carbohydrate	Wt of carbohydrate in 30 c.c. of reaction mixture	Amount oxidized in neutral solution	Percentage oxidation in neutral solution	Amount oxidized in a solution containing 0·15 gr. of alkali in 30 c.c.	Percentage oxidation in alkaline solution
Starch	0·1050	0·0690	65·7	0·0936	89·1
Maltose	0·1097	0·0494	45·0	0·1000	91·1
Arabinose . . .	0·1000	0·0462	46·2	0·0978	97·8
Laevulose . . .	0·09185	0·0342	37·1	0·0868	94·5
Galactose . . .	0·08613	0·0315	36·5	0·0754	87·5
Glucose	0·0962	0·0224	23·2	0·0897	93·2
Lactose . . .	0·0997	0·0189	18·9	0·0753	75·5
Cane sugar .	0·1080	0·0126	11·7	0·0174	16·1

In view of these experiments, it appears that there occur in the tissues of the animal body readily oxidizable substances; and that the oxidation of these substances by atmospheric oxygen induces the oxidation of carbohydrates in the animal body, directly into carbon dioxide and water. It further appears that intermediate compounds are formed only in certain pathological conditions, and not in normal health. It is interesting to note that Chakravarti and Dhar (*Indian J. Med. Research.* **17**, 430 (1929)) have obtained intermediate products of aldehydic and ketonic nature in the rapid oxidation of sugars by hydrogen peroxide and ferric chloride.

GLYCOLYSIS IN CANCER AND OTHER PATHOLOGICAL CONDITIONS OF THE TISSUES.

Miniami (*Bioch. Zeit.* **142**, 334 (1923)) working with Warburg's technique (*ibid.* **142**, 317 (1923)) has found that the glycolytic activity of cancer tissue, as measured by lactic acid production, is many times that of an equal weight of normal tissue. These results have been confirmed by Waterman (*Arch. Neerland Physiol.* **9**, 573 (1924)). The addition of an extract of cancer tissue to the medium in which the normal tissues were kept caused an increase in the formation of lactic acid. Addition of phosphates or hexose-phosphate to normal tissues did not raise the glycolytic activity of normal cells.

Warburg (*Naturwiss.* **50**, 1131 (1924)) has shown that whereas the lactic acid produced by normal tissues under anaërobic conditions

disappears to a large extent when oxygen is admitted, that produced by cancer cells is removed only to a small extent. He also observed that if the oxidation processes in embryonic tissues be depressed by small amounts of hydrocyanic acid or by prolonged exposure to nitrogen, the tissues when placed under aërobic conditions behave like malignant tumours. He advances tentatively the idea that cancer may arise as a result of a chronic oxygen starvation, leading certain cells to show abnormal metabolism.

The inability of the tumour cells to oxidize fully the lactic acid formed by glycolysis is well illustrated by the experiments of Warburg, Wind, and Negelein (*Klin. Woch.* **5**, 829 (1926)). Blood loses about 57% of its glucose in passing through the vessels of a tumour as compared with 18% on passing through the liver. The inefficiency of the oxidizing mechanisms for degrading lactic acid in tumour cells is such that 66 per cent of the glucose that disappears in passing through a tumour appears in the venous blood in the form of lactic acid. Little is yet known of the cause of the lowered oxidation in cancerous tissues. *Holmes* (*Bioch. J.* **20**, 812 (1926)) *has found that in general they contain abnormally small amounts of reduced glutathione,* and may be slow in reducing the dipeptide added in its oxidized form. Rat sarcoma and carcinoma tissue were found, on spectroscopic evidence, to be deficient in the respiratory pigment cytochrome. Less definite findings are recorded by Bierich and Kalle (*Z. physiol. Chem.* **158**, 1 (1926)) and Bierich and Rosenbohm (*ibid.* **155**, 249 (1926)), who conclude that the amounts of reduced glutathione and cytochrome in cancerous tissues may show wide variations, whereas Thomson and Voegtlin (*J. Biol. Chem.* **70**, 801 (1926)) found tumours to be richer in glutathione than most normal tissues.

In support of the work of Warburg and his colleagues, Bierich (*Z. physiol. Chem.* **155**, 245 (1926)) has found that the lactic acid content of tumour tissues shows a wide variation, the absolute limits lying about 100 per cent higher than those for normal tissues.

Warburg (*Über die katalytischen Wirkungen der lebendigen Substanz,* Berlin, 1928) states that whilst normal tissues under aërobic conditions metabolize sugars by making use almost exclusively of an oxidative process, they may under anaërobic conditions form lactic acid, thus exhibiting a fermentative function. A peculiarity of the cancer cell is its power under aërobic conditions of making use of both these processes in metabolizing carbohydrates. Recently Warburg (*Bioch. Zeit.* **204**, 482 (1929)) has stated that aërobic glycolysis is not specific for tumours. When normal cells undergo aërobic

glycolysis they die, whereas cells of tumours undergoing glycolysis live and grow without limit, while using the chemical energy of glycolysis.

Recently Crabtree (*Bioch. J.* **22**, 1289 (1928)) has obtained interesting results on the carbohydrate metabolism of pathological overgrowths in the cases of fowl pox, vaccinia lesions in young chickens and rabbits, and human warts. The general conclusion of Crabtree is that the magnitude and relationships of the respiratory and glycolytic processes, found by Warburg to be characteristic of malignant tissues, are shared in common with the latter by pathological overgrowths generally. It has also been concluded by Crabtree that the peculiar metabolic phenomena observed with pathological overgrowths are a result rather than the cause of pathological overgrowths. Very recently Crabtree (*ibid.* **23**, 536 (1929)) has determined the carbohydrate metabolism of several strains of mouse tumours, and has observed great deviations from the standard value found for tumours of rat, fowl, and a limited series of human tumours. There was a number of cases of high respiration, both in its absolute value and in its relation to the aërobic and anaërobic glycolysis. The respiratory quotients with one exception were found to be below unity. Crabtree's work suggests that the glycolytic activity of tumours exerts a checking effect on their respiration. The carbohydrate metabolism of tumours is, to some extent, influenced by the environment in which they grow.

These researches are interesting, and may probably lead to important results regarding the treatment of cancer and malignant growths.

If the contention of Holmes that cancerous tissues contain abnormally small quantities of reduced glutathione is correct, this will give strong support to the author's view that biological oxidations are really induced oxidations.

CHAPTER IV

Metabolism of Fats.

The fats undergoing catabolism in the animal body are mainly derivatives of straight chain normal fatty acids containing an even number of carbon atoms. The number of carbon atoms in the fatty acids that occur in the animal body varies from four (butyric) to twenty four (carnaulic acid) or more. Palmitic, stearic, and oleic acids, with sixteen and eighteen carbon atoms, are quantitatively predominant. Formic, acetic, and probably propionic acids also occur in the animal body, but not in the form of fats. All these fatty acids undergo complete oxidation in the animal body with the formation of carbon dioxide and water. With the exception of formic acid, all the fatty acids enumerated above are very resistant to the action of the ordinary oxidizing agents; yet, in the animal body they undergo oxidation with extraordinary ease. What is the mechanism by which this oxidation is brought about?

Since the publication of Knoop's important paper on *"Der Abbau aromatischer Fettsäuren im Tierkörper"*, in 1904, it has been believed that the oxidation of the saturated fatty acid molecules in the living body occurs at the β-carbon atom, so that the carbon chain is progressively shortened by losing two carbon atoms at a time. This theory was based mainly on the researches of Knoop, in which the fate of the phenyl derivatives of a series of fatty acids in the animal body was studied. If the theory of β-oxidation is correct, then, as was first pointed out by Knoop and Embden, a degradation of the long fatty acid chain step by step will result in the successive formation of a number of fatty acids, each containing two carbon atoms less than its precursor, since the fatty acids occurring in the body contain an even number of carbon atoms. Finally, butyric and from it acetic acid should be produced. Important evidence for the formation of butyric acid is obtained from the fact that two derivatives of this acid, namely β-hydroxybutyric acid and aceto-acetic acid, are excreted in the urine of diabetics. That aceto-acetic acid, β-hydroxybutyric acid, and acetone are derived from fat seems to be well-established

from the quantitative study of the excretion of these substances in severe diabetes, first carried out by Magnus-Levy (*Arch. exp. Path. Pharm.* **42**, 149 (1901)) and extended by others.

Evidence of a different nature in favour of Knoop's theory has been put forward by Dakin (*J. Biol. Chem.* **4**, 77, 221, 227, 419 (1908)). This worker showed that if ammonium salts of fatty acids are warmed to 40° with hydrogen peroxide, oxidation takes place at the β-position. Thus ammonium butyrate gives rise to aceto-acetic acid, acetone, and other products, principally lower fatty acids and carbon dioxide. This reaction demonstrates the occurrence of β-oxidation *in vitro*; β-hydroxy butyric acid has not been detected as an intermediate product, probably because this substance is readily oxidized by hydrogen peroxide to aceto-acetic acid, acetone, etc.

Further evidence in favour of Knoop's hypothesis is furnished by the liver perfusion experiments of Embden and co-workers (*Beitr. z. Chem. Physiol. u. Path.* **8**, 129 (1906); *ibid.* **11**, 318 (1908)). When the liver of a freshly-killed dog is perfused with defibrinated blood, small quantities of acetone and aceto-acetic acid are produced. The addition of different neutralized fatty acids to the blood with which the liver was perfused, has shown that acids containing an even number of carbon atoms increase the formation of aceto-acetic acid, whereas those with an odd number do not. This is because acids with an uneven number of carbon atoms ultimately give rise to propionic acid, which has been shown not to yield aceto-acetic acid in liver perfusion experiments. The work of Embden is, however, open to some objections.

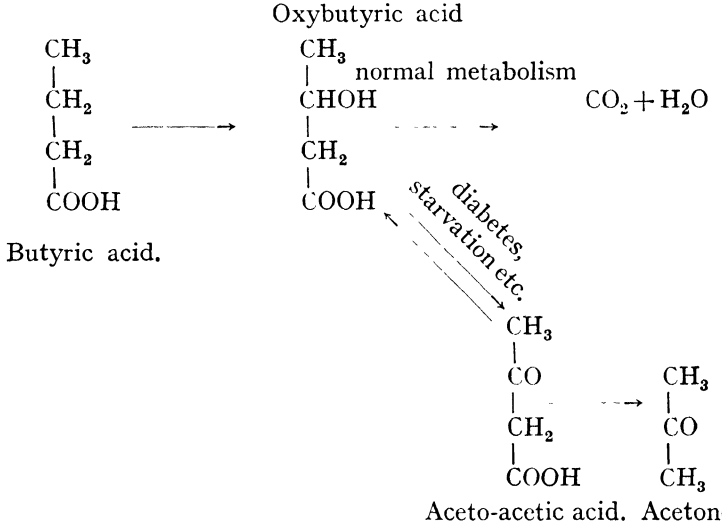
Recent work in this line is that of Kahn (*Amer. J. Med. Sci.* **166**, 826 (1923)), and of Sevringhaus (*J. Biol. Chem.* **59** (1924)); *Proc. Amer. Soc. Biol. Chem.* **XI**, ix)). Kahn administered a tri-glyceride of margaric acid, which contains an odd number of carbon atoms, to a fasting normal man, or to several diabetics with ketosis, and noticed the disappearance of the acetone bodies from the urine. Sevringhaus failed, however, to find any diminution of "acetone body" excretion, when glyceryl margarate in amounts up to 100 grms. daily was taken by a normal individual exhibiting ketosis owing to a deficiency of carbohydrate in the diet. Further work in this line will be awaited with interest. The theory of β-oxidation demands that fatty acids containing an odd number of carbon atoms should not give rise to acetonuria.

Unfortunately the experiments of Knoop, Dakin, and Embden were only qualitative, and later work has revealed that the theory of β-oxidation does not yield quantitative results. Clutterbuck and

Raper (*Bioch. J.* **19**, 384 (1925)), using conditions similar to, but not identical with those of Dakin, obtained products which showed that oxidation could occur at the γ- and δ-carbon atoms, and possibly also at the α-. So far as could be determined in the case of stearic, palmitic, and myristic acids, γ- and δ-oxidations could occur to the same extent. Hurtley (*Quart. J. Med.* **9**, 301 (1916)), from a careful study of the 4-carbon atom acids of diabetic urine, concludes that the theory of β-oxidative degradation of fatty acids in the animal body is inadequate. This view was supported by an experimental investigation of the oxidation of butyric acid by hydrogen peroxide, in which over 50 per cent of the theoretical yield of succinic acid was obtained. This fact is of particular interest because Clutterbuck and Raper, although as yet uncertain as to the further stages in the oxidation of the γ- and δ-keto acids produced by the oxidation of the higher fatty acids with hydrogen peroxide, found that both form succinic acid. This means that the carbon chain breaks at the γ- and δ-carbon atoms to yield the 4-carbon acid. The formation of succinic acid as an intermediate product of fatty acid oxidation *in vitro*, and the probability that it also occurs *in vivo*, suggest a possible path by which fatty acids give rise to sugar; for succinic acid is known to be easily converted into fumaric and maleic acids in the tissues, and the latter is converted into glucose in an animal exhibiting diabetes.

Attention was drawn to the statement that if the theory of β-oxidation were to hold good, the long chain fatty acids containing an even number of carbon atoms should ultimately give butyric acid, and from it finally acetic acid. But the view that acetic acid is the final product is not generally accepted. Most physiologists appear to believe that butyric acid undergoes oxidation to β-hydroxy-butyric acid, then to aceto-acetic acid, and finally to carbon dioxide and water. In normal metabolism, aceto-acetic acid is not considered to yield acetone by splitting off carbon dioxide, for acetone is not utilized in the human body, and is excreted as such in the urine. Only in diabetes does aceto-acetic acid give rise to acetone. Again, there is considerable difference of opinion whether or not butyric acid passes through the intermediate stage of β-hydroxy-butyric acid. One view is that in healthy individuals β-hydroxy-butyric acid, resulting from butyric acid, undergoes direct oxidation to carbon dioxide and water, the aceto-acetic acid not appearing as an intermediate product. In the case of interference with normal catabolism of oxybutyric acid, however, a false route would be interposed—

oxidation into aceto-acetic acid, and then formation of acetone by splitting off carbon dioxide. This school of thought is represented by Otto Neubauer (*27th Internat. Kongr. Wiesbaden,* 566 (1910)), who proposes the following schematic representation:—



On this scheme it can easily be conceived why in a healthy individual (as contrasted with diabetic subject) even large quantities of oxybutyric acid do not give rise to aceto-acetic acid production (Schwarz, Zeehuyzen, Araki, and others), and why in perfusion experiments on the normal liver (in contrast to a phlorhizin liver) the amounts of oxybutyric acid decomposed are in absolute disproportion to the newly formed aceto-acetic acid (Przibram. *Zeit. f. exper. Pathol.* **10** (1912)).

On the other hand, Blum (*Münch. med. Woch.* **57**, 683 (1910)) has advanced the view that the normal path for the catabolism of butyric acid is by way of aceto-acetic acid, without the intermediate formation of β-hydroxy-butyric acid. The evidence, adduced by him in support of this contention that β-hydroxy-butyric acid is not an intermediate product in the oxidation of butyric acid to aceto-acetic acid, is mainly based on the fact that he failed to produce a marked acetonuria by the subcutaneous administration of sodium β-hydroxy-butyrate to a dog, which under normal conditions developed ketosis when sodium butyrate or sodium aceto-acetate was administered. The actual de-

monstration of the formation of aceto-acetic acid from β-hydroxy-butyric acid by Embden (*Beitr. chem. Physiol. Path.* **11**, 318, 333 (1908)), and by Dakin and Wakeman (*J. Biol. Chem.* **6**, 375 (1909), **8**, 105 (1910)) was referred by Blum to a pathological condition of the liver cells. According to Blum and others of his way of thinking, the presence of β-hydroxy-butyric acid can be ascribed to two causes. It has been found independently by Blum (*Münch. med. Woch.* **57**, 683 (1910)), Dakin and Wakeman (*J. Biol. Chem.* **6**, 375 (1910)), Friedmann and Maase (*Bioch. Zeit.* **27**, 474 (1910)), and Neubauer (*Verhandl. Kongr. finn. Med.* **1**. 566 (1910)) that the liver can effect the reduction of aceto-acetic acid to β-hydroxy-butyric acid. Apparently an equilibrium is established

$$\beta\text{-hydroxy-butyric acid} \underset{\text{Reduction}}{\overset{\text{Oxidation}}{\rightleftharpoons}} \text{aceto-acetic acid.}$$

Secondly, β-hydroxy-butyric acid may also result from unsaturated acids taking up the elements of water. Thus Dakin showed that cinnamic acid may yield phenyl-hydroxy-propionic acid. This is further supported by the observation of Leathes and Wedell (*J. Physiol.* **38** (1909)) that the liver can render saturated acids unsaturated. Then, again, there is the irreconcilable fact that enzymic catalysts are known to exist in the tissues, which are capable of oxidizing oxybutyric acid to aceto-acetic acid (Dakin and Wakeman, *J. Biol. Chem.* **6**, 373 (1909); **8**, 105 (1910)) and of converting the latter into acetone by splitting off CO_2 (Pollak. *Hofmeister's Beitr.* **10**, 232 (1907)).

Thus the attempts to find in the organism the intermediate products of fat metabolism have resulted in a mass of conflicting results. No single observation recorded above can easily be disposed of. What seems most probable is that all the observations are correct, and that they cannot be reconciled is due to the fact that they can only be obtained in various pathological conditions. It should be emphasized here that the results obtained by administering foreign substances should be cautiously utilized in establishing any theory of the metabolism of nutrient materials in normal individuals. Thus, for example, Knoop postulated his theory of β-oxidation from a study of the fate of phenyl derivatives of saturated fatty acids in the animal body; the animal body, however, is not accustomed to such substances. Then, again, the liver perfusion experiments of Embden and collaborators are open to criticism. In these experiments fully oxy-

genated blood must be used, whereas the liver is normally supplied with a mixture of oxygenated and venous blood *via* the hepatic artery and the portal vein respectively. Further, in these experiments, the blood with which the liver is perfused is incorporated with large quantities of sodium salts of fatty acids, whereas under normal conditions the liver gets a supply of little fat and more sugar. Embden (*Bioch. Zeit.* **27**, 1 (1910)) himself has shown that it is also possible in the experimental perfusion of the liver to inhibit the formation of acetone bodies by adding easily oxidizable substances to the perfusion fluid. For example, we may prevent the production of aceto-acetic acid from caproic acid by introducing into the liver valerianic acid. Also substances of varied character like xylose, gluconic acid, mannite, glycerol, alcohol, tartaric acid, lactic acid, propionic acid, citric acid, glycocoll, alanine, glutamic acid etc., which readily undergo combustion in the living body, manifest antiketogenetic power. So it appears evident that the experiments of Embden and Knoop were not carried out under normal conditions.

ARE THE "ACETONE BODIES" PRODUCTS OF NORMAL METABOLISM?

It is still an open question whether β-hydroxy-butyric acid, aceto-acetic acid, and acetone are products of normal metabolism or not. Most physiologists believe that either one or both of the first two substances are intermediate products of normal metabolism, that the diabetic cannot further oxidize them, and that as such they are excreted in the urine.

It is established beyond doubt that the excretion of acetone bodies in the urine occurs only in conditions in which carbohydrate is either not being utilized owing to metabolic deficiency, or is missing from the food. To the former category belong diseases like diabetes or cases of poisoning with drugs like phlorhizin; to the latter, cases of continued vomiting or voluntary abstention from carbohydrate food. When acetonuria has made itself manifest as a result of lack of carbohydrate material in the food, the administration of such material causes the prompt disappearance of ketosis. This antiketogenetic effect of carbohydrates was pointed out long ago by Hirschfeld (*Zeit. klin. Med.* **28**, 176 (1895)). It appears that for the complete combustion of fats carbohydrates should be metabolized at the same time.

Various explanations have been given of the antiketogenetic effect of carbohydrates. Geelmuyden (*Zeit. physiol. Chem.* **41**, 135

(1904)) has supposed a combination of acetone bodies and carbohydrates to occur before the oxidation of the former can proceed, and suggests that the compound formed by acetone bodies with carbohydrates is possibly a glycuronate. No experimental evidence exists in favour of such a combination, and the uncertain position of glycuronic acid as an intermediate product of glucose metabolism (loc. cit.) renders the view of Geelmuyden unlikely. Woodyatt (*J. Amer. Med. Assoc.* **55**, 2109 (1910); *ibid.* **66**, 1910 (1916)) suggests that antiketogenesis is due to a reaction of glucose, glyceric aldehyde, or some simple sugar with aceto-acetic acid, which is of the nature of a coupled reaction, the sugar being oxidized and the aceto-acetic acid simultaneously reduced. There is no experimental evidence for this view, nor for that of Ringer (*J. Biol. Chem.* **17**, 107 (1914)) who assumes a hypothetical combination of glucose and β-hydroxy-butyric acid in order that the latter may be oxidized. Shaffer (*J. Biol. Chem.* **54**, 399 (1922)), on the basis of experiments on the ratio of antiketogenetic substances to ketogenetic substances required to prevent acetonuria in cases exhibiting it, has arrived at the conclusion that two molecules of aceto-acetic acid enter into reaction with one of glucose. Shaffer, however, makes assumptions for which there is no sufficient justification.

The author (*J. Phys. Chem.* **29**, 376, 799 (1925)) has suggested the following explanation:—Under normal conditions the heat and energy of the body are derived from the simultaneous combustion of carbohydrates, proteins, and fats. When, as a result of special conditions carbohydrate is not burning, more fat and protein must be burnt in order to maintain the body temperature at its normal level. Hence, in unit time more fat would be burning than under normal conditions, and combustion would be incomplete, so that acetone bodies would be formed. It has been observed that wherever there is rapid combustion of fat, as for instance, in hyperthyroidism, there is elimination of acetone bodies. The same is the case *in vitro* too. Chakravarti and Dhar (*J. Indian. Chem. Soc.* **6**, 617 (1929)) have observed that in the rapid oxidation of fatty acids by hydrogen peroxide and ferric salts there is the formation of acetone bodies. They have also observed that if the rate of oxidation of fats be slowed down, as is the case when carbohydrates (e.g. glucose) are present, the amount of acetone bodies formed diminishes. Palit and Dhar (*J. Phys. Chem.* **34**, 710 (1930)) further find that, in the slow and induced oxidation of fats, acetone bodies are not formed at all, but only carbon dioxide and water, the products of normal metabolism.

It is evident, therefore, that acetone bodies are not products of normal metabolism, but result from abnormal conditions. The author has suggested that under normal conditions, fats are mainly burned in the animal body directly to carbon dioxide and water, without the formation of intermediate products. An objection may be raised as to how a fatty acid containing 18 carbon atoms can be directly oxidized to carbon dioxide and water without the formation of intermediate products. Many physiologists are of opinion that aceto-acetic acid, with four carbon atoms in its molecule, undergoes complete oxidation to carbon dioxide and water. If a molecule with four carbon atoms can be directly oxidized, then the direct oxidation of more complex acids is not impossible.

SLOW AND INDUCED OXIDATION OF FATTY ACIDS AND FATS.

The experimental procedure was the same as that described in connection with the oxidation of carbohydrates. Potassium and sodium salts of fatty acids, and even butter, have been oxidized to carbon dioxide and water by air in presence of inductors like sodium sulphite, colloidal phosphorus, and freshly precipitated cerous, manganous, ferrous, uranous, and other hydroxides. Whilst oxidation takes place to an appreciable extent in neutral solution, it has been shown that alkalinity increases the amount of oxidation. Indeed, the greater the amount of alkali present the more carbohydrate is oxidized. Oxidation takes place even in the absence of inductors, if alkali is present. In the case of aqueous solutions of sodium and potassium salts of fatty acids, the small amount of alkali derived from hydrolysis is sufficient to cause oxidation. The following table shows the experimental results with cerous hydroxide as inductor (compare Palit and Dhar. *J. Phys. Chem.* **32**, 1663 (1928); **34**, 710 (1930); *Z. anorg. u. allg. Chem.* **191**, 150 (1930)).

Volume of air passed 75 litres in 11 hours.

Substance used	Percentage oxidation as determined by carbon dioxide estimation	Percentage oxidation as found by direct estimation
Potassium oleate	7·4	10
Lecithin	7·8	8
Cholesterol	14·3	14·8
Glucose	12·0	12·6
Potassium stearate	—	68·9
Butter	—	29·9

No intermediate products have been detected, and the liberated carbon dioxide accounts for practically the whole of the substance disappeared.

COMPARATIVE EXPERIMENTS ON THE OXIDATION OF PROTEINS, CARBOHYDRATES, AND FATS.

Inductor—cerous hydroxide; Volume of air passed 60 litres in 9 hours.

Substance	Percentage amount of substance oxidized
Egg-white	83·3
Egg-yolk	69·4
Starch	57·2
Glucose	36·5
Butter	29·9

These substances fall into the following order according to their ease of oxidation:—

Egg-white > Egg-yolk > Starch > Glucose > Butter.

In other words, proteins are more readily burned than carbohydrates, which in their turn are burned with greater ease than fats. This order is the same as that given by Voit, who stated that "the metabolism in the body is not proportional to the combustibility of the substances outside the body; but protein which burns with difficulty outside, metabolizes with the greatest ease, then carbohydrates, while fat which readily burns outside is the most difficultly combustible in the body". This conclusion was arrived at by Voit from actual feeding experiments.

These results show with what great success biological oxidations have been imitated outside the body.

INDUCED OXIDATION OF GLYCEROL.

Volume of air passed was 66·5 litres in 10 hours, and the amount of glycerol taken was 0·125 grm. p_H before experiment = 7·5.

Substance used as inductor	Amount of inductor used in grm.	Percentage oxidation of glycerol as found by carbon dioxide estimation	Percentage oxidation of glycerol as found by direct estimation	p_H after the experiment
Ferrous hydroxide .	0·06476	11·8	11·6	6·8
Cerous hydroxide .	0·1069	6·7	6·6	7·0
Sodium sulphite . .	0·2000	4·03	4·8	7·3

In the case of sodium sulphite, the amount of oxidation as shown by estimation of carbon dioxide was a little lower than by direct estimation; and this low value is due to the absorption of carbon dioxide by the alkali set free by hydrolysis of sodium sulphite. Exactly similar results were obtained in other cases in the presence of sodium sulphite as inductor.

CHAPTER V

Oxidation of Mixtures of Carbohydrates, Fats, and Proteins.

Dhar (*Proc. k. Akad. Wet. Amsterdam*, **29**, 1023 (1921); *Z. anorg. Chem.* **144**, 289 (1925); also compare Moureu and Dufraisse *Compt. rend.* **179**, 237 (1924)) has observed that in the presence of sodium arsenite, which becomes oxidized when added to sodium sulphite, the velocity of the oxidation of sodium sulphite by air is markedly retarded. Similarly, the presence of carbohydrates, which are themselves oxidized when mixed with sodium sulphite, ferrous hydroxide, or cerous hydroxide, markedly diminishes the velocity of oxidation of the last-named substances. Moreover, we have arrived at the generalization that in oxidation reactions the phenomenon of negative catalysis is observed when the catalyst is a reducing agent.

Palit and Dhar (*J. Phys. Chem.* **32**, 1663 (1928); **34**, 710 (1930)) have carried out experiments on the induced oxidation of mixtures of carbohydrates, fats, and proteins with each other, and have arrived at the important conclusion that the presence of any one of these substances which is itself undergoing oxidation, retards the oxidation of the other. A few of the experimental results are given below to illustrate this point of view:—

Oxidation of potassium stearate.
Inductor—ferrous hydroxide.

In the absence of any retarding agent the percentage oxidation of the stearate is 63·8.

Carbohydrate used	Percentage oxidation of stearate in the presence of carbohydrate.	Percentage oxidation in the presence of urea and the corresponding carbohydrate
Arabinose . . .	58·6	56·9
Galactose . . .	56·9	55·2
Glucose	51·7	41·3
Laevulose . . .	50·0	44·8
Lactose	48·2	39·5
Cane sugar . .	53·4	39·5
Maltose	50·0	43·1
Starch	58·6	43·1

The above results show that in the presence of both urea and carbohydrate there is greater retardation than in the presence of carbohydrate alone. Similar results are obtained with potassium oleate and palmitate.

In exactly the same way the presence of fats retards the oxidation of carbohydrates. (Compare Palit and Dhar, *J. Phys. Chem.* **32**, 1676 (1928)).

Carbohydrate	Percentage oxidation with no retarding agent	Percentage oxidation with stearate as retarding agent
Starch	49·0	41·3
Maltose	31·0	21·5
Lactose	16·5	14·4
Laevulose . .	17·0	11·5
Arabinose . . .	4·0	3·5
Cane sugar . .	21·0	10·5
Galactose . . .	12·0	10·0

Now in normal health the heat and energy of the body are supplied to the system from the simultaneous combustion of carbohydrates, fats, and proteins. The experimental results conclusively prove that the oxidation of fat is retarded by carbohydrates, and perhaps less powerfully by proteins . . . It seems fairly certain now that the presence of either one or both of the above substances which are undergoing oxidation, retards the oxidation of the third. It is therefore evident that in the presence of a large excess of carbohydrate and fat, little protein should be burnt. The protein-sparing qualities of carbohydrates and fats were discovered from feeding experiments by some of the earliest students of metabolism, and it is believed that carbohydrate is the more efficient of the two in sparing proteins. It has also been proved by Lusk, Landegren, and others (Compare Lusk: *Science of Nutrition*, p. 269 (1919)) that the withdrawal of carbohydrates from the food increases the protein metabolism. Moreover, in diabetes, where the glucose passes out unoxidized, there is considerable waste of tissue protein. The experimental results of Palit and Dhar, recorded above for the first time, provide a scientific explanation of these empirical observations of physiologists.

Moreover, on the basis of the above results, a satisfactory explanation can be given of the onset of glycosuria, following the intake of phosphorus, arsenic, phlorhizin, or ether. All these substances are marked reducing agents, and therefore in accordance with the general

principle laid down in the opening paragraph of this section, they produce retardation of the oxidation of glucose in the animal body. Hence some of the glucose escapes unoxidized in the urine, and more protein should be metabolized in order to keep up the normal supply of body heat and energy. In this connection it is interesting to note that Ray, McDermott, and Lusk (*Amer. J. Physiol.* **3**, 139 (1899)) have found that phosphorus injections raise the protein metabolism of fasting dogs to 250, 260, 283, 248, 183, and 164 per cent of normal. They have contrasted this increased protein metabolism with that obtained in phlorhizin glycosuria, which is represented by increases of 540, 450, and 340 per cent. Moreover, Allen and Du Bois (*Arch. Intern. Med.* **17**, 1010 (1916)) find indications that the administration of whisky favours the production of glucose in a diabetic.

METABOLISM IN DIABETES

Glycosuria. — Diabetes is the condition in which the organism fails to utilize one of the principal food-stuffs — glucose, an intermediate product not only of carbohydrate, but in certain conditions of protein and fat diet as well. The diabetic organism is apparently able to utilize a certain quantity of glucose as well as the non-diabetic; but when the lower limits of utilization have been exceeded, the percentage of blood sugar rises, and, having reached a certain value, is excreted in the urine. In severe forms of diabetes, all the ingested glucose may be quantitatively eliminated in the urine.

It is interesting to note that galactose is utilized more than fructose, and fructose still more than glucose by the diabetic; this is the reverse of the order obtaining in normal health. The fact that fructose is assimilated with greater readiness in the animal economy than dextrose has led to the employment of its polysaccharide, inulin (which occurs in the Jerusalem artichokes, black salsify, and artichokes) as an article of food for diabetics; as inulin yields fructose on hydrolysis in the body, Straus of Berlin (*Berl. Klin. Woch.* 1912 (1912)) strongly advocates inulin treatment.

Acetonuria. — In normal health, the heat and energy of the body are derived from the simultaneous combustion of carbohydrates, fats, and proteins. More than half the number of calories are derived from the easily burnt carbohydrate. It seems evident, therefore, that if glucose (i. e. carbohydrate) escapes unburnt in the urine of the diabetic, the heat loss must be made up from the other classes of food-stuffs, principally the fats with their nine calories per gram

as against the four calories of protein. There will thus be considerable waste of tissue fat and protein, especially of the former.

From the foregoing, it will be seen that fat should be burnt at a greater speed in the diabetic than in the normal healthy individual. The author believes that this rapid oxidation of fats yields not carbon dioxide and water, but the intermediary products β-hydroxybutyric acid and aceto-acetic acid, the latter splitting off carbon dioxide to yield acetone. These are excreted in the urine. Apparently, the easily oxidizable carbohydrates, which act as negative catalysts in the oxidation of fats, are necessary for the complete combustion of the latter.

If the acetone bodies are to be ascribed to the rapid but incomplete combustion of fats which is shown to take place in the diabetic, it follows that, not only in diabetic but also in all other conditions where there is rapid combustion of fats and little oxidation of carbohydrates, there should be marked acidosis and acetonuria. Indeed, these conclusions are confirmed by experiments on human beings and animals carried out by numerous physiologists. Thus J. Bär (*Arch. f. exp. Pathol.* **54**, 153 (1905)) has reported that a simple withdrawal of carbohydrates is followed in normal human beings accustomed to a mixed diet, and also in apes, by an acidosis, i. e. excretion of oxybutyric acid, aceto-acetic acid, and acetone, with coincident increase in ammonia elimination. Deprivation of carbohydrate increases the body's need of fat, and this flooding of the metabolic mill with fat results in its incomplete oxidation with the consequent production of acetone bodies. In starvation, or as a result of continued vomiting, acetonuria is observed after some days from the start, when most of the body store of glycogen has been utilized. In all such cases, where acetonuria sets in, as a result of simple carbohydrate deficiency in the food, the administration of carbohydrates causes the disappearance of ketosis. In hyperthyroidism, too, where there is rapid oxidation of fat, glycosuria and sometimes actual manifestations of diabetes have been observed.

If the oxidation of fats can be retarded somehow, then in diabetes there should be marked decrease in the elimination of acetone bodies. Thus xylose, gluconic acid, citric acid, propionic acid, glutamic acid, glutaric acid, etc. have been found to diminish the formation of acetone bodies in diabetes. All these substances are utilized by the diabetic. Hence smaller amounts of fat need to be burnt. The author is of opinion that all these reducing substances, which are themselves oxidized, act as negative catalysts in the oxidation of fats, thus

favouring slow but complete oxidation of the latter with the resulting decrease of acetone bodies.

Proteins also exert antiketogenetic properties, although to a slight extent. Very little carbohydrate and a great deal of fat are taken by the Eskimos; but they eat an abnormal amount of protein; in their case the proteins act like carbohydrates in retarding the oxidation of fats, and thus assist their complete oxidation.

ACIDOSIS AND DIABETIC COMA

As is well known, diabetic coma, a symptom complex clearly described many years ago by Kussmaul, has been recognized, especially from the investigations of Stadelmann and the Naunyn School, as an acid intoxication caused by the accumulation of acetone bodies in the system. Magnus-Levy, who took a prominent part in these investigations, showed that very large amounts (twenty to thirty grams in the course of twenty-four hours) of butyric acid appear in the urine in all severe cases of diabetes, even in the absence of coma. In cases of coma, the formation of this acid rises to an abnormal level. Under such circumstances enormous quantities, as much as 160 grs. of butyric acid in twenty-four hours, appear in the urine. In the body of a subject dead from diabetic coma, two hundred grams of the acid were found. The coma is regarded as an acid intoxication, in which all the individual phenomena are dependent directly or indirectly on the accumulation of the acid. As the supply of alkali, which the system keeps ready for the neutralization of the acids, is not large, these acids are for the most part neutralized by ammonia which is thus withdrawn from urea formation. In these cases, therefore, the urinary ammoniacal elimination serves as an approximate index of the acid excretion.

Magnus-Levy observes that the diabetic does not die in coma because of the neutralized acid which is eliminated in the urine, but rather on account of that which is retained in the body; the acid thus retained neutralizes the alkalis of the tissues and of the body fluids.

ALKALINE TREATMENT OF DIABETIC COMA.

In the opinion of most physiologists the lowering of the alkalinity of the blood and tissues should be anticipated by a prophylactic alkaline treatment. Umber recommends the administration of sodium bicarbonate even up to 200 grs., in those cases where acetoacetic

acid in the urine can be recognized by the ferric chloride reaction. Umber further states: "There is no question as to our ability to save severe cases of diabetes by alkaline treatment in the face of an acidosis of an year's duration, although without this treatment this would be a matter of impossibility." C. von Noorden also believes strongly in the efficacy of alkaline treatment, and is of opinion that there is no other effective remedy.

Apart from neutralizing the acids generated in the diabetic, alkali, according to the writer, has another and perhaps more important effect. From experiments on the induced oxidation of carbohydrates, fats, and nitrogenous substances carried out in the author's laboratories, it has been repeatedly and consistently observed that alkalis increase the oxidation of the above substances. Therefore there appears to be no doubt that alkali treatment increases the metabolism of glucose in the diabetic, with concomitant decrease in acid production. Murlin and Kramer (*J. Biol. Chem.* **27**, 517 (1916)) have obtained results which indicate that if depancreatized dogs be given alkali, they are able to oxidize some glucose. Small quantities of iron which also increase metabolism have likewise been found to be efficacious in diabetes.

The author has recommended alkaline treatment, not only in diabetes, but in all other cases of metabolism diseases like gout, beriberi, and rickets.

HIGH FAT DIET IN DIABETES.

The fundamental defect of the diabetic is his inability to utilize one of the principal food-stuffs, carbohydrate. Consequently the energy and heat of his body are derived from the combustion of fats and proteins, principally the fats with their nine calories per gram as against the four calories per gram of the proteins. Thus a considerable waste of tissue fat and protein is noticed in diabetes. To prevent this, Newburgh and Marsh (*Arch. Intern. Med.* **26**, *December* **1920**) recommend the ingestion of large quantities of fat. The high fat content of the diet gives a total number of calories which it would be difficult to obtain with carbohydrates in quantities not sufficient to cause glycosuria, or with protein; and it is asserted by the above authors that in the case of some of the younger patients the capacity for work under this treatment was really astonishing. In India certain old fashioned physicians actually utilize the "High fat diet" in the treatment of diabetes. (Also, compare Maignon, *Compt. rend. Soc. Biol. Jan.* 21 (1922)).

There are some dangers following this treatment based on "High fat diet". In such cases as these, there would be a rapid combustion

of fat which would lead to the production of β-hydroxybutyric acid, acetoacetic acid, acetone, etc. Thus acidosis would set in and lead to diabetic coma. Maignon in France (*loc. cit.*) also proposes a diet rich in fat and oil, and to guard against acidosis gives sodium bicarbonate concurrently. However, this treatment still seems unsatisfactory; and the author would recommend attempts at effecting the cure of the fundamental defect, namely, the inability to metabolize carbohydrates. In some cases iron preparations, alkalis, and substances rich in vitamin B have proved of great value.

THE RÔLE OF PANCREAS AND INSULIN IN CARBOHYDRATE METABOLISM AND DIABETES.

Till the year 1889 nothing definite was known about the connection of the pancreas with carbohydrate metabolism. In that year there were published the results of the memorable investigation of Minkowski who worked in the laboratory of Prof. von Mering. Minkowski successfully removed the pancreas from a dog and observed that the animal passed a high proportion of sugar in the urine after the operation. Simultaneously and independently, an Italian worker, De Dominicis, made a similar observation. The work of these two independent investigators has thus clearly revealed the dependence of carbohydrate metabolism on the pancreas. It is natural, therefore, to presume that diabetes is due to a derangement of the pancreas, and its failure to secrete a hormone which is essential for the combustion of sugar. It is not proposed to give here an historical account of the investigations in this field which culminated in the isolation, in 1922, by Banting and Best, of a pancreatic hormone, which has been shown to control sugar metabolism. An excellent historical survey of the subject is given by Dale, in *Certain Aspects of Biochemistry* (University of London Press, 1926).

Of recent years, a vast literature has grown around the subject of insulin, the internal secretion of the pancreas. Attention has been directed mainly along two channels; on the one hand, the centres of production and distribution of insulin; on the other, the fundamental action of insulin in promoting sugar metabolism. With regard to the first question, we can conclude, from the existing evidence, that apart from its exclusive production and store in the pancreatic islets, insulin is normally circulating in the body and deposited in other organs. With regard to the second aspect of the question the evidence is more varied and conflicting. The preparation of Banting and Best gives the following results in diabetes:—

(1) Blood-sugar is markedly reduced, even to normal value.
(2) Glycosuria is abolished.
(3) Acetone bodies are made to disappear from the urine.
(4) The "Respiratory quotient" gives evidence of increased utilization of carbohydrates.
(5) Definite improvement is observed in the general condition of the patients, and in addition the patients themselves report a subjective sense of well-being, and increased vigour for a period following the administration of the preparation.

The author advanced the opinion five years ago (Dhar. *Chemie der Zelle und Gewebe* **12**, 217 (1925); *J. Phys. Chem.* **29**, 376 (1925)) that insulin acts as a promoter mainly of the oxidation of glucose. As has already been stated above, the diabetic patients feel a sense of well-being, and this can only arise when there is increased oxidation of sugar, which in its turn leads to a complete oxidation of the fats, thus supplying the body with an extra amount of energy. In this connection it must be noted that a similar sense of well-being is felt on the inhalation of small quantities of oxygen, especially on the tops of mountains. This has been repeatedly observed in the Mount Everest expeditions. An overdose of oxygen just like an overdose of insulin produces violent symptoms. This is corroborated by the following observations of Professor L. Hill (*Nature* **125**, 415 (1930)):—"Experiments have shown that animals can safely breathe two atmospheres of oxygen for two hours, but during this period both the use of oxygen by the body and the output of carbonic acid diminish, the body temperature drops, and the animals become drowsy. If the exposure is more prolonged, or in shorter times if the oxygen tension is higher, the animals fall into a comatose condition and finally have convulsions."

Direct evidence in favour of an increased oxidation of sugar is furnished by the experiments of Hepburn and Latchford (*Amer. J. Physiol.* Sept. 1 (1922)):—The average consumption of sugar by the isolated rabbit heart, perfused with Locke's solution, was found to be 0·87 mgm. per hour. When insulin of proved potency was added to the perfusion fluid, the average consumption of sugar rose to 3·06 mgms. per hour.

Some unpublished experiments carried out in the author's laboratory indicate that insulin acting as an inductor can also promote the slow and induced oxidation of sugar *in vitro*. Further work is in progress.

The disappearance of acetone bodies from the urine of the diabetic on the injection of insulin has been explained by the author as follows:— When insulin is injected, the glucose in the system is burnt; and this oxidation of glucose inhibits the oxidation of fats. Moreover, it has been pointed out in the previous pages that the complete oxidation of fats to carbon dioxide and water is only possible when the oxidation is slow. On the other hand, if the oxidation of fats takes place rapidly, as in the case of the diabetic, β-hydroxy-butyric acid, aceto-acetic acid, acetone, etc. are likely to be formed. Hence it appears that the complete oxidation of fats and the consequent disappearance of acetone bodies is not due to any direct action of insulin on the metabolism of fats; but is only a necessary consequence of the increased oxidation of sugar brought about by insulin.

Best, Hoet, and Marks (*Proc. Roy. Soc.* (B), **100**, 52 (1926)) conclude that the immediate effects of insulin in the diabetic are an accelerated combustion of sugar, and the synthesis of a further quantity of glycogen. In order to make a balance sheet of the glycogen-sugar system in the insulin-treated animal, Best, Dale, Hoet, and Marks (*Proc. Roy. Soc.* (B), **100**, 55 (1926)) were able to show that the reducing sugar, which disappears from an eviscerated spinal preparation under the action of the hormone, is equal to the sum of the glycogen deposited in the muscles and the dextrose equivalent of the oxygen absorbed. The balance is preserved, whether the blood-sugar is maintained at a high level by the infusion of dextrose, or allowed to fall to a low level by restricting the supply. Similar conclusions have been reached by Bessinger and Lesser (*Biochem. Z.* **168**, 398 (1926)).

So it appears that in a diabetic, the injection of insulin causes some of the sugar to be stored as glycogen, while most of it is oxidized.

It is now generally recognized that insulin is a powerful drug, and that an overdose of it produces violent symptoms. The patient feels hungry, flushes, and grows pale, sweats profusely, becomes tremulous and unsteady, and suffers from impairment of vision. In some cases unconsciousness and convulsions are produced. Whatever their exact meaning and origin, there can be no doubt that these symptoms are conditioned by the fall of blood-sugar, since they are promptly removed by introducing more glucose. In the rabbit this is usually done by injection, while in human beings it is usually sufficient to give sugar by the mouth; orange juice has an effect similar to that of glucose in relieving the violent symptoms produced by an overdose of insulin.

It is interesting to note that this action of insulin is more prolonged and profound in cases where the thyroid has been removed. Conversely, the effect of administering thyroid substance is to increase greatly the resistance to insulin. These results are easily explained on the view that thyroid secretion is responsible for fat metabolism. In the absence of thyroid there is little oxidation of fat. When fat is being metabolized in the body, the oxidation of carbohydrate is retarded. From this it will be seen that in the cases of thyroid removal there will be rapid oxidation of glucose; added to this is the accelerating effect of insulin on glucose oxidation, if insulin is injected. Therefore, there will be a greater lowering of blood sugar in cases of thyroid removal than in cases where the thyroid is functioning normally. Hence the greater violence of symptoms following an overdose of insulin.

It is interesting to note that "thyroid protein is differentiated from other proteins in an obvious manner by its iodine content; and the specific constituent being a simple amino-acid, can be readily separated from the rest of the molecule; the insulin protein bears no such chemical earmark, unless it be the high content of sulphur" (Harington and Scott. *Bioch. J.* **23**, 384 (1929)). What seems to be a peculiarity of insulin protein has been stated by Bertrand and Mâcheboeuf (*Compt. rend.* **182**, 1305, 1506; **183**, 5, 257, 326 (1926)). They have shown that in comparison with other organs, the pancreas of all the species investigated contained relatively large quantities of nickel and cobalt salts, and that preparations of insulin contain these elements. Injection of small doses of nickel and cobalt salts intensified the insulin effect in rabbits and dogs, and it is now claimed that injection or oral administration of small amounts of nickel and cobalt salts in human cases of diabetes has sometimes been followed by an alleviation of symptoms. This work is interesting and is yet to be confirmed by others.

The work of Abel (*Proc. Nat. Acad. Sci.* **12**, 132 (1926)), Abel, Geiling, and others (*J. Pharm. Exp. Therm.* **31**, 65 (1927)), and Harington and Scott (*Biochem. J.* **23**, 384 (1929)) on crystalline insulin seems to show that it is the true active principle of the gland in so far as the function of the latter in relation to carbohydrate metabolism is concerned. In view of this, the claims of Funk (*Science* **65**, 39 (1927)) need confirmation. This investigator states that insulin may be fractionated to yield insulin A and insulin B. The former, which is present in larger proportion, decreases the blood-sugar of normal rabbits, and animals having high initial blood-sugars. On the other hand, insulin B is said to increase the blood-sugar, and to cause dilution of the blood with arge retention of water.

SIMILARITY BETWEEN THE FUNCTIONS OF VITAMINS AND INTERNAL SECRETIONS.

General considerations.—The internal secretions are fundamentally concerned in the growth process of the organism; the vitamins are also essential for growth, reproduction, and well-being of organized beings. The lack of internal secretions, as well as of vitamins, produces various diseases which finally result in death. To give an example, the absence of the pancreatic secretion, insulin, leads to diabetes; even pernicious anæmia is, of recent years, being attributed to the lack of an internal secretion of the liver. Admirable cures of pernicious anæmia have been effected in innumerable cases by liver preparations (compare Minot and Murphy. *J. Amer. Med. Assoc.* **87**, 470 (1926); **89**, 759 (1927); Cohn, Minot, Alles and Salter. *J. Biol. Chem.* **77**, 325 (1928)). Similarly, it is well known that lack of vitamin B leads to beriberi, that of vitamin D to rickets and so on. Again vitamins, like the hormones, produce their effects in the most minute quantities.

Overdoses of internal secretions produce violent symptoms. To give a few examples, it is well known that on administering an overdose of insulin, the patient feels hungry, flushes, and grows pale, sweats profusely, becomes tremulous and unsteady, and suffers from impairment of vision. In some cases unconsciousness and convulsions are produced, which may lead to death in case of neglect. It is also established now that the continued administration of large doses of thyroxin in man is attended with rapid, irregular pulse, flushing of the skin with feeling of heat, increase of perspiration, loss of weight, nervous excitability, greatly increased metabolic rate, and nitrogen loss. Similar violent symptoms follow the administration of overdoses of other internal secretions. Several years ago, the author (*Chemie der Zelle und Gewebe* **12**, 217 (1925)) put forward the view that the function of vitamins and internal secretions are similar. If this similarity is pushed further, overdoses of vitamins should also prove dangerous. It is very gratifying to the author to note that evidence is accumulating to substantiate his views.

Hypervitaminosis.—It is now recognized that excessive doses of irradiated ergosterol, or other preparations rich in vitamin D, may exercise a very damaging influence on the growing animal. Attention was first directed to this problem by Pfannensteil (*Klin. Woch.* **6**, 2310 (1927)) and Kreitmair (*Münch. med. Woch.* **75**, 637 (1928)). Harris and Moore (*Bioch. J.* **22**, 1461 (1928)) find that young rats lose weight and die on a synthetic diet containing 0·1% of irradiated ergosterol. Some indication was obtained that a considerable increase in the

allowance of vitamins B and C served to alleviate the condition. Dhar (*Chemie der Zelle und Gewebe* **13**, 119 (1926)) has shown that vitamin D accelerates the oxidation of fats, and vitamins B and C act as promoters of the oxidation of carbohydrates. Hence in presence of an overdose of vitamin D, fats will be very rapidly oxidized, causing serious trouble in the animal body; but if there is also a large increase of vitamins B and C, carbohydrates also will be burnt very readily. In the foregoing pages, it has been emphasized that when carbohydrates are readily oxidized, the oxidation of fats is greatly retarded. Hence a considerable increase in the allowance of vitamins B and C will nullify the effect of an overdose of vitamin D, and thus hypervitaminosis will be avoided. Similar alleviation of symptoms arising from an overdose of insulin is brought about by injecting adrenalin secretions. Dhar (*Chemie der Zelle und Gewebe*, **13**, 119 (1926)) has advanced the view that insulin accelerates the oxidation of glucose, and adrenalin secretions promote the oxidation of fats. In an overdose of insulin, glucose will be very readily burnt, causing violent symptoms in the animal body; but if adrenalin secretion is administered at this stage, fats will also be burnt readily. Now the oxidation of fats also retards the oxidation of carbohydrates. Hence the violent symptoms due to an overdose of insulin can be decreased by administering thyroid, or adrenalin, or vitamins A and D, which accelerate the oxidation of fats. Further, Harris and Stewart (*Bioch. J.* **23**, 206 (1929)) find that a synthetic diet containing 0·1% of irradiated ergosterol produces a marked rise in the inorganic phosphate of the blood, and a considerable rise in the serum calcium. The former effect is eventually obtained even in the adult animals, although in these the serum calcium does not increase. A general post-mortem finding as a result of "hypervitaminosis" is the wide-spread occurrence of abnormal deposits of calcium throughout the body, particularly in the vascular system, spleen, kidney, liver, and lungs. Collazo, Rubino, and Varela (*Bioch. Z.* **204**, 347 (1929), and Holtz and Brand (*Z. physiol. Chem.* **181**, 227 (1929)) have obtained similar results on administering excessive doses of "vigantol", a potent preparation of vitamin D. Other dietetic factors considerably influence the results.

It is probable that excessive doses of other vitamins will also produce specific ill effects.

PANCREATIC SECRETION AND VITAMIN B.

Some years ago the author advanced the view that insulin acts as a promoter in the oxidation of carbohydrate food materials. On receiving

an injection of insulin the diabetic patient feels a sense of well-being, and this can only arise when there is increased metabolism of sugar, and indirectly of fat, thus furnishing the body with an extra amount of energy.

It was also stated that vitamin B is most likely a promoter of the oxidation of carbohydrate food materials; consequently the view was advanced that diabetes should be treated with food materials rich in vitamin B. It is held, also, that beri-beri is mainly caused by the lack of proper metabolism of carbohydrate food materials, due to the absence of the promoter, vitamin B. It is now well known that pancreatic secretion helps greatly the combustion of sugar in the animal body. Consequently the part played by vitamin B is more or less identical with that played by the pancreatic secretion. The author ventures to suggest that cases of beri-beri should also be treated with very small doses of insulin. Food materials rich in vitamin B have been found to be efficacious in the treatment of diabetes.

VITAMINS A AND D AND THYROID SECRETION.

The author (Dhar. *Chemie der Zelle und Gewebe* **13**, 119 (1926)) has repeatedly emphasized the fact that vitamins A and D, associated with fatty food materials like butter, cod-liver oil etc., act as promoters of the oxidation of fats by oxygen. It is also well known that many diseases are now definitely associated with lack of vitamins A and D. Thus rickets, osteomalacia, and various forms of eye-troubles are caused by the want of vitamins A and D. Moreover, growth is definitely influenced by the vitamins A and D.

It will now be shown that the internal secretion of thyroid plays practically the same rôle as that of vitamins A and D. There is strong tendency in hypothyroidism to deposit fat, and as a rule obesity follows; in such cases thyroid feeding is of great value. Halsey (*Endocrinology and Metabolism*, Vol. I page 102 (1922)) remarks "It is my belief that in any case of obesity not responding to diet, purgation, and exercise, it is well to make a trial of thyroid feeding, while continuing the same regime." From this it seems fairly certain that thyroid acts as a promoter of the metabolism of fatty food materials.

In the lack of development and growth in children, both mental and physical, thyroid is of general value. Thyroid is useful in diseases due to low general nutrition of all tissues, including the bones, such as osteomalacia, rickets, etc.

Constipation has been shown to be a characteristic of hypothyroidism, and thyroid is of marked benefit in constipation. It is also well

known that for avoiding constipation butter fat, which contains vitamins A and D, should be given.

This similarity between the functions of vitamins A and D and of thyroid secretion was put forward by the author several years ago; and it is gratifying to learn that keratomalacia, night blindness, and some other eye-troubles, associated with the lack of vitamins A and D, are now being treated by thyroid secretion. The author further suggests that rheumatism, senility, toxemia, haemorrhage, skin diseases, and several infectious diseases which are now treated by thyroid, should also be treated by the intake of food materials rich in vitamins A and D.

OTHER INTERNAL SECRETIONS.

From a survey of the existing literature on internal secretions, the author (Dhar. *Chemie der Zelle und Gewebe* **13**, 119 (1926)) has suggested that one of the functions of adrenal secretions is also to act as a promoter of the oxidation of fatty food materials. The effect of epinephrin, the active principle of adrenal glands, in the quantity in which it is used in pharmacological investigation, is definitely to raise metabolic rate and body temperature, possibly owing to the increased oxidation of fatty materials.

On the other hand, the function of pituitary secretions is mainly to increase the protein metabolism by acting as promoter; moreover the secretions from gonads also act as promoters of the oxidation of fat.

It is well-known that large doses of thyroxin or epinephrin injected into the circulatory system produce hyperglycemia and glycosuria.

Hence it appears that the hyperfunction of the thyroid, adrenals, and pituitary leads to increased metabolism but lowered carbohydrate tolerance.

The author (*Chemie der Zelle und Gewebe* **13**, 119 (1926)) has explained the above facts by the following considerations:—

(1) From our experiments on induced oxidation we have demonstrated that the oxidation of substances like sodium sulphite, ferrous hydroxide, etc. by air or oxygen is greatly retarded by the presence of sugars, sodium arsenite, etc. which, in their turn, are oxidized in presence of sodium sulphite, ferrous hydroxide etc. Similarly, we have proved that the oxidation of fats is retarded by carbohydrates and *vice versa*.

(2) It is well-known that the heat and energy of the animal body are supplied by the oxidation of fats, carbohydrates, and proteins. In

normal health, and in normal conditions, when a mixed diet is taken, definite proportions of the various substances are oxidized for the supply of heat and energy. In persons who have hyper-functioning thyroids, or when thyroxin is injected, the fatty food material is burnt very rapidly, the oxidation of proteins seems to be also accelerated, and this combustion mainly supplies the necessary heat to the body. Now, because the necessary heat and energy for the maintenance of the body temperature and the functions are supplied by the combustion of fats, and protein to a smaller extent, in presence of the promoter thyroxin; the other food product, namely carbohydrate, need not be oxidized. Consequently the hyper-function of the thyroid will lead to slight oxidation of carbohydrates, which will be eliminated, and glycosuria will result. An exactly similar explanation can be given of the production of glycosuria due to hyper-function of the adrenals. In the case of adrenal secretions, we assume that the oxidation of fat is also accelerated by the promoter action of this secretion, and consequently in this case also the main store of energy for the body comes from the oxidation of fat, and so the glucose is not oxidized and glycosuria results.

With pituitary the case is different; for we assume that in presence of this secretion it is mainly the oxidation of protein matter which is increased; and consequently the energy supply comes from this oxidation, leaving glucose and fats unoxidized. From this point of view it will be clear that the function of the pancreas secretion, which mainly accelerates the oxidation of glucose, need not be considered antagonistic to that of the thyroid, pituitary, or adrenals. We assume that the matter merely rests on the preferential oxidation of one variety of food material due to the presence of the promoter. In hyper-functioning of the pancreas the glucose will be readily oxidized, and will supply the energy. Hence, according to our point of view, the pancreas need not inhibit the action of the thyroid, or the adrenals; moreover, the adrenal or thyroid need not inhibit the action of the pancreas. We are strongly of the opinion that in normal health pancreas, thyroid, adrenal, and pituitary perform their proper function by promoting the oxidation of carbohydrates, fats, and proteins in the proper proportions. Consequently a combination of the respective hormones, especially thyroid and pituitary, should yield better results in many diseases, than either singly.

Injection of a solution of post-pituitary causes a lowering of carbohydrate tolerance, and glycosuria results. It is interesting to observe that in the experiments conducted to ascertain the effect of oral ad-

ministration of the pituitary on the nitrogen, sugar, and alkaline reserve of the body, Hammett, Patten, and Suitsu (*Amer. J. Physiol.* **51**, 588 (1920)) found the only consistent change to be an increase of uric acid in the blood.

Injection of testicular extracts was described by Brown-Sequard as resulting in a feeling of increased vigour and general well-being, and similar results have since been recorded by other workers after careful experimental observation.

Biedl (*Intern. Secretory Organs*, pp. 386, 396 (1913)) believes that the increased metabolism resulting from administration of ovarian and orchic substance is due to the consumption of non-protein matter, as it has been shown that protein metabolism is not affected by the gonads. The author has advanced the opinion that this increased metabolism is possibly due to the increased oxidation of fats by the promoter action of the internal secretions from the sex glands. The internal secretions are fundamentally concerned in the growth process of the organism; the vitamins, especially vitamin A, are also essential for growth, reproduction, and well-being. Like hormones, vitamins produce their effects in the most minute quantities. Moreover, vitamins themselves are apparently only synthesized by plants, and the animal organism is unable to produce them from simpler materials. In this connection the important observations made regarding the action of light on the development of animals and human beings, and on calcium deposition during growth, are of interest.

CHAPTER VI

Actino-therapy.

Finsen may be looked upon as the father of modern light-therapy. He began his pioneering work in 1893, and achieved great success in the treatment of lupus, a tubercular skin disease very difficult to cure. Over 1100, out of about 1200 cases, were greatly improved as a result of light treatment. The next important step in light-therapy was taken in 1903, when Rollier, who has been described as the "High Priest" of modern sun-worshippers, established a sanatorium at Leysin, in Switzerland, for the treatment of tuberculosis by sunshine. Great success was obtained, especially with surgical tuberculosis, and since then light-therapy has become an important feature of medical practice. Both Finsen and Rollier attributed their success in the treatment of diseases to the ultraviolet portion of the solar spectrum. Light-therapy has been more successful in the high Alps than elsewhere, not only because of the large amount of ultraviolet radiation available, but also because the snow absorbs heat rays and reflects ultraviolet rays. On a clear day the amount of radiation at 6000 feet is roughly one and a half times that reaching the earth at sea-level. It is not always necessary to expose a patient to direct sunlight, as there is a sufficient amount of indirect ultraviolet radiation in the diffused light from the blue sky.

The quartz mercury vapour lamp is very convenient in studying the influence of ultraviolet rays on the human body in general treatment, and it seems to have been established that ultraviolet light plays the preponderant rôle in light-therapy, so that its application is constantly increasing. Recent years have witnessed a great expansion in light-therapy, and at present the provision of lamps and accessories has become quite an industry. Two types of lamp are in use for the treatment of diseases; the aircooled "sun lamp" for general application or for the treatment of large areas, and the water-cooled apparatus for local applications. The air-cooled lamp seems to be adapted to yield a radiation containing a larger proportion of rays of wavelength exceeding 3000 Å; whilst the water-cooled lamp is designed to give out an intense

radiation, much of the energy of which is in the form of rays of wavelength less than 3000 Å. To secure more effective action, optical sensitizers are sometimes applied to the surface to be irradiated. These sensitizers may be dilute solutions of eosin or some other dye, or of calcium chloride solution.

Regarding the comparative influence of light on different individuals, Pacini (*Outlines of Ultraviolet Therapy*, Chicago 1923) writes: "Speaking generally, light people respond more promptly than dark; females than males; the young sooner than the old; and the regions usually protected from the light and of high nervous sensibility."

If general irradiation is intended, it is advisable to begin by fractional exposures. A fresh part is exposed at each sitting, and the previously treated portions are exposed for longer periods.

Ellis and Wells (*Chemical Action of Ultraviolet Rays*, p. 297 (1925)) state: "To ascertain the effect upon the skin, eyes, and general condition, Bach resorted to an heroic test. He applied the ultraviolet light with great intensity upon his own person without protecting his eyes. An exposure of 30 minutes was given at a distance of 16—20 inches from a 3000 candle-power lamp. The head and body were treated alternately. After three minutes, there was a sensation of warmth, and after 10 minutes, a burning sensation at the place of exposure. An intense reddening appeared in 2 or 3 hours after the treatment. This coloration disappeared in three days. There was no blistering, but the epidermis became hard and dry, and peeled within a week, being replaced by a new epidermis, which was elastic and brown. The conjunctiva commenced to redden in about 2 hours after treatment, being inflamed and painful, but the inflammation disappeared in about three days without treatment. Immediately after the treatment, and for days thereafter, there was a feeling of freshness and increased energy. This effect perhaps may be expressed as a case of over-stimulation of a person in good health.

In actual practice the ultraviolet radiation is used either as the principal remedial agent, or as an adjuvant to other therapeutic measures."

According to L. Hill (*Proc. Roy. Soc.* B. **102**, 119 (1927)) sunlight focussed on the skin through a solution of 3% quinine sulphate, which absorbs rays of the mercury vapour lamp shorter than 4200 Å, does not produce erythema if the skin is kept cool by running water. The rays effective in producing erythema seem to lie in the ultraviolet.

From rough experiments, Hill concludes that the erythema-producing rays of the sun lie mainly between 3300—3000 Å. There also appears to be a correspondence between the fading of acetone-methylene blue and the erythema producing power of the sun's rays. The experiments of Hausser and Vahle (*Strahlentherapie* Vol. 13, p. 59 (1922)) seem to be more precise. Using the mercury vapour lamp radiations of equal intensities as measured by a thermopile, they have found the maximum erythema production to be at 2967 Å. At 3131 Å, the erythema production was only 4·5% of that at 2967 Å. While the erythema producing power of rays of wavelength 3131 Å is relatively weak, the intensity of sunlight in this region is sufficient to be effective. The rays of the sun which exert an antirachitic influence, and which can synthesize vitamin D from ergosterol, lie between 3200—2900 Å.

What an important place light therapy occupies in modern medicine can be seen from the following quotation from an "Interim Report on Artificial light and X-ray Therapy" by Cruickshank and Watt (published by the Scottish Board of Health, 1925):

"Hitherto where growth has been deficient or function defective we have had recourse to material remedies. In ultraviolet radiation a new form of treatment is at command. The extent of its therapeutic uses is as yet unknown, but experimental investigation has shown that its therapeutic properties are in the main limited to conditions of growth or of function that are below normal. For example, it has been found to increase body weight, to increase the rate of growth, to improve the mineral content of the blood, to increase the functional activity of the endocrine glands, to increase the bactericidal power of the blood, etc., where they are below normal; but to have no corresponding effect on normal individuals. We found in the course of our enquiry that without exception every patient undergoing light treatment experienced an improvement in his or her general feeling of well-being, apart altogether from improvement or otherwise in the disease for which the treatment was being given."

Cruickshank and Watt report that visible light leads to a dilatation of the cutaneous vessels, stimulating the sweat glands, and aiding the relief of deep-seated congestion.

The rays of shorter wavelength are absorbed by the thinnest layers of the epidermis. On the other hand, rays of longer wavelength can penetrate to appreciable depths. The following results of Glitscher and Hasselbach (*Brit. J. Actinotherapy I*, September (1926)) illustrate this point of view.

Transmission by Epidermis.

Wavelength in A U.	Percentage transmission by	
	0·1 mm. thickness	1 mm. thickness
4360	59	0·5
4050	55	0·3
3660	49	0·08
3540	42	0·02
3130	30	—
3015	8	
2990	2	
2970	0·01	

Compare *The Penetration of Ultra-violet Rays into Live Animal Tissues*, by Macht, Anderson, and Bell (J Amer. Med Assoc. **90**, 161 (1928)).

WHAT IS THE FUNDAMENTAL ACTION OF THE LIGHT RAYS?

From their researches on the photo-oxidation of food materials by air in sunlight at the ordinary temperature, Palit and Dhar have concluded that the light absorbed by the system accelerates the metabolism of food materials in the body. The person thus has a sense of well-being, and disease is avoided. Sunlight is appreciably transmitted by the epidermis, and by absorption of light the body cells are activated, and hence increased oxidation of carbohydrates, fats, and proteins takes place. It seems to be accepted on all hands that several diseases are caused by defective metabolism, and therefore sunlight should prove efficacious in the treatment of these diseases.

PHOTO-OXIDATION OF CARBOHYDRATES, FATS, AND NITROGENOUS SUBSTANCES BY AIR IN SUNLIGHT.

It has been shown by Dhar and Sanyal (*J. Phys. Chem.* **29**, 926 (1925)) that methyl alcohol, ethyl alcohol, and glycerol are oxidized by passing air at the ordinary temperature in the presence of sunlight.

Palit and Dhar (*J. Phys. Chem.* **32**, 1263 (1928); **34**, 993, (1930)) have made a systematic investigation of the oxidation of various substances by air in sunlight at the ordinary temperature. They have shown that different carbohydrates, glycogen, urea, glycine, hippuric acid, α-alanine, sodium urate, potassium palmitate, stearate, oleate, sodium formate, tartrate, oxalate, lecithin, cholesterol, butter, milk, egg-white, egg-yolk etc. can be oxidized photochemically by passing air. Some of the experimental results are recorded below:—

Substance used in the experiment	Weight of substance taken grm.	Amount oxidized grm.	Percentage amount oxidized
Arabinose	0·1000	0·0075	7·5
Galactose	0·0861	0·0067	7·8
Cane sugar	0·0964	0·0098	10·2
Glucose	0·0962	0·0144	14·9
Laevulose	0·0918	0·0159	17·3
Lactose	0·0977	0·0197	19·7
Maltose	0·1097	0·0285	25·9
Starch	0·1027	0·0399	38·8
Glycogen	0·0987	0·0195	19·7
Glycerol	0·2500	0·045	18·0
Urea	0·2000	0·0175	8·7
Glycine	0·0999	0·0096	9·6
α-alanine	0·0997	0·0365	36·6
Hippuric acid	0·0483	0·0069	14·2
Sodium urate	0·0420	0·0082	19·6
Potassium stearate	—	—	40·0
,, oleate	—	—	31·5
,, palmitate	—	—	36·7
Sodium tartrate	0·0989	0·0679	31·3
Sodium formate	0·0737	0·01467	19·9
Potassium oxalate	—	—	29·8

Moreover, it has been shown that in the presence of zinc oxide, and ferric and uranium nitrates, which act as photo-sensitizers, the amount of oxidation of the foregoing substances is greatly accelerated.

It is interesting to note that Einstein's Law of photochemical equivalence is applicable to the photo-oxidation of glucose, lactose, and alanine. In the case of glycine, however, seven molecules are oxidized per quantum of light absorbed. All the above substances are completely oxidized to carbon dioxide and water, without the formation of intermediate products. Hence light accelerates the oxidation reactions on which animal life depends. Palit and Dhar have also carried out comparative experiments on the oxidation of egg-white, egg-yolk, starch, butter, glucose, cane sugar, and glycogen in sunlight, and the following are the results:—

Egg-yolk	60·9 per cent
Egg-white	31·25 ,, ,,
Starch	38·2 ,, ,,
Butter	31·8 ,, ,,
Glucose	13·6 ,, ,,
Cane sugar	7·8 ,, ,,
Glycogen	7·5 ,, ,,

It appears therefore that egg-yolk is the most easily oxidizable substance in presence of light; then comes butter, which is oxidized with greater ease than sugars, which are the least oxidized. These experiments are close imitations of the biological oxidations; and a similar order regarding ease of oxidation in the animal body was observed by Carl von Voit, the great physiologist. These results on the oxidation of food materials by air in sunlight are suggestive, and the beneficial influence of light in the treatment of disease may be due to an increased metabolism in light.

Dhar (*Chemie der Zelle und Gewebe* **12**, 217, 225, 317 (1925); **13**, 209 (1926)) has emphasized the importance of sunlight in the treatment of deficiency diseases; and it seems likely that rickets, osteomalacia, beriberi, pellagra, diabetes, pernicious anæmia, cancer etc. would have been more prevalent in poor tropical countries like India and China, had not the compensating agent, sunlight, been present.

Pincussen (*Bioch. Z.* **150**, 36 (1924)), in his investigations on the effect of solar radiation on rabbits, noticed a stimulation of the protein metabolism, as shown by an increase in nitrogenous excretion. Sensitizers like the various dyestuffs and potassium iodide still further increase the nitrogen excretion.

Moreover, Dhar has obtained a markedly beneficial effect of sunlight in the treatment of diabetes, which is caused by the defective metabolism of glucose; although Sampson (*Physiotherapy Technic*, St. Louis (1923)) considers that diabetes is not amenable to ultraviolet light therapy; yet he admits that this has not been the experience of others. Bach (*Ultraviolet Light*, New York (1916)) has successfully used ultraviolet light in the treatment of diabetic gangrene.

Ellis and Wells (*Chemical Action of Ultraviolet Rays*, page 270 (1925)) make the following interesting statement regarding the comparative position of light therapy in medical science: "So far from serious burns being caused by ultraviolet rays, exposure to these very rays is becoming a favourable measure to relieve pain and to promote healing in ordinary burns, in X-ray dermatitis, and in sun-burn.

Much of the practice in ultraviolet radiation is empirical. This is, however, true of most important advances in therapeutics. Quinine in malaria, mercury in syphilis, iron in chlorosis, ipecacuanha in dysentery, and the majority of our most valuable remedies entered the pharmacopœia, not through the portals of the pharmacologist's laboratory, but in consequence of accidental discoveries, of lucky guesses, and not infrequently with credentials endorsed by alchemists, astrologers, magicians, and the medicine men of savage races."

PHOTO-SYNTHESIS OF VITAMINS.

Huldschinsky (*Deut. Med. Wochschr.* **45**, 712 (1919)) proved that infantile rickets could be cured by ultraviolet radiation from a quartz mercury vapour lamp. Hess and Unger (*Amer. J. Dis. Children* **22**, 186 (1921)) correlated the seasonal variation in the occurrence of rickets with the change in the intensity of sunlight. Hess and collaborators (*J. Amer. Med. Assoc.* **74**, 217, (1920); **77**, 39 (1921)); *Proc. Soc. Exp. Biol. Med.* **18**, 298 (1921)) have further noticed that rats fed on a diet deficient in vitamins A and D do not develop rickets if they have access to light. Thus the original observations of Huldschinsky, followed by the work of Hess and the extensive researches of Miss Chick and her colleagues in Vienna (*Medical Research Council*, Report No. 77 (1923)), have proved conclusively that rickets can be cured as effectively by exposure to ultraviolet radiations of the quartz-mercury vapour lamp as by the administration of anti-rachitic foods, e.g. cod liver oil; whilst secondly, stimulation of growth in animals deprived of vitamin A had been noticed to follow exposure to these radiations (compare Miss Hume. *Lancet* **2**, 1318 (1923)). Further, Miss Hume and Smith (*Bioch. J.* **17**, 304 (1923); **18**, 1334 (1924)) observed that rats kept in glass jars, which were exposed to a quartz lamp for 10 seconds on alternate days, maintained normal health and growth longer than control rats fed on a diet deficient in the same factors.

Shortly afterwards Steenböck and Black (*J. Biol. Chem.* **61**, 405 (1924), and Steenböck and Nelson (*ibid.* **62**, 209 (1924)) discovered that food stuffs deficient in vitamins may have growth-promoting and calcifying properties conferred on them by exposure to ultraviolet light. The work of Hess and Weinstock (*J. Biol. Chem.* **62**, 301 (1924); **63**, 297 (1924)) confirms the observations of Steenböck and collaborators. A wide range of materials can thus be activated, e.g. wheat, rolled oats, corn, starch foods, meat, milk, egg-yolk, and vegetable oils. According to most workers, physiologically active substances are produced from inactive precursors in the foods by the photochemical action of the radiations. The precursor of the active substance found in foodstuffs was first traced to the oils and fats present, and finally identified with the sterols, phyto-sterol, and cholesterol (compare Steenböck and Daniels. *J. Amer. Med. Assoc.* **84**, 15, 1093 (1925); Hess and Weinstock (*J. Biol. Chem.* **64**, 181, 193 (1925); Drummond, Rosenheim, and Coward, (*J. Soc. Chem. Ind.* **44**, 123 (1925)).

Soon afterwards Drummond (*Chemistry and Industry*. 994, December (1926)) observed that by hydrolyzing cod liver oil, which is rich

in vitamins A and D, by means of a boiling solution of caustic alkali in absence of air, the full vitamin content was concentrated in the unsaponifiable fraction, which represented about 1 per cent of the original oil; and that all the cholesterol could be removed without affecting the vitamin activity of the residual red brown oil, which is 0·4 per cent of the original cod liver oil. This indicated that the vitamins resembled cholesterol in chemical nature, but that cholesterol itself was inactive. A further observation was made that when cholesterol, obtained from the brain and rigorously purified, is subjected to irradiation, this previously inactive material becomes anti-rachitic.

Hess and Weinstock (loc. cit.) noticed that cholesterol and phytosterol undergo marked chemical changes on exposure to ultraviolet light, being converted into pale yellow waxy products with melting points greatly below those of the original materials. These changes are probably of the same nature as those observed by Schulze and Winterstein (*Zeit. physiol. Chem.* **43**, 316 (1904); **48**, 546 (1906)) in sterols exposed to daylight for long periods, or those caused by X-rays (compare Roffo, *Compt. rend.* **180**, 228 (1924)). Rosenheim and Webster (*Lancet* **1**, 1025 (1925) have shown that the anti-rachitic factor is formed both in presence and in absence of oxygen. Hess, Weinstock, and Sherman (*J. Biol. Chem.* **70**, 123 (1926)), however, report that in presence of oxygen, oxidation of the active substance may occur during exposure, and result in a reduced yield of the anti-rachitic substance. These facts throw doubt on the correctness of the view of Vollmer and Serebrijski (*Bioch. Zeit.* **176**, 84 (1926)) that the antirachitic activation of cholesterol is essentially a peroxide formation.

The careful experiments of Channon (*Bioch. J.* **19**, 424 (1925)) prove that the animal organism possesses the power of synthesizing cholesterol. The experiments of Gardner and Fox (*Proc. Roy. Soc.* **98**, B, 76 (1925)) afford further evidence in favour of this.

The elucidation of the action of ultraviolet radiations on cholesterol and closely related sterols has progressed so far as to make it evident that it is the anti-rachitic factor (vitamin D) and not the growth-promoting, anti-xerophthalmia vitamin A that is produced (compare *Lancet* **1**, 1025 (1925)).

Within recent years considerable evidence has accumulated in favour of the view that it is not cholesterol but an impurity associated with it which is the precursor of vitamin D. Rosenheim and Webster have not been able to activate by ultraviolet light the cholesterol regenerated from the dibromide. It has been suggested that the labile pro-vitamin had been destroyed in the course of the treatment by

different reagents. In repeating the experiments of Schultz and Ziegler (*J. Biol. Chem.* **69**, 415 (1926)), Heilbron, Kamm, and Morton (*J. Soc. Chem. Ind.* **45**, 932 (1926); and *Bioch. J.* **21**, 78 (1927)) were able to show in a spectrographic study of cholesterol, before and after irradiation with ultraviolet light, that purified cholesterol contains, in small amount, another substance which can be accumulated in the least soluble fraction on crystallization from ethyl acetate. This substance possesses absorption bands in the ultraviolet at 2930 Å, 2800 Å, and 2690 Å, whereas cholesterol itself shows only general absorption in the ultraviolet. These bands disappear on irradiation, with the concomitant appearance of anti-rachitic potency. It was suggested that this unknown substance and not cholesterol itself was closely related to the precursor of vitamin D. While this work was in progress, Rosenheim and Webster (*Bioch. J.* **21**, 127, 389 (1927), and Windaus and Hess (*Nach. Ges. Wiss. Göttingen* **175**, 84 (1927)), were making a detailed examination of the effects of irradiating a large number of cholesterol derivatives and related compounds. In the course of this work it was shown that the trebly unsaturated and highly labile ergosterol gave rise to a strongly potent antirachitic substance. These investigators reported that ergosterol showed the three absorption bands at 2930 Å, 2800 Å, and 2690 Å with great intensity, and that on illumination by a quartz mercury vapour lamp the bands disappeared with a concomitant appearance of antirachitic properties. According to Pohl (*Nach. Ges. Wiss. Göttingen*, 142, 185 (1926)), the ultraviolet absorption spectrum of ergosterol is essentially coincident with that of the provitamin, which is present in cholesterol to the extent of about 0·017 per cent. In view of the high sensitivity of ergosterol to the oxidative process, of its ultraviolet spectrum similar to but more intense than that of impure "activatable" cholesterol, and of the very high degree of antirachitic potency developed by ergosterol on irradiation, both Rosenheim and Webster and Windaus suggest that provitamin D is ergosterol or some closely related sterol. They suggest that it is the presence of small amounts of the latter, in the proportion of about 1 part in 2000, in all specimens of cholesterol prepared from natural sources, that is responsible for the development of antirachitic properties. Irradiated ergosterol is certainly the most potent anti-rachitic substance known, and it is estimated that about 5 mgs. are equivalent to a litre of good cod liver oil. Rosenheim and Webster state that the curative dose for rickets, developed in rats, is of the order of 1/10,000 to 1/20,000 mg. per diem. These workers record further the observation that the comparatively long-wave radiations of solar

ultraviolet light are capable of activating ergosterol. The high content of cholesterol present in human skin (13 to 24 per cent), and the presence in this cholesterol of some substance possessing the same ultraviolet absorption as ergosterol being borne in mind, this observation is of importance in relation to the curative effect of sunlight in rickets. It seems probable that the ultraviolet light of the solar radiation, absorbed by the skin photochemically, produces vitamin D from the provitamin contained in the cholesterol of the skin; and it is this photosynthesized vitamin D, which produces the antirachitic effect. Thus the curative effect of ultraviolet light, either artificial or solar, seems to be indirect rather than direct. On the other hand, Palit and Dhar suggest that the beneficial effects of sunlight are due to increased metabolism.

In view of the results above described, it would seem that there is no serious obstacle to the belief that provitamin D is identical with ergosterol. Nevertheless, the observations of Jendrassik and Keményffi (*Bioch. Z.* **189**, 180 (1927)) lead these authors to suggest an alternative view. They have failed to confirm the observation that cholesterol, after bromination and subsequent reduction, cannot be activated by irradiation. It is also asserted, that in a series of fractionation experiments, inactive cholesterol, after removal of the active fraction by recrystallization and washing, can be reactivated repeatedly. The successive development of antirachitic potency in active fractions, from which the active substance developed in previous irradiations has been removed, and especially their success in activating cholesterol after bromination, lead Jendrassik and Keményffi to the remarkable conclusion that although cholesterol itself is not the provitamin, it gives rise to the latter in presence of water. They also state that on withdrawal of the last traces of water from cholesterol, the provitamin is destroyed and the possibility of its re-formation is lost altogether, with a concomitant disappearance of the characteristic absorption bands, unless it be again treated with water. These interesting observations need further confirmation. MacNair (*J. Biol. Chem.* **76**, 251 (1928)) finds that ergosterol is not entirely removed from cholesterol by bromination, some 3 per cent still remaining. Further work has shown that ergosterol and its esters alone can be activated. Isoergosterol and neoergosterol cannot be activated (compare Windaus and Borgeaud. *Annalen* **460**, 235 (1928)). Oxidation or reduction destroys the capacity of ergosterol to form the vitamin, since Windaus and Brunken (*Annalen* **460**, 225 (1928)) have demonstrated the inactivity of irradiated ergosterol peroxide.

Morton, Heilbron, and Kamm (*J. Chem. Soc.* 2000 (1927)) examined the absorption spectra of ergosterol before and after illumination for different intervals of time, and observed that after illumination lasting for about 150 minutes, the absorption in the region 2600 to 3000 Å disappears, but absorption in the region 2300—2600 Å with a maximum at 2470 Å appears. This new band itself disappears on further illumination, and after six hours of illumination all traces of selective absorption completely disappear. It is well known that cod-liver oil, on long irradiation, loses its antirachitic power. Morton, Heilbron, and Kamm conclude that the whole of the incident energy is absorbed by ergosterol and becomes photochemically active. The nature of the change which gives rise to vitamin formation is at present unknown. If a satisfactory yield in the reaction

$$\text{Ergosterol} + (\text{Ultraviolet light}) \rightarrow \text{Vitamin D}$$

is desired, a prolonged exposure to ultraviolet light screened by "vita glass", which cuts off radiations shorter than 2750 Å, is necessary (compare Heilbron and Sexton, *J. Chem. Soc.* 47 (1928)).

Webster and Bourdillon (*Bioch. J.* **22**, 1223 (1928)) have stated that the temperature coefficients of the changes causing production and destruction of vitamin D are similar and small, and that both reactions are directly photochemical in nature. Reerink and von Wijk (*Bioch. J.* **23**, 1294 (1929)) irradiated ergosterol, in solution in hexane, exposing it to wavelengths longer than 2750 Å and to wavelength 2540 Å. They calculate that, with long wave irradiations, for the first 15 minutes the conversion of ergosterol into vitamin D is the only reaction taking place, and that in this time half the ergosterol is converted. More prolonged irradiation resulted in the slow destruction of the vitamin. The product of short irradiation was highly active and was obtained in the crystalline state, free from ergosterol, provided oxygen was excluded throughout; the crystals had a melting point below 0°. Very recently Asken, Bourdillon, Bruce, Jenkins, and Webster (*Proc. Roy. Soc.* B **107**, 76, 91 (1930)) have obtained, from irradiated ergosterol, by double distillation in a vacuum, followed by crystallisation from aqueous alcohol, a crystalline product showing high antirachitic power and a melting point of 113° to 115°. They have not suggested that these crystals are pure vitamin D. These crystals may consist of an inactive substance contaminated with an extremely active vitamin, or of crystalline vitamin forming mixed crystals with an inactive substance. It is probable that several antirachitic substances are formed by the irradiation of ergosterol, and that the crystals

are a relatively pure specimen of one of these substances. The above authors have also studied the effects of a second irradiation by short waves on the substances formed by a first irradiation of ergosterol by long waves. Their results show that the product with high absorption at 2800 Å is not vitamin D as previously suggested. The substance with high absorption at 2800 Å can be formed by the action of short-wave radiation on some product of a previous action of long waves on ergosterol. The authors have concluded that the initial effect of long-wave radiation on ergosterol is the simultaneous production of at least two substances, only one of which is vitamin D. It appears, therefore, that the exact nature of the change leading to the formation of vitamin D on irradiation of ergosterol is far from being clear.

Heilbron, Kamm, and Morton (*Bioch. J.* **21**, 1279 (1927)) examined spectroscopically a large number of oils, and observed well-defined ergosterol absorption bands in some of them.

Kon, Daniels, and Steenböck (*J. Amer. Chem. Soc.* **50**, 2573 (1928); compare Kon, *Rocz. Chem.* **8**, 502 (1928)) have shown that cholesterol purified by fractional oxidation with permanganate in acetone cannot be activated antirachitically by irradiation. For the photochemical formation of vitamin D from ergosterol, the minimum amount of radiation energy to produce sufficient vitamin to cause deposit of calcium in the bones of a rachitic rat is 700—1000 ergs, corresponding to the formation of 6×10^{-8} grms. of vitamin D. For monochromatic light, from mercury lines 2560 Å, 2650 Å, 2800 Å, and 2930 Å, the quantum efficiency is independent of the wavelength, and of the continuous or intermittant nature of the irradiation. The same value is obtained with ergosterol, solid or in alcoholic solution, and with ergosterol acetate. The quantum efficiency of ordinary cholesterol is variable, and may be nearly as high as that of ergosterol.

Recently, Marshall and Knudson (*J. Amer. Chem. Soc.* **52**, 2304 (1930)) have shown that the velocity of production of vitamin D from ergosterol is directly proportional to the light intensity, and to the number of quanta absorbed by ergosterol, and independent of the wavelength of the light used. The quantum yield of this reaction is 0·3 molecules of vitamin D per quantum of light absorbed. The highest concentration of vitamin D which can be produced by direct irradiation of ergosterol is 35 per cent.

Vitamin D absorbs in the same wavelength region as ergosterol, and is destroyed by light of the same wavelength as that which forms it.

Despite many ingenious attacks on the problem, it cannot be said that the mechanism of the formation of vitamin D from ergosterol

has been elucidated. Heilbron, Morton, and Sexton (*J. Chem. Soc.* **47**, (1928)) have studied the absorption spectra of a number of cholesterol derivatives with the object of determining the class of compound to which vitamin D may be assigned. Selective absorption occurs only when at least two double linkings are present in the molecule. It is shown that the absorption spectrum of cholesterilene resembles that of ergosterol, and it seems probable that two of the three double linkings of ergosterol occupy the same positions as in cholesterilene. Cholestenone and vitamin D also appear to be correlated. The course of the various changes which occur when ergosterol is irradiated has been the subject of a spectrographic investigation by Bourdillon, Fischmann, Jenkins, and Webster (*Proc. Roy. Soc. B.* **104**, 561 (1929)). The antirachitic activity of irradiated ergosterol solutions has been compared with their absorption spectra, both before and after removal of unchanged ergosterol, and evidence has been obtained which points to the successive formation of three substances or groups of substances. The first of these shows an absorption band similar to that of ergosterol with a maximum at 2800 Å, but more than twice as intense. This substance is believed to be vitamin D. The second product which is formed by further irradiation of the first has a maximum absorption at 2400 Å, and has no antirachitic activity. The third substance, formed in turn from the second, possesses neither antirachitic activity nor specific absorption. Reasons are given for supposing that ergosterol, when irradiated, changes directly into the vitamin without the loss of any large part of its molecule. More recently Bourdillon, Jenkins, and Webster (*Nature* **125**, 635 (1930)) state that they have now come to the conclusion that the absorption band at 2800 Å is not that of the vitamin D, but that of a decomposition product; the vitamin shows low absorption at this wavelength. The absorption spectrum of ergosterol does not seem to have been established beyond doubt.

Knudson and Moore (*J. Biol. Chem.* **81**, 49 (1929)) have brought forward some evidence to show that a certain degree of antirachitic potency can be developed in ergosterol by irradiation with soft X-rays. Cluzet and Kofman (*Compt. rend.*. **189**, 45 (1929)) have reported that sterols exposed to ultraviolet light, X-rays, or radium rays, darken a photographic plate. Similar observations have been made by Hugounenq and Couture (*ibid.* **189**, 47 (1929)).

Notwithstanding the efforts of numerous workers, the mechanism of the formation of vitamin D from ergosterol remains unsettled. The most recent work (compare *Nature* **125**, 428 (1930)) indicates that vitamin A can be formed in the complete absence of light, except the

minimum required to see, feed, and handle the animals, and collect the etiolated shoots. Moore found that wheat seeds contained no vitamin A, that etiolated shoots, fed to rats in a diet containing vitamin D but no A, stimulated growth, although given to the animals after dark. It is certain that light can accelerate the synthesis of vitamin A, since green plant tissues are better sources than white.

The activation of edible substances on irradiation has not been properly explained. Recently, Dhar and Palit advanced the view that when substances like cholesterol, olive oil, etc. are exposed to light in presence of air, peroxides are formed, and that these induce the oxidation of food materials mixed with them. Hence the beneficial properties of substances not containing the necessary vitamins are due to the presence of peroxides, which help the oxidation of the food materials in the body. The following observations support the above views:—

A current of air free from carbon dioxide was passed through solutions or suspensions of the substances exposed to sunlight for 5 hours. After exposure, an excess of potassium iodide and acid was added to the mixture and the liberated iodine was titrated against N/100 thiosulphate.

Substance	Amount taken in c.c. or g.	Amount in c.c. of N/100 thiosulphate required for iodine liberated
Olive oil	3 c.c.	3·2
Mustard oil	3 ,,	1·4
Cocoanut oil	3 ,,	1·00
Mahua oil	3 ,,	1·2
Castor oil	3 ,,	0·95
Til oil	3 ,,	1·2
Linseed oil	3 ,,	0·9
Butter	0·92 g.	2·25
Starch	0·10 ,,	0·20
Glycogen	0·10 ,,	0·05
Dextrin	0·10 ,,	0·45
Glucose	0·10 ,,	0·05

The irradiated products had the property of oxidizing other substances when mixed with them. The extent to which they oxidized glucose was carefully determined, and some of the results are recorded below:—

	percentage of glucose oxidized		percentage of glucose oxidized
Mustard oil	5·5	Castor oil	4·1
Cocoanut oil	3·7	Til oil	2·3
Mahua oil	3·3	Linseed oil	0·8

From the foregoing observations it is clear that when food materials are exposed to sunlight in the presence of air they take up oxygen, probably forming some peroxide type of compound which can oxidize other food materials when mixed with them. Consequently the addition of the exposed substances to ordinary foodstuffs facilitates the proper assimilation of food materials, and produces efficacious results.

Moreover Chakravarti and Dhar (*Indian. J. Med. Research* **17**, 430 (1929); Palit and Dhar (loc. cit.)) have carried out feeding experiments with pigeons and have drawn the following conclusions:—

Pigeons do not get polyneuritis in 5 weeks when fed on Rangoon rice alone, provided they have sunlight. Leafy vegetables like palak, bathua, and tomatoes (substances containing vitamins), when mixed with polished rice, appear less effective than sunlight in the prevention of polyneuritis and eye-troubles. Colloidal ferric hydroxide and ferric chloride in very small doses are as effective as the above vegetables. Comparative experiments show that sunlight is the best preventive of diseases like polyneuritis and beriberi. Olive oil exposed to light in the presence of air comes a close second, whereas unexposed oils are harmful. In tropical countries many deficiency diseases are avoided by sunlight. It is interesting to note in this connection that Delore (*Bull. Soc. Chim. biol.* **11**, 74 (1929)) has observed a gain in weight owing to absorption of oxygen, when olive, linseed, and specially cod liver oils are exposed to air in absence of light. Preliminary irradiation of the oils by ultraviolet light enhances the rate of auto-oxidation up to a limit, beyond which it is decreased. In comparison with other oils, cod liver oil seems to behave as if it had been already irradiated.

Recently, Rousseau (*Compt. rend.* **189**, 37 (1929)) has shown that 0·5 per cent of ergosterol dissolved in olive oil and alcohol has a greater power of oxidation when exposed to sunlight in the presence of air than cholesterol under the same conditions.

SUNLIGHT AND ARTIFICIAL LIGHT IN THE REARING OF FARM ANIMALS, POULTRY, PLANTS, ETC.

Orr, Henderson, and Crichton (*Trans. Highland and Agric. Soc. Scotland* (1926)) observed that, when pigs nine weeks old were subjected to light treatment from a carbon arc at a distance of three feet for one hour daily, the amount of calcium and phosphorus which the pigs were able to retain in their body was markedly increased, and the amounts excreted in the fæces were correspondingly diminished. Fairhall (*Amer. J. Physiol.* **84**, 378 (1928)) has shown that on a normal diet, irradiated

rats had a somewhat higher concentration of calcium in the serum than did normal rats of the same age. Irradiation increased calcification in rats both on a normal and on a calcium-poor diet.

Irradiation strengthens the weak legs of young chickens. Hughes and Payne (*J. Biol. Chem.* **66**, 595 (1925) and Hart, Steenböck, and Elvejem (*ibid.* **62**, 117 (1924)) have shown that ultraviolet light increases the egg production and improves the quality of the eggs of hens. Orr, Henderson, and Crichton state: "It is suggested that there should be a fuller utilization of sunshine for farm animals, and the employment of artificial means of irradiation in winter is put forward as worthy of trial. The agriculturist should realize that the beneficial influence of sunshine falls not alone upon his crops but on all farm animals."

Tsuji (*Louisiana Planter* **60**, 413 (1918)) has investigated the influence of ultraviolet light in the sugar cane, pine apple, and banana industries, and has noted a stimulation in the growth of sugar-cane and an increase in the percentage of sugar. Maquenne and Demoussy (*Compt. rend.* **149**, 756 (1909)) have reported that when ultraviolet rays are applied in a proper manner, growth of flowers and vegetables is enhanced.

Sheard and Higgins (*Science* **65**, 282 (1927)) have shown that radiations in the region 3200—3900 Å help, whilst radiations of wavelengths 2700—3200 Å from a quartz mercury vapour lamp retard the germination and subsequent growth of seeds. The region 2700—3200 Å, though effective therapeutically, appears to be harmful in the growth of seeds.

ACTION OF LIGHT ON ENZYMES AND ANTIBODIES.

Agulhon (*Compt. rend.* **153**, 979 (1911)) divided the ordinary enzymes into three classes on the basis of their sensitiveness to light.

(a) Enzymes oxidized by air in presence of light of all wavelengths, but destroyed in a vacuum only by ultraviolet radiations:— sucrase, tyrosinase, laccase.

(b) Enzymes destroyed by all radiations with or without oxygen:— emulsin, catalase.

(c) Enzyme destroyed in a vacuum by ultraviolet light, and somewhat weakened by visible rays:— rennet.

Pincussen and collaborators (*Bioch. Z.* **134**, 459 (1923); **144**, 366 (1924); **152**, 406, 416 (1924)) reported that diastase of all concen-

trations loses its activity on exposure to ultraviolet light, but not in sunlight. They observed that sodium chloride protects diastase and maltase in malt diastase from destruction on exposure to sunlight. Other salts also exert protecting action. The actions of pancreatic, salivary, and takadiastase are increased, whilst that of malt diastase on soluble starch is retarded by ultraviolet light in presence of potassium iodide. These authors showed that the active enzyme in urease solutions is enfeebled in sunlight and destroyed in ultraviolet light. Lüers and Lorinsen (*Bioch. Z.* **144**, 212 (1924)) compared the inactivation of malt amylase by heat with that by ultraviolet radiation. The ultraviolet light inactivation does not follow the mono- or bimolecular equation, nor the Schütz law. The authors conclude that the two methods of inactivation are different. Pincussen (*Bioch. Z.* **171**, 1 (1926)) has observed that diastase, which has been rendered almost inactive by exposure to ultraviolet light, is partly reactivated by the addition of a small quantity of non-irradiated diastase. Pincussen and collaborators (*Bioch. Z.* **195**, 79, 87, 96 (1928)) have stated that the lipase action of the serum of rabbits is considerably injured by irradiation.

Albela (*Deut. Med. Wochschr.* **48**, 1347 (1922)) could not detect any change in phagocytic action with rabbits exposed to ultraviolet light for long periods. Heuer and Potthoff (*Centr. Bakt. Parasitenk. I. Abt.* **88**, 299 (1922)) observed that the exposure of animals to ultraviolet rays has only a slight influence on the formation of antibodies. Pigmented animals show this influence more than unpigmented ones.

Heuer (*ibid.* **88**, 380 (1922)) states that agglutinins for cholera and para-typhoid-A are less resistant to ultraviolet radiations than those of typhoid, and the bacteriolysins less resistant than the agglutinins. Friedberger and Scimone (*Z. Immunitäts* **37**, 341 (1923)) have stated that a decrease in amboceptor is observed when hemolytic serum is exposed to ultraviolet rays. Trypanosomes lose their infectivity much sooner than motility on irradiation. Wassermann serums and Sachs-Georgi positive serums of man and rabbits become negative on illumination with ultraviolet light.

Balderrey and Barkus (*Amer. Rev. Tuberculosis* **9**, 107 (1924)) observed an alkalinity of the blood when the human body was exposed to sunlight. Koopmann (*Deut. Med. Wochschr.* **50**, 277 (1924)) has reported that the concentration of erythrocytes, hæmoglobin, viscosity, and coagulation point of human blood remain unchanged on illumination of the body with ultraviolet rays. According to Clark (compare Pacini, *Outlines of Ultraviolet Therapy* 36 (1923)), radiations of

wavelengths 3000—2000 Å increase the white corpuscles of lymphatic origin. Kroetz (*Bioch. Z.* **151**, 449 (1924)) has stated that in most patients the water content of the serum increases after irradiation with ultraviolet or X-rays, but becomes normal again after 24 hours.

Recently Risler, Philibert, and Courtier (*Compt. rend.* **186**, 1152 (1928)) have described the great bactericidal action of light produced by electric volatilization of aluminium wire. They have shown that B. tuberculosis is partly destroyed by the light of the neon lamp in the presence of many dyes, especially those of the pinacyanol class.

STERILIZATION BY LIGHT.

It has been known for nearly half a century that ultraviolet radiations have bactericidal properties. The following results have been obtained:—

(1) Ultraviolet radiations of wavelengths 2970—2100 Å are most active in killing bacteria; radiations of wavelengths 3800—3000 Å do not exert marked germicidal action, but have greater penetrating power through human skin than radiations of wavelengths 2970 to 2100 Å.

(2) The active rays are those absorbed by the substance; the total light effect depends on the intensity of the radiation and the time during which it reacts.

(3) Light acts mainly on the protein constituents of the organisms, and possibly the aromatic amino acids are chiefly attacked.

(4) Atmospheric oxygen is not necessary for the germicidal action of light. The germicidal effect is due to the direct action of the light on the bacteria, and not to the formation of ozone, hydrogen peroxide, or any other germicidal substance.

(5) Turbidity of the liquid to be irradiated greatly reduces the sterilizing action of the rays.

Different bacteria show varying photo-sensitivity. It has been found that under standard conditions Sp. choleræ, B. typhosus, B. dysenteriæ, B. coli are very readily destroyed, and B. anthracis (sporogenous), pneumobacillus and sarcina alba are less readily affected. Organisms of the following group are twice as resistant as those of the first group:—

B. tetani, B. megatherium, B. phleole, B. subtilis and sarcina lutea. Paramecium requires 10 times and yeast 20 times as long as B. coli for destruction. This variation of photosensitivity has been practically utilized. Thus when milk is sterilized by ultraviolet light, it is observed

that the harmful bacteria are more affected than the desirable lactic acid bacilli. Moreover, vaccinia virus can be sterilized by ultraviolet light because the harmful bacteria are much more sensitive to light than the more desirable organisms.

Cernovodeanu, Henri, and Barorni (*Compt. rend.* **151**, 724 (1910)) observed that an emulsion of tuberculosis bacilli was readily sterilized, and tuberculin was inactivated by ultraviolet light. Other workers observed that the oxidases of milk, the lipolytic ferments of oils, the complement of serum, diphtheria toxin, and the toxin of lamprey serum can be inactivated by ultraviolet light.

Coblentz and Fulton (*Bureau of Standards, Scientific papers* nos. 330, 378 and 495) exposed atomized suspensions of B.coli to ultraviolet radiations from a quartz mercury vapour lamp, and concluded that the germicidal action of ultraviolet radiations extends up to 3650 Å, decreasing with increasing wavelength. Prolonged exposure is needed with radiations from 2970—3650 Å. The lethal action of waves shorter than 2800 Å is about ten times more rapid than that of radiations of wavelengths greater than 3050 Å, despite the much lower intensity of the shorter waves. Coblentz and Fulton calculated the energy required of wavelengths 1700—2800 Å to kill B.coli bacteria to be about 19×10^{-12} watt.

Dreyer observed that the middle region of the spectrum, normally harmless, can be made to exert a lethal action on sensitizing the bacteria by suspending them in 1/5000 M aqueous solution of erythrosin.

Although according to Bernard and Morgan (*Brit. Med. J. Nov.* 14 (1903)) sterilization *in vivo* by ultraviolet light is an impossibility, subsequent research has shown that, in certain types of open wounds, the germicidal action of light can be successfully utilized.

Lesure (*J. Pharm. Chim.* (vii) 10, 569 (1910)) attempted to sterilize by ultraviolet rays medicinal preparations, especially those intended for injection. Olive, cotton-seed, and almond oils can be effectively sterilized by exposure to ultraviolet light for about 3 minutes. Fats and butter can be sterilized by exposing them in thin layers. Insulin solutions and bacterial vaccines are also sterilized by ultraviolet radiations.

Regarding sterilization of milk, Helbronner and von Recklinghausen (*U. S. Patent* 1,141,046, May 15 (1915)) have stated that complete sterilization by light alone would be difficult, and the prolonged exposure required would have harmful effects on the flavour and digestive qualities of milk. They propose irradiation of milk kept at a temperature of 60°. It is probable that illumination of milk results in the formation of vitamin D, but the amount of vitamin A may be reduced.

The photo-sterilization of water has several advantages:—

(1) The water itself undergoes no change. The dissolved gas and solids, which give to the water, as a drink, its pleasing qualities, are almost wholly retained.

(2) Sterilization can be carried to completion, and the water can be utilized conveniently for surgical purposes, for washing butter, or in the margarine industry, and generally whenever water is to be added to food.

Turbidity of the water must be removed before ultraviolet light sterilization can be effective. Deposits on the quartz may diminish the efficiency of the lamps. Removal of turbidity is effected by sedimentation with the use of coagulants like alum, or by coarse filtration.

CHAPTER VII

Deficiency Diseases, Internal Secretions, Vitamins, and Light.

PERNICIOUS ANÆMIA AND WANT OF VITAMINS B AND C

In publications (*Chemie der Zelle und Gewebe* **12**, 217, 225, 317 (1925); **13**, 119, 209 (1926); *J. Phys. Chem.* **33**, 1897 (1929)) from these laboratories, it has been suggested, that pernicious anæmia — the nature of which is still unknown — is a deficiency disease, due to the lack of vitamins, specially B and C. The symptoms of pernicious anæmia are very nearly the same as those of scurvy and pellagra. The primary stage in scurvy, pernicious anæmia, and pellagra is characterized by a peculiar paleness of the skin, apathy, melancholy, muscular weakness, dizziness, sleeplessness, and slight dyspnea. The skin becomes yellowish, dry, and scaly, and is covered usually with large livid spots, due to sub-cutaneous hæmorrhages. In addition, there are frequently pains in the lumbar regions and legs. In all these diseases there is disturbance of digestion, and consequent loss of appetite. The tongue is apt to be thickly coated, and in later stages, the patients suffer very much from dyspnea and palpitation. They faint easily, and heart weakness progresses.

It has been found that sunlight is very efficacious in the treatment of pernicious anæmia. Rickets, which is known to be a deficiency disease, can be treated with sunlight and ultraviolet light. Probably scurvy, beriberi, pernicious anæmia, pellagra, etc. are really caused by the want of proper metabolism in the animal body, due to the absence of the activators — vitamins B and C. It has been proved that many chemical changes are accelerated by sunlight, and that the metabolism of carbohydrates, fats, and proteins, is increased thereby. In pernicious anæmia sunlight is helpful because the normal metabolism, which becomes defective due to the onset of the disease, is accelerated by sunlight.

In recent years, Minot and Murphy (*J. Amer. Med. Assoc.* **87**, 470 (1926); **89**, 759 (1927) and Cohn, Minot, Alles, and Salter (*J. Biol. Chem.* **77**, 325 (1928)) have shown that in cases of pernicious anæmia

the feeding of large amounts of liver is followed by an increase in the red blood cell count. The curative effect is produced by some substance present in normal mammalian liver, and presumably absent from the systems of persons suffering from pernicious anæmia. The latter authors have published an account of the preparation and properties of the active material obtainable from liver.

CANCER AND WANT OF VITAMINS B AND C

The author (Dhar, *Chemie der Zelle und Gewebe* **12**, 317 (1925)) has advanced the view that cancer, especially of the mouth and stomach, is due to defective metabolism, and is very likely a deficiency disease like scurvy, beriberi, and pellagra. Most likely cancer is caused in the first instance by the lack of vitamins B and C.

Recently, there has been considerable discussion with regard to the bacterial nature of this disease, but the problem is far from being solved.

In this disease also, in the beginning we find loss of appetite, repulsion from food, flatulence, acidity, and other stomachic troubles. There are certain symptoms which are similar to those in pernicious anæmia. In course of time the surface of the tongue is to an increasing degree denuded of epithelium, and the mucous membrane becomes dry and fissured. All these symptoms show that cancer of the mouth and stomach is very likely caused by the want of proper assimilation, due to the lack of vitamins and sunlight, which would act as activators in metabolism. It is a significant fact that in tropical countries, where there is plenty of sunshine, though the people are illfed, cancer is less prevalent than in western lands where living conditions are more favourable. We are strongly of opinion that this is due to the beneficial influence of sunlight towards metabolism. In presence of sunlight the oxidation of fats, proteins, and carbohydrates is greatly accelerated. Hence the absence of the necessary amount of vitamin in the food of the people living in tropical countries cannot cause deficiency diseases because of the beneficial effects of sunlight on metabolism.

We have advanced the view that vitamins A and D, which are associated with fatty food materials, act as accelerators in the metabolism of fat. When the fat is not properly metabolized in the animal body troubles of digestion, acidosis, rickets, eye-troubles, etc. may arise. We are of opinion that vitamin B acts as an accelerator in the oxidation of carbohydrate food-materials, and that beriberi is a

metabolism disease due to defective carbohydrate assimilation caused by the lack of vitamin B in the diet. This view is supported by the experimental results of Funk and Dubin (*Sciene* **54**, 447 (1920)). These authors have shown that when autolyzed yeast is added to the diet of rats, there is greater percentage increase in the weight of the rats when the diet consists of carbohydrates than when it is made up of proteins, because the vitamin B, which is present in yeast, acts as a marked accelerator in the metabolism of carbohydrates; and hence yeast is more efficacious for a carbohydrate diet than a protein one. Moreover, from the experiments of Findlay (*Bioch. J.* **15**, 104 (1921)), it appears that beriberi is caused by the disturbance of carbohydrate assimilation. The view that beriberi is a metabolism disease is supported by the observation of Okada and co-workers (*Japan. Med. world* **3**, III, 102 (1923)) that starvation of vitamin B leads to decrease of basal metabolism in human beings.

In our experiments with pigeons, we (Chakravarti and Dhar, *Indian J. Med. Research* **17**, 430 (1929)) have found that when polished rice is given to them, there is always digestive trouble in the beginning, and that later on polyneuritis follows. War œdema has been associated with troubles of digestion; so are the initial stages of beriberi. Hence defective diet leads to stomachic troubles, severe forms of diarrhœa, beriberi, scurvy, pellagra, pernicious anæmia, and in some cases cancer. These are really metabolism diseases, and should prove amenable to treatment by sunlight and ultraviolet light.

EYE DISEASES AND WANT OF VITAMIN A.

In different parts of India night-blindness is very common, especially amongst the poorer people. We have observed that in Behar, Chotanagpur, and several hill districts of the United Provinces, a large percentage of the poorer people, who have usually an inadequate supply of food materials, suffer from night-blindness and other eye troubles. In Chotanagpur there is a common belief amongst the poorer people that sheep or goat-liver is the best remedy for night-blindness. These poor people cannot afford eggs, butter, or milk, and because there are no rains for several months there is great scarcity of leafy vegetables, which are convenient sources of vitamin A for the poor. Hence the poor people suffer from night-blindness, kerato-malacia, xerophthalmia, etc., which are certainly caused by the lack of vitamin A.

The author (Dhar, *Chemie der Zelle und Gewebe*, **13**, 209 (1926)) has attempted to show that leukæmia, which is supposed to be a mysterious

blood disease, concerning whose causation and intimate nature we have but little precise knowledge, is also a deficiency disease due to lack of vitamins. The author has indicated that several of the so-called tropical diseases are caused by bad food and lack of vitamins.

DEFICIENCY DISEASES AND BOWEL DISORDER.

It has been shown that there are some common features in the symptoms of pernicious anæmia, pellagra, and scurvy. It has also been stated that stomachic troubles like gastro-intestinal symptoms, vomiting, diarrhœa, etc. are commonly observed in scurvy, pellagra, and pernicious anæmia. Moreover, Hamilton Wright has reported that in beriberi, patients at the outset of the disease invariably complain of loss of appetite and epigastric pain or discomfort, and that those who die in the early stage of the disease invariably show injection, punctiform or more extensive hæmorrhages, and swelling of the duodenal mucosa.

In epidemic dropsy also we observe disorder of the bowels, vomiting, diarrhœa, dysentery, and pronounced anæmia. McCarrison (*Indian. J. Med. Res.* VI, 257, 550 (1919)) has observed gastric, intestinal, biliary, and pancreatic disorders as a result of a diet deficient in vitamins, especially B and C. Such deficient dietaries give rise to congestive and atrophic changes in all the coats of the intestine, to the impairment of its digestive and assimilative functions, and to failure of its protective resources against bacterial infections. Pigeons fed exclusively on a diet of polished rice frequently suffer from diarrhœa, and this can be regarded as a symptom of vitamin deficiency, since it can, in many cases, be caused to disappear at once by the administration of alcoholic extracts of egg yolks. Diarrhœa or colitis is almost a constant precursor or concomitant of war œdema, which is in all probability due to the continued deficiency in the diet of protein and antineuritic substance. At least this fact is true in the wet form of beriberi. Continued dietetic deficiency may lead to a state of chronic gastro-intestinal catarrh, and sometimes to cancer.

RICKETS AND FAT ASSIMILATION.

Rickets has long been known to be a disease of improper nutrition, and to be caused by faulty feeding. Chronic diarrhœa, which may itself result from unsuitable food, frequently precedes this disease. The fault in the diet upon which the occurrence of rickets depends is

poorness in animal proteins and fats, and excess of carbohydrate, especially starch. Deficiency of good class fats is decidedly more important than deficiency in protein, and some of the harm caused by excess of carbohydrate may be due to their interference with the digestion of fat. It has already been emphasized that vitamins A and D, which are associated with fatty food materials, act as promoters in the oxidation of fats in the body. When vitamins A and D are absent from foods insufficient oxidation of fats takes place, and rickets may result.

It (Mittra and Dhar. *J. Phys. Chem.* **29**, 376 (1925)); Palit and Dhar, *ibid.* **29**, 799 (1925)); **30**, 939 (1926); **32**, 1663 (1928)) has also been proved that the oxidation of various substances by air or oxygen can be markedly retarded by the presence of carbohydrates, which in their turn are also oxidized by air in presence of the above substances. Moreover, the oxidation of fats is retarded by the presence of carbohydrates. Consequently, when too much of carbohydrate food is given to children, the fatty food material is not properly burnt, especially when vitamins A and D are absent; and hence rickets may result.

Moreover Chittenden and Underhill (*Amer. J. Physiol.* **44**, 13 (1917)) fed dogs exclusively on vegetable foods, and observed that they did not thrive. One diet, consisting of crackers (bolted wheat flour), peas, and cotton seed oil, produced in dogs restricted to it for several months a condition regarded by these investigators as equi valent to pellagra in man. The animals developed inflammation of the mouth with sloughing of mucosa, diarrhœa, and skin changes of a nature regarded as analogous to those seen in pellagra.

From the foregoing facts we can conclude that almost all deficiency diseases, namely scurvy, beriberi, pellagra, malnutritional œdema, rickets, etc. are associated with a state of gastro-intestinal disturbance. Moreover, from Mc Carrison's researches, we find that stomachic troubles arise through the lack of vitamins, and this conclusion supports the author's contention that deficiency diseases are caused mainly by the failure of proper metabolism or oxidation of food materials through the want of accelerators or promoters like vitamins, internal secretions, etc. which accelerate the oxidation of food materials by air.

The author has shown that the internal secretion from thyroid gland plays practically the same rôle as that of vitamins A and D. Thyroid stimulates all metabolism, and specially the oxidation of fats is greatly accelerated. In the lack of development and growth of children, mental and physical, thyroid is of general value. Thyroid is useful in diseases due to lower general nutrition of all tissues, including the bones, such as osteomalacia, rickets, and osteomyelitis. Many eye

troubles are also ascribed to the lack of thyroid. It is well known that vitamins A and D play the same part as thyroid in the promotion of growth, and the avoidance of rickets, osteomalacia, osteomyelitis, and eye troubles like night-blindness, keratomalacia etc. It is clear therefore that vitamins A and D, and thyroid secretion, are likely to act as promoters in inducing the oxidation of fats and maintaining health. It is interesting to note that in many cases of rickets Miss Hodgson (*Lancet* II, 945 (1921)) found acidosis developing at the height of the disease. Pritchard (*Brit. Med. J.* I, 887 (1923)) considered the cause of rickets was a relative excess of acid substances produced in the system. Burgess and Osman (*Lancet* I, 281 (1924)) reported three cases of acute rickets in which they found severe acidosis.

It has already been emphasized in the previous pages that cancer of the tongue and stomach, and pernicious anæmia, are associated with want of proper digestion or assimilation of food materials, probably due to the want of activators like vitamins (mainly B and C); and the author has included both these diseases in the category of deficiency diseases.

LEUKÆMIA AND HODGKIN'S DISEASE.

Leukæmia is a mysterious blood disease, the origin of which is still unknown. This disease is characterized by a persistent increase of white blood corpuscles. The symptoms of the disease come on, as a rule, insidiously; progressive abdominal enlargement from splenic hypertrophy, or enlargement of superficial lymphatic glands, is first noticed, accompanied by pallor, dyspnœa, palpitation, and other signs of anæmia. There is a great tendency to hæmorrhage from internal organs, and especially to epistaxis, and retinal hæmorrhages also are common. Gastro-intestinal symptoms, nausea, vomiting, and grave diarrhœa are frequent. As in pernicious anæmia, an irregular pyrexia is often observed, and the temperature may rise at night from time to time as high as 103°, or even higher. With regard to the treatment of this disease the best results seem to have been derived as in pernicious anæmia from the use of (1) arsenic, (2) sodium cacodylate, and (3) salvarsan. Hodgkin's disease, which is supposed to have some pathological affinity with leukæmia, is characterized by progressive enlargement of the lymphatic glands, together with symptoms of anæmia. The cause of this disease is as obscure as that of the preceding. As in leukæmia, there is a tendency to hæmorrhage, and the temperature of the body too often rises to 100 to 103° F or higher. In this disease, too, arsenic has been found to be efficacious. As in pernicious anæmia,

milk and milk food, with eggs, meat juice, and jellies are recommended as good things for leukæmia. Grawitz has recommended a diet consisting of milk and vegetables. The author is of opinion that, like pernicious anæmia, leukæmia and Hodgkin's disease are caused by the lack of vitamins, mainly B and C.

ADDISON'S DISEASE AND VITAMINS A AND D.

The author has suggested that the function of adrenal secretion is to act as a promoter in the oxidation of fatty food materials. The effect of epinephrin, the active principle of adrenal glands, is definitely to increase metabolic rate and body temperature, possibly owing to the increased oxidation of fats. Moreover, like vitamins A and D, epinephrin has been found useful in the treatment of rickets and osteomalacia. Osteomalacia, indeed, is considered by some to be caused by adrenal hypofunction.

Addison's disease is today regarded by most investigators as due to hypofunction of the adrenal glands. It develops gradually, with general asthenia, lack of interest, and malaise; digestive disturbances are common, with vomiting or diarrhœa and constipation, which may be alternating in character. Addison's disease usually resists all methods of treatment. Organotherapy with adrenal substance offers great hope, and in the hands of numerous practitioners has proved far more effective than all other therapy. The symptoms of actual extirpation of the adrenals include rapidly developing asthenia, convulsions, psychic depression, cardiac weakness, dyspnea, etc. Acute gastrointestinal symptoms, vomiting, diarrhœa, may be marked. In Addison's disease all the above symptoms are prominent. Emphasis has already been laid on the function of adrenal secretion as an activator in the oxidation of fatty food materials, and in this respect the function of adrenal secretion is more or less allied to that of vitamins A and D. Just as adrenal secretion is a remedy for Addison's disease, so vitamins A and D should be particularly useful in its treatment. The author would urge medical men to treat Addison's disease with food materials rich in vitamins A and D.

BERIBERI, EPIDEMIC DROPSY, AND PANCREATIC SECRETION.

Pellagra, beriberi, epidemic dropsy, sprue (psilosis), dysentery, infantile biliary cirrhosis, pinos, hill diarrhœa, etc. are regarded as tropical diseases. We now know definitely that beriberi and pellagra are deficiency diseases.

It is now well established that beriberi is due to the lack of vitamin B in food materials. In the foregoing pages it has been shown that vitamin B is likely to act as a promoter in the oxidation of carbohydrate food materials. Moreover, it is well known that pancreatic secretion is very helpful in the treatment of diabetes, and hence the view has been advanced that, like vitamin B, pancreatic secretion acts as a promoter in the oxidation of carbohydrate food materials. Moreover, the experiments of Findlay (*Bioch. J.* **15**, 104 (1921)) seem to prove that beriberi is due to a definite breakdown in the carbohydrate metabolism. Hence the suggestion that insulin should prove to be of great value in the treatment of beriberi. Moreover, food materials rich in vitamin B should prove of great value for diabetes. It should be emphasized that diabetes is very common in tropical countries, especially amongst people of sedentary habits. Most of the tropical countries are extremely poor, and the majority of the population have to depend on cheap carbohydrate for sustenance. Moreover, if the normal food lacks vitamin B, either beriberi or diabetes results. We must not neglect the fact that the pancreas may be taxed too much by the intake of large quantities of carbohydrate food materials, and this will certainly facilitate diabetes. It is only fair that, like beriberi, diabetes should be classed as a tropical disease.

Though Magnus-Levy (*Spez. Path. Ther. inn. Krankh.* **1** (1913)) was unsuccessful in isolating an antidiabetic principle from oatmeal, Boruttau (*Bioch. Zeit.* **88**, 420 (1918)) found that pancreas extracts, yeast, and yeast extracts inhibit the cleavage of glycogen in the isolated heart, while extracts from the peripheral oat layer decrease the sugar elimination in diabetic dog and man. In India, owing to the preponderance of carbohydrates in normal diet, conditions set in which gradually lead to diabetes. These conditions strongly resemble the experimental glycosuria which has been described in pigeon beriberi by Funk (*Zeit. physiol. Chem.* **89**, 378 (1914)).

Funk and von Schönborn (*J. Physiol.* **48**, 328 (1914)) found that whereas in normal pigeons the glycogen and blood sugar content of the liver are 1·17 per cent and 0·1 per cent respectively, on an artificial vitamin-free diet the glycogen content drops to 0·48 per cent and the blood sugar rises to 0·15 per cent. A pigeon fed on an excess of sugar showed 4·5 per cent glycogen and 0·15 per cent blood sugar. With an excess of starch, there is no glycogen and 0·26 per cent blood sugar. In the last case, if vitamin B is added, the values for glycogen and blood sugar approach normal. These results were later repeated several times, and corroborated by Funk (*J. Physiol.* **53**, 247 (1919)). The finding

of glycosuria in pigeon beriberi has not yet been confirmed in human beriberi. Besides this, Funk also studied the influence of substances having a known action on carbohydrate metabolism. Among them the effect of glucose, phlorhizin, adrenaline, pituitrin, thyroid, and parathyroid on pigeon beriberi was tested on normal and rice-fed pigeons. Of special interest was adrenaline, which shortened the life of the animal; this was also true of thyroid. The influence of parathyroid was quite different. While thyroid raised the blood sugar content of the liver, the addition of parathyroid increased the glycogen and amino-acid contents, but had little effect on the blood sugar. The occurrence of glycosuria on the addition of thyroid and adrenaline has already been explained in previons pages. The discovery of glycosuria in pigeon beriberi may evidently be connected with some observations of Mc Carrison (*J. Indian. Med. Research* **5**, 2755 (1919)), who found that in avian beriberi, the suprarenals undergo considerable enlargement, and are characterized by a high adrenaline content. It is clear that in beriberi carbohydrate metabolism becomes defective, and consequently the animal has to depend on fat and protein, so that adrenaline, which acts as an accelerator of fat metabolism, is increased.

Epidemic dropsy is characterized by the sudden appearance of anasarca preceded in most instances by fever, vomiting, diarrhœa, or by irritation of the skin; and often accompanied by a rash, by fever of a mild remitting type, by disorder of the bowels, and by pronounced anæmia. Œdema is a constant feature of the attack. This disease, like several malnutrition œdemata, is really a deficiency disease caused by the lack of vitamin B. In this disease also pancreatic preparations should prove helpful. The author (Dhar. *J. Phys. Chem.* **30**, 277 (1926)) has advanced the view that want of vitamin B would lead to insufficient metabolism and acidosis, and that the presence of acids will help in the retention of water in the animal economy. It is well known that in the wet form of beriberi, water is retained in various parts of the body and causes swelling. It appears, therefore, that the swelling in beriberi is due to the presence of acids, caused by the deficiency of metabolism of carbohydrates; and that alkalis ought to be beneficial for patients suffering from beriberi. It is very interesting to note that beriberi is most common amongst people who have to depend mainly on foods rich in carbohydrates, chiefly rice.

From a critical examination of typical cases of epidemic dropsy occurring in Bengal and the United Provinces, and the corresponding diet, it appears that the attack is due to malnutrition and want of vitamins, aided by humidity and insanitary living conditions. No

cases of epidemic dropsy were observed in those families which were taking germinating gram and other seeds. It is interesting to note that the working classes in India, who have more or less an outdoor life, and eat several raw things like onions, radishes, cucumbers, grams, maize, etc. do not suffer from deficiency and metabolism diseases, bad teeth etc., as much as the lower middle classes, who have to lead an indoor life, because of clerical and other duties, and do not get as much exercise and sunlight as the working classes do. Moreover, these lower middle class people cannot afford to have really good meals consisting of eggs, milk, meat, butter, fish, bread, etc. Unlike the working classes, they cannot eat raw food, owing to indigestion, and suffer constantly from metabolism and deficiency diseases.

According to Appleton (*Amer. J. Pub. Health*, **11**, 617 (1921)), the people of Labrador suffer constantly from deficiency diseases like beriberi, œdema, scurvy, night blindness, etc. because they are poor and cannot afford to have really good meals. Moreover, there is not much sunshine in Labrador.

PELLAGRA AND VITAMINS B AND C.

There has been considerable controversy with regard to the origin of pellagra, which is recognized as a typical tropical disease. One group of investigators believes that pellagra is an infectious disease, but admits that lowered vitality from faulty nutrition is a predisposing factor. Another group holds that this disease is caused by the lack of vitamin and is therefore analogous to beriberi and scurvy. There is yet no definite indication as to the lack of which vitamin leads to pellagra. It has also been suggested that the cause of pellagra lies in the absence of tryptophan, and perhaps also of lysine from zein, the chief protein of maize. The metabolic importance of the former amino-acid was established by Willcock and Hopkins (*J. Physiol.* **35**, 88 (1906)) in the case of young mice, that of the latter by Osborne and Mendel (*J. Biol. chem.* **17**, 325 (1914)). Occasional outbreaks of pellagra in institutions, camps, etc. have always been overcome by the inclusion of more milk, meat, eggs, or cheese in the dietary; but whether the cure was due to tryptophan in caseinogen is not thoroughly established. Miss Chick and Miss Hume (*Bioch. J.* **14**, 135 (1920)) describe experiments with monkeys on a diet rich in all known vitamins, but with zein as its principal protein. Symptoms closely resembling those of pellagra were produced. In one case, they cleared up rapidly when caseinogen was administered as well, but the crucial point, whether

it was tryptophan which made the difference, could not be established with certainty. The amino-acid appeared to have a beneficial effect, but no cure was effected with it alone.

The author has already pointed out that the symptoms of pellagra are allied to those of scurvy and pernicious anæmia. The researches of Goldberger and co-workers (*Pub. Health, Reports*; Wash. D. C. Sept. 11, 1914; Nov. 17, 1916; Nov. 12, 1920) have shown that this disease is most likely a deficiency disease, and Voegtlin (*ibid.* June, 1920) holds the view that pellagra is caused by a combination of the deficiencies of some of the well-recognized food factors.

Pellagra begins with a feeling of weakness and consequent disinclination to work. The patient has a pale and a peculiar staring look, and complains of headache, giddiness, and vague but often severe pains in the back and joints. He becomes irritable, stupid, and morose.

At first the tongue is coated, later it loses its epithelium and becomes sore. The gums swell and bleed easily. There may be eructations of gas with nausea and vomiting. The appetite is variable. The lower parts of the abdomen are sometimes tense and painful. These symptoms and many others are exactly similar to those of scurvy and pernicious anæmia. Moreover, as Vedder (*Arch. Intern. Med.* **18**, 137 (1916)) has pointed out "the gastro-intestinal lesions in pellagra and scurvy are analogous". Diarrhœa, enteritis, ulceration of the intestines and hæmorrhage in mucous membranes are observed in scurvy, pellagra, and pernicious anæmia. Moreover, there are similar nervous symptoms in pellagra and scurvy.

Suggestive similarities exist between pellagra and beriberi. It has been repeatedly demonstrated that in beriberi there is degeneration of many of the motor cells in the cord. Roberts describes the extensive degenerations which have been observed in the spinal cord in pellagra. These results conclusively show that pellagra, in certain respects, has many symptoms which are common to beriberi and scurvy, and it appears therefore that this disease is something like a combination of beriberi and scurvy. Consequently from the above arguments the author is of opinion that pellagra is caused by the lack of vitamins B and C. He would also urge medical men to treat the initial stages of pellagra with sunlight, which is very likely to be beneficial. In several parts of India the poorer classes have to depend mainly on maize for their food. Moreover they consume only a little protein of inferior quality. Though the food is so inadequate in quality and deficient in vitamins, yet cases of pellagra are not common; few cases were reported in Northern Behar. This is believed to be due to the fact

that we get a lot of sunshine in this country, which largely helps to prevent pellagra. Some have regarded pellagra as sunstroke of the skin. "Sun disease" was the old popular name for pellagra. We are, however, convinced that sunlight is efficacious in the treatment of pellagra, because in its presence the metabolism of food materials in the animal body is increased. In Italy and other countries, pellagra is more frequent in the beginning of spring. The author (Dhar, *J. Phys. Chem.* **33**, 1897 (1929)) has stated that this is due to the shortage of vitamins in food-stuffs in winter, and to the lack of sunshine, the animal body suffering from pre-pellagral conditions.

SPRUE AND VITAMINS B AND C.

Sprue or psilosis is a chronic catarrhal inflammation of the alimentary canal, characterized by a peculiar ulcerative condition of the tongue and the mouth, and large, pale, frothy stools. Dr. Miller of China expresses the belief that the disease is due to the consumption of canned and preserved meat. In this disease isolated patches of inflammation appear on the tongue, to disappear again completely. In course of time the surface of the tongue is denuded of epithelium, the mucous membrane becomes dry and fissured, the gums and the rest of the buccal mucous membrane are sympathetically affected by inflammation. These symptoms are more or less allied to those obtaining in pellagra, pernicious anæmia, scurvy, etc.

Milk cure, fruit treatment, meat juice, and underdone meat have been tried with success. The author has advanced the view that this tropical disease is, like pernicious anæmia, a deficiency disease caused by the lack of vitamins B and C in food materials.

LACK OF VITAMINS AND STOMACHIC DISORDERS.

We have already pointed out that disorders of the bowels are usually associated with lack of vitamins in food materials. Dysentery is very common in tropical countries, where semi-starvation and intake of inferior food materials are almost the natural order of things amongst the majority of the population, and in many cases dysentery is mainly due to lack of vitamins in food materials. It is needless to mention that the origin of dysentery is in some cases bacterial, and that in many cases bacterial infection and shortage of vitamins occur together.

Hill-diarrhœa is a gastro-intestinal catarrh seen in tropical countries, and marked by liquid, pale, frothy stools passed in the early morning. It can be distinguished from sprue by the absence of mouth

symptoms. A milk diet, as in sprue, is advised. It seems likely that this disease is also caused by the lack of vitamins in food materials.

In tropical countries, when partial famine conditions prevail, the poorer people have to consume inferior food materials, which in normal time nobody will eat. It has been repeatedly observed that bowel disorders are very common at such times, even when abnormally large amounts of food materials are not partaken of.

In several parts of India the poorer people have to depend mainly on maize for food, and in such places diarrhœa, dysentery, and other bowel troubles are frequent.

During the French Revolution food was scarce, and gelatine was extracted from bones and made into soup for the poor and the hospitals in Paris. It was very soon found that the continued use of such soups caused indigestion, nausea, burning thirst, and diarrhœa. Dogs fed with gelatine and bread suffered from profuse diarrhœa. During the last European war bowel disorders and œdema were frequent in many countries, where insufficient and inferior food materials had to be consumed.

These results show that the quality of the food taken is of great importance. It seems very probable that substances like gelatine, maize, etc. cannot be properly assimilated, and leave a residue which may cause irritation, produce gastric catarrh, and so lead to bowel disorders.

INFANTILE BILIARY CIRRHOSIS, PONOS AND WANT OF VITAMINS.

In India, a disease called infantile biliary cirrhosis is observed in large towns, especially amongst children under one year. In this disease nausea, occasional vomiting, sallowness, feverishness, constipation, irritability of temper, thirst, and languor are observed. On examination, the liver is found to be abnormally large. The stools are pale, and the urine is dark. At Allahabad (India) several cases of this disease in an acute form were studied, and the children could be cured after several months by administering food materials rich in vitamins. The children are fed with milk, which in India is always boiled, and thus the amount of vitamins is reduced. Consequently it appears that the food given to the children contains very small quantities of vitamins, specially of B, and hence this disease is very probably caused by the lack of vitamins in the food of the children.

Under the name "Ponos" Karamitsas and Stephonos have described a peculiar disease in the insanitary and poverty-stricken islands of

Spezia and Hydra in the Grecian archipelago. Ponos is analogous to the infantile biliary cirrhosis of Indian cities. Like the latter it is confined to very young children. In this disease the earliest symptoms are languor and pallor of the skin, which rapidly acquires a sallow tint. Fever of an irregular character sets in, and the spleen is enlarged. Constipation, diarrhœa, dysentery are common. When the disease has been established for some time œdema sets in, and there may be hæmorrhages in or from various organs, specially the gums. In certain cases boils and superficial gangrene are met with. It seems very likely that this disease is caused by the lack of vitamins, especially B, in food materials.

PURPURA AND HÆMOPHILIA, AND WANT OF VITAMIN C.

By purpura is meant a disorder of the system by which spontaneous hæmorrhages arise in the skin and from the mucous membranes.

The mucous membranes may bleed, sometimes hæmorrhages occur from nearly all the mucous surfaces, and the disease may run a fatal course.

Large hæmorrhages may thus occur from the bladder or the kidneys, from the intestines, from the stomach, and from the lungs, imperilling the life of the patient from sheer loss of blood.

Hæmophilia may be defined as a diathesis, hereditary or otherwise, characterized by a predisposition to hæmorrhages, which are either induced or spontaneous.

The cause of this disease is still shrouded in mystery. Milk is recommended because of its calcium content, and especially if the blood loss be excessive.

It seems very probable that both hæmophilia and purpura are caused by the lack of vitamins, especially vitamin C in food materials.

Recently, some favourable reports have appeared of the use of thyroid in hæmophilia.

TROPICAL SLOUGHING SORES.

The multitude of sores, ulcers, and gangrenous lesions, which meet the physician on every side in tropical countries among poorly nourished people, living under unsanitary conditions, is little short of bewildering. Chronic ulcers seem to be connected with malnutrition. In rapid tropical sores death usually results, otherwise excision in the first stages might be practised if the condition could be diagnosed early.

In the case of indolent ulcers, good results have been obtained from certain antiseptics. Skin grafting is valuable when the denuded surface is large. Good food and tonics seem to be the only treatment. It seems very probable that by malnutrition through lack of vitamins and foods of good quality, the system becomes highly susceptible to the invasion of all sorts of bacteria.

LIGHT AND DEFICIENCY AND METABOLISM DISEASES.

It is now definitely established that rickets can be healed by sunshine and ultraviolet light. Moreover, rickets is practically unknown in tropical countries, though, due to poverty, the food supply to children in the tropics is quite inadequate. It has been observed that pernicious anæmia can be treated by sunshine with success. It appears that sunshine ought to do good in other diseases like beriberi, scurvy, pellagra, etc., which are definitely known to be caused by the lack of vitamins. In tropical countries like India and China, owing partly to poverty and partly to lack of education, the food of the majority of the population certainly lacks vitamins in proper proportions. Consequently, we find that scurvy, beriberi, pellagra, and other vitamin-shortage diseases occur a great deal, but it is certain that these diseases would occur in much larger proportion were the compensating agent, namely sunlight, absent. It is urged that medical men should try the effect of sunshine and other forms of radiation on a larger scale than has been hitherto done, in the treatment of scurvy, beriberi, pellagra, and possibly cancer and other forms of deficiency diseases.

Ehrstrom of Finland (*Lancet* **1**, I, 1278 (1924)) states: — "We are well in the autumn after the light and open air in the summer, and we feel unwell in spring after the darkness of the winter". Possibly it is the lack of vitamins in the winter diet and the absence of sunlight in winter which causes the feeling of tiredness in the beginning of spring. A similar explanation holds good for the same feeling in the spring reported by the early settlers of America.

Huldschinsky (*Z. Kinderk.* **26**, 207 (1920)) has used in tetany the sunray therapy with which he was successful in the treatment of rickets. Hart, Steenböck, Lepkovsky, and Halpin (*J. Biol. Chem.* **58**, 33 (1923)) observed the beneficial effect of sunlight on the growth of chicks. Hughes (*Science* **59**, 213 (1924)) found that a condition identical with rickets developed in chicks when the birds were given satisfactory food but received sunlight filtered through glass. Chicks receiving the

same food but exposed to direct sunlight for a few hours daily developed normally. Cramer and Drew (*Brit. J. Expt. Path.* **4**, 271 (1923)) kept rats in darkness on a diet poor in vitamin A and found a diminution of blood platelets as compared with rats kept in well lighted room on a similar diet. Exposure to the mercury vapour lamp increased the number of platelets to normal.

It seems certain from the foregoing results that sunlight and artificial light are very favourable for growth, and can stop rickets. Hence light plays the same rôle as that of vitamins A and D.

In presence of light, the oxidation of fats is likely to be increased more than that of carbohydrates, and that is why light is more efficacious in rickets than in diabetes, beriberi, and scurvy; but as we have proved experimentally that light can accelerate the oxidation of three classes of food materials — carbohydrates, fats, and proteins — by air, we are convinced that light should prove efficacious towards deficiency of metabolism, and other diseases like beriberi, epidemic dropsy, scurvy, pellagra, cancer, diabetes, gout, etc.

The experiments on pigeons and rats carried on in the author's laboratories (Chakravarti and Dhar, *Indian. J. Med. Research,* **17**, 430 (1929), Palit, Kaul, and Dhar (*J. Phys. Chem.* **34**, 737 (1930)) show that these animals do not get polyneuritis and eye troubles when fed on Rangoon rice (polished) alone for about 24 days, provided they get some sunshine daily. Leafy vegetables when mixed with Rangoon rice appear less effective than sunlight in the prevention of polyneuritis and eye troubles. Colloidal ferric hydroxide and ferric chloride in very small doses are as effective as leafy vegetables.

From the foregoing discussion, it appears very likely to the author that vitamins, internal secretions, and light play more or less the same rôle in animal metabolism; and that one can replace either of the other two factors under suitable conditions. One or other of these three factors seems to be indispensable for normal growth, reproduction, and well-being. A proper realization of this principle will lead to a greater utilization of the inexhaustible wealth of sunlight which forms the priceless heritage of animal life on this planet.

CHAPTER VIII

Normal Ossification, and the Formation of Crystalline Deposits in Diseases.

The following views on normal bone formation were advanced some years ago by the author (Dhar, *Z. anorg. u. allg. Chem.* **162**, 243 (1927)). It is well known that a freshly prepared precipitate of barium sulphate is capable of adsorbing and coagulating colloidal solutions of gold, ferric hydroxide, arsenious sulphide and several other substances. Moreover, barium sulphate is capable of adsorbing ions like oxalate, iron, copper, etc. (*Koll. Zeit.* **35**, 144 (1924)). In papers published from these laboratories we (*Koll. Zeit.* **34**, 270 (1924); **37**, 2, 89 (1925); *J. Phys. Chem.* **28**, 41 (1926)) have shown that freshly obtained precipitates of silver chromate, lead chromate, ferric hydroxide, etc. can adsorb and coagulate their respective sols. Thus when a sol of lead-chromate is obtained by the interaction of a dilute solution of lead acetate and potassium chromate in presence of agar, this sol can be adsorbed and coagulated readily when shaken with freshly precipitated lead chromate. These results on adsorption and coagulation have been utilized by us in explaining periodic precipitation obtained in the laboratory as well as in nature. In a recent paper Ganguly (*J. Chem. Soc.* **129**, 1381 (1926)) has shown that when a solution of calcium bicarbonate, in presence of gelatine, is allowed to evaporate in air, iridescence, owing to a fine periodic deposition of calcium carbonate, is seen. These observations have been utilized in offering an explanation of normal bone formation, and abnormal calcification in certain diseases.

NORMAL OSSIFICATION AS A COLLOID-CHEMICAL PROCESS.

It is well known that bones are formed either by ossification of connective tissue or by the replacement of cartilage by bone tissue. The osteoblasts are supposed to hollow out the cartilage, and, after destroying cartilage cells, deposit further formless material upon the remains of the matrix, and so promote its calcification.

Through food the human body absorbs calcium salts and phosphates. These react in body fluids, viz. blood, sera, etc. forming calcium

phosphate. It has been proved that many sparingly soluble substances, e.g. silver chloride, silver bromide, mercurous chloride, calcium phosphate, etc. can remain in the colloidal state in presence of gelatine, albumin, gum arabic, etc. We are of the opinion that the calcium phosphate in the human body exists mainly in a colloidal form due to the peptizing influence of serum, albumins, proteins, and other body colloids. It is well known that phosphoric acid is a weak acid; so that calcium phosphate can be readily dissolved by mineral acids. Hence if there is an accumulation of hydrogen ions in the body fluids, the phosphate ions will be removed according to the following scheme:—

$$H^{\cdot} + PO_4''' \rightarrow HPO_4''$$
$$H^{\cdot} + HPO_4'' \rightarrow H_2PO_4'$$
$$H^{\cdot} + H_2PO_4' \rightarrow H_3PO_4$$

So the presence of acids in the body fluid in quantities greater than p_H 7·45 will cause a dissolving of calcium phosphate existing in a colloidal state in the body.

Similarly it is probable that small quantities of calcium carbonate exist in the body fluid in a colloidal state, due to the interaction of calcium ions and carbonate or bicarbonate ions. This carbonate can be dissolved by the carbonic acid present in the body; moreover it can also be very readily dissolved by hydrogen ions:

Carbonic acid.

$$CaCO_3 \text{ (colloid)} + H_2CO_3 \rightarrow Ca(HCO_3)_2 \text{ (dissolved)}$$

Calcium Carbonate — Calcium bicarbonate.

$$H^{\cdot} + CO_3'' \rightarrow HCO_3'$$
$$H^{\cdot} + HCO_3' \rightarrow H_2CO_3.$$

Hence it is clear that the dissolving or precipitation of calcium carbonate or phosphate is susceptible to slight changes of hydrogen ion concentration in the body fluid. In normal health the blood is slightly alkaline. We have already said that barium sulphate can adsorb and coagulate various colloidal solutions, and also that lead chromate can adsorb and coagulate its own sol. Consequently it appears that the semi-solid calcium phosphate existing in cartilage adsorbs and coagulates the sol of calcium phosphate existing in the body fluids, and thus a layer of calcium phosphate is deposited on the cartilage. Similarly

the calcium carbonate present in the cartilage is also capable of adsorbing and coagulating its own sol, and hence a layer of calcium carbonate is formed on the cartilage. As both these processes go on simultaneously there is a deposit containing both calcium phosphate and calcium carbonate, more or less uniformly distributed. Now along with this precipitation of calcium phosphate and carbonate on cartilage, some of the peptizing media, viz. serum, proteins, etc. will also be simultaneously precipitated on the cartilage. This process will repeat itself under normal conditions, producing normal ossification. Hence it is pretty certain that there is a definite equilibrium between the amounts of calcium carbonate and calcium phosphate that remain in the peptized condition, and the amounts that are obtained as bones. It should be emphasized that the tissues consisting of organic materials in the cartilage can also adsorb the colloidal solutions of calcium phosphate and calcium carbonate, just as barium sulphate adsorbs a sol of ferric hydroxide. Consequently the presence of solid or a semi-solid matter is essential for the coagulation of inorganic colloids like calcium phosphate and calcium carbonate. Moreover, this precipitation is very susceptible to the changes in hydrogen ion concentration in the body fluids. Hence it is not surprising to find that the mode of growth of bones is extremely sensitive to disturbances of metabolism.

POROSITY OF BONES IN OLD AGE.

It is well known that in old age the bone becomes thin and less compact, and it is also known that metabolism in old age is not so good as in youth. It seems probable also that in old age the hydrogen ion concentration in the body fluids may be slightly greater than in youth, and hence in course of time the amounts of calcium phosphate and calcium carbonate which were present in youth will decrease due to the dissolving action of the increased hydrogen ion concentration in the body fluids in old age.

There is another probable way of explaining the porosity of bones in old age. It is well known that with age the amount of water present in the body is decreased. Consequently the concentration of the body fluids which act as peptizing agents for the calcium carbonate and phosphate is increased. We have repeatedly observed that the amount of a sparingly soluble substance which can be kept in suspension is greatly increased when the concentration of the peptizing agent is increased. Consequently in old age, the concentration of the body colloids being greater than in youth, the amounts of calcium carbonate

and phosphate which can remain in suspension appear to be greater; thus the equilibrium between the amounts of calcium carbonate and phosphate held in suspension and those deposited is displaced. In other words, a part of the calcium phosphate and carbonate in the bones will pass into the colloidal state, and hence the bones will be thinner and more porous.

Renal calculi may be composed of calcium oxalate, uric acid, urates, phosphates, cystein, or a mixture of these. Pure uric acid stones may occur in quite young children, because in them a definite deposit of uric acid crystals in the pyramids and pelvis of the kidney is almost a normal event. Renal calculi may occur at any age, but are very rare in the old. Alcohol and lead acetate are said to predispose to renal calculi.

These facts will be readily understood from the following considerations:—It is well known that the percentage of water which enters into the composition of the animal body decreases with the age of the animal. Consequently in children the concentration of the body colloids being less than that in old age, the amounts of calcium oxalate, uric acid, phosphates etc. which can remain in suspension in childhood is much less than in old age. There is greater likelihood, then, of the precipitation of sparingly soluble substances like calcium oxalate, uric acid, etc. occuring as deposits of renal calculi in childhood than in old age. Hence renal calculi are more common in the young than in the old.

It seems likely that alcohol and lead salts act as precipitants for substances like uric acid and calcium oxalate which exist as colloids in the body, and hence alcohol and lead salts help the deposition of the calculi.

DEPOSITS ON DEAD TISSUES.

Nothing is commoner than to find necrotic tubercles in the lung or elsewhere, which have become encapsulated within a dense connective tissue, and in the central parts of which rough granules of lime have appeared in the cheesy substance. The lymph glands of the mesentery undergo the same changes after tubercular infection, and may be finally converted into rough white masses like pebbles. Sublimate poisoning and anæmic necrosis in the kidneys are frequently followed by an abundant precipitation of calcium. Moreover it is extremely common to find calcified plates in tumours. Consequently we may look for dead tissues as the basis for deposition of lime. It is probable that in dead tissues the organic matter is partly destroyed and becomes

less. Hence the inorganic calcium carbonate and phosphate which remain become more prominent, due to the loss of their covering organic matter, and are capable of adsorbing and coagulating the sols of calcium phosphate and carbonate present in the body fluids. Hence deposits of calcium carbonate and phosphate generally occur round about the dead tissues, which are certainly thicker in consistency than living tissues, and thus act as coagulating agents of calcium phosphate and carbonate sols.

In the case of tumours, where the circulation has become deficient, the sols of calcium carbonate and phosphate remain in contact with the thick growth for a longer interval because of the deficiency of circulation, and hence coagulation and precipitation are facilitated.

RICKETS AND DEFECTIVE METABOLISM.

We have already shown that rickets is very probably due to the incomplete metabolism of fatty food materials. We have also stated that the metabolism of fat is accelerated by the presence of either vitamins A and D or thyroid or adrenaline secretions. Moreover, metabolism in general can be accelerated by sunlight; and hence rickets can be partially or wholly avoided by sunlight or suitable artificial light. It is well known that the incomplete metabolism of fat leads to acidosis in diabetes and in later stages of starvation. Similarly, defective metabolism leads to the generation of acids, and precipitation of uric acid in gout. Hence alkalis are given with great advantage as medicine in the treatment of diabetes, gout, digestive disorders, and gastric catarrh. The bicarbonates of sodium and potassium have been used, and also the citrates and tartrates. We have proved experimentally that the presence of alkalis is associated with greater oxidation of carbohydrates, fats, and proteins. Hence addition of alkalis is likely to increase metabolism in the animal body. It is well known that pancreatic secretion is very helpful in carbohydrate metabolism, and that this secretion contains OH ions.

IMPORTANCE OF MILD ALKALIS IN HEALTH AND DISEASE.

Sodium citrate in small quantities is usually added to milk to improve digestion in infant feeding. The function of the citrate is probably two-fold, and it acts in the following way:—Milk is a negatively charged colloid, and in presence of citrates the negatively charged sol adsorbs some citrate ions so that the stability of the colloid is increased.

Consequently coagulation and clotting of milk are markedly retarded by the addition of citrates. Milk contains together with calcium appreciable quantities of citric acid. Hence the view that the addition of citrate acts as a decalcifier of milk seems incorrect. The particles of fats, proteins, and calcium citrate present in the milk are stabilized by the preferential adsorption of citrate ions, and coagulation and clotting are retarded. There is another function of the sodium citrate added to milk: citric acid being a weak acid, a sodium citrate solution is slightly alkaline; hence the addition of citrate makes the milk slightly alkaline and liable to be oxidized more readily in the human body. Moreover it is known that citrates retard the clotting of animal blood. This is also due to the preferential adsorption of citrate ions by the negatively charged colloid, blood, and its consequent stabilization towards coagulation. Exactly similar results are obtainable with tartrates. The clotting of blood is also retarded by the presence of albumin, which is adsorbed by blood and acts as a marked peptizing agent.

It has been proved that along with the defective metabolism and stunted growth, which follow destruction of the thyroid, general retardation in the development of bone also occurs. We are of the opinion that in rickets, which is probably a disease caused by defective metabolism of fats, there is a slight increase in the concentration of hydrogen ions in the body fluids. Hence according to the views already expressed, deposition of calcium carbonate and phosphate on the cartilages will be decreased, and bone formation will be defective. Thus in rickets and osteomalacia, thyroid and alkali treatment should prove beneficial. *Hence in normal ossification and in calcification in several diseases, coagulation, peptization, and concentration of hydrogen ions are likely to play an important rôle.*

The foregoing views on bone formation may be summarized thus:— Normal calcium phosphate exists in the serum mostly in the colloidal condition, due to the peptizing influence of serum-albumin and other organic colloids. Similarly, small amounts of calcium carbonate also exist in the colloidal condition in the serum, some of it also being present as bicarbonate. This view easily explains the fact that serum contains more calcium salts than can be dissolved in the form of calcium phosphate or carbonate by the same volume of water. That at least some of the calcium exists in the colloidal condition is shown by the researches of Cushny (*Chem. Soc. Ann. Reports* **17**, 161 (1920)). This investigator found that when serum is filtered through collodion all the crystalloids pass through except some of the calcium and mag-

nesium. Further work has supported his observations, so that it is now generally recognized that both these elements are present to a certain extent in the serum in a form which will not pass through a dialyzing membrane with ease. It has also been found that the proportion of non-dialyzable calcium is variable. The writer has also stated that there seems to be a definite equilibrium condition in the body as represented by the following scheme:—

$$3\,Ca^{\bullet\bullet} + 2\,PO_4''' \rightleftarrows Ca_3(PO_4)_2 \text{ (colloidal)} \rightleftarrows \underset{\text{Solid in bones.}}{Ca_3(PO_4)_2}$$

A similar equilibrium may also exist in the case of calcium carbonate. In a recent paper Loeb and Steinberger (*J. Gen. Physiol.* **61**, 452 (1924)) have shown that the proportion of calcium passing through the membrane is as low as 55, when serum is dialyzed against water; but that when it is dialyzed against hydrochloric acid at p_H 2·5 or against 0·8 per cent sodium chloride solution at p_H 7·4, all the calcium passes through the membrane. These results would appear to show that an equilibrium exists between the dialyzable and non-dialyzable calcium serum, such as that indicated above. Clark (*Amer. J. Hyg.* **3**, 481 (1923)) found the proportion of diffusible calcium raised after exposure of serum to ultraviolet light. The above observation of Loeb and Steinberger that all the calcium can be made to pass through the membrane when dialyzed against hydrochloric acid at p_H 2·5 can easily be explained as follows:—Owing to the increase in the H-ion concentration, some of the calcium phosphate and calcium carbonate existing in the colloidal condition will dissolve and pass out. This process will continue until all the calcium in the colloidal condition passes out.

Some physiologists believe that, even after subtracting the amount of non-dialyzable calcium present, there still remains that present in diffusible forms, the concentration of which is considerably greater than that of saturated solutions of calcium carbonate and phosphate. This has led some authorities to view the serum as a metastable supersaturated solution of calcium salts (compare Rona and Takahashi, *Bioch. Zeit.* **49**, 370 (1913)). It may be pointed out here that the exact amounts of diffusible and non-diffusible calcium cannot be determined with accuracy from dialysis experiments for the following reasons:— It has already been pointed out that an equilibrium exists in the serum:—

$$\underset{\text{in solution.}}{Ca_3(PO_4)_2} \rightleftarrows \underset{\text{colloidal state.}}{Ca_3(PO_4)_2}$$

As soon as some of the calcium phosphate existing in true solution passes through the membrane the above equilibrium is disturbed, and hence some of the salt in the colloidal condition goes into true solution; this, in turn, passes out through the membrane. This process continues, and theoretically it is possible, by prolonged dialysis with constant replacement of the dialyzing liquid, to remove all the calcium. In the light of these considerations, there is no wonder then that the proportion of dialyzable calcium is found to be variable.

Granted then that most of the calcium phosphate and carbonate present in serum exists in the colloidal condition, how can the process of normal ossification be explained? The author has suggested that the calcium phosphate present in cartilage coagulates and adsorbs the colloidal calcium phosphate. Similarly the colloidal calcium carbonate is adsorbed and precipitated by the calcium carbonate present in cartilage. Thus normal ossification takes place. Such an adsorption of a substance in the colloidal condition by a freshly formed precipitate of the same substance is a phenomenon of common occurrence (compare Dhar and Chatterji, *J. Phys. Chem.* **28**, 41 (1924); Sen and Dhar, *Kolloid. Zeit.* **34**, 270 (1924); Dhar and Chatterji, *ibid.* **37**, 2, 89 (1925); *Zeit. anorg. u. allg. Chem.* **159**, 186 (1927)).

If the food lacks either calcium or phosphate, bone formation cannot take place normally because the proper concentration of calcium phosphate in the colloidal state to be adsorbed by the cartilage is not attained. If the food is rich in phosphorus and lacks calcium there might be a tendency to form acid phosphate of calcium, which is soluble. Recent experiments show that even though the food is adequate in its calcium and phosphorus contents, but lacks vitamins A and D, rickets and osteomalacia and other bone troubles follow. We can explain these results by the following considerations:—

If by any means there is a slight increase in the H-ion concentration of the blood, the calcium phosphate and carbonate existing in the colloidal state will partly dissolve, and hence their concentrations in the colloidal state will decrease, giving rise to a disturbance of bone formation. It is interesting to note that Miss Hodgson (*Lancet.* **2**, 945 (1921)) found in many cases of rickets that acidosis developed at the height of disease. Pritchard (*Brit. Med. J.* **1**, 887 (1923)) stated his belief that the true cause of rickets is probably a relative excess of acid substances produced in the system. Burgess and Osman (*Lancet* **1**, 281 (1924)) reported three cases of acute rickets in which they found severe acidosis. This acidosis may be the result of improper oxidation of fats, due to the lack of vitamins A and D. Sunlight, iron preparations,

and vitamins aid the complete combustion of food materials, and hence prove efficacious in the cure of rickets. Hess and Lundagen (*J. Amer. Med. Assoc.* **79**, 2210 (1923)) note that there may be seasonal variations in the amount of calcium present in children's blood; and that minimum values are usually observed in March, when the incidence of rickets is also at its height. The values may rapidly be brought again to normal by exposing the child to short wavelength radiation, or by administering cod liver oil.

It is of interest to note that Tisdall and Harris (*J. Amer. Med. Assoc.* **79**, 884 (1922)) found the inorganic phosphorus content of normal serum from birth to twenty years to be remarkably constant at 5·6 milligrams per 100 c.c. of serum. At twenty years of age the phosphorus content drops sharply to 3·75 mgs., and remains constant throughout adult life. During the period of union of fractures in adults, these observers found that the phosphorus content of the serum should be raised to about the same level as in childhood. Peterson (*Johns Hopkins Hosp. Bull.* **35**, 378 (1924)) found it possible by dietetic management to lower the phosphate content of the blood to a point where the product of calcium and phosphorus was less than thirty. He concluded from his study that in the healing of fractures a definite relationship exists between the concentration of inorganic bone forming elements in the serum and the rate of repair. If the phosphorus —calcium product is again raised to its normal level, the fractured bones will unite. He applied these conclusions to the case of a man aged 47, who had a fractured bone, and by improvement of diet and exposure to light, he could easily cause the fracture to join. These experimental results clearly follow from the consideration of the equilibrium:—

$$3\,Ca^{\cdot\cdot} + 2\,PO_4^{\prime\prime\prime} \rightleftarrows Ca_3(PO_4)_2 \text{ (colloidal)} \rightleftarrows Ca_3(PO_4)_2 \text{ solid in bones.}$$

The author believes that the decrease in the amount of calcium phosphate existing in serum with increase of age is due to a gradual but slow decrease of the alkalinity of the blood with old age. In this connection it is of interest to note that the requirement of vitamin A decreases with age.

For a number of years Freudenberg and György have investigated the influence of various conditions on the adsorption of calcium salts from solution by pieces of cartilage *in vitro*. Their results cannot be said to have advanced, to any appreciable extent, our knowledge of the process of calcification. Freudenberg and György (*Bioch. Zeit.* **142**, 407 (1923)) have put forward a hypothesis, according to which calcifica-

tion takes place in the following stages:—(a) Cartilage protein + calcium = Ca-cartilage-protein; (b) phosphate + Ca-cartilage-protein = Ca-cartilage-protein-phosphate; (c) Ca-cartilage-protein-phosphate = Calcium phosphate + cartilage-protein.

The above views of Freudenberg and György seem to throw very little light on the process of normal ossification.

ROBISON'S WORK ON BONE FORMATION.

In investigating the products of fermentation of sugar by yeast, Harden and Robison discovered the presence of a hexose monophosphoric ester. Robison isolated the compound and described its properties (compare *Bioch. J.* **16**, 809 (1922)). It is entirely different from the compound prepared by Neuberg (*Bioch. Zeit.* **88**, 432 (1918)), and yields calcium and barium salts which are readily soluble in water. Robison was led to investigate the action of enzymes on solutions of these salts, and found that in some cases the ester was hydrolyzed; and the progress of the hydrolysis was shown by the deposition of sparingly soluble calcium or barium phosphate. These results at once suggested to him the possibility that such a soluble calcium salt might be the form in which the mineral was carried to ossifying cartilage, there to be broken down by an appropriate enzyme into calcium phosphate and free sugar. Experiments soon showed that ossifying cartilage of young animals actually possessed the power rapidly to hydrolyze this hexose monophosphoric acid, whereas of all other tissues the only ones showing this power to any significant degree were the kidney and the intestines.

A further task was to demonstrate that compounds of the type of hexose monophosphoric ester do occur in the circulating blood. Goodwin and Robison (*Bioch. J.* **18**, 1161 (1924); also Robison and Soames *ibid.* 740; Kay and Robison, 755)) have isolated two esters of this type. One of these shows reducing properties, is optically laevorotatory, and is rapidly hydrolyzed by the enzyme found in ossifying cartilage, the teeth, the kidney and the intestines. In the young animal, its activity would appear to be more marked in the first two positions than in later life, but its activity in the kidney and the intestines seems to be less variable with age. At body temperature the enzyme shows greatest activity at H-ion concentration between p_H 8·4 and 9·4. Recently Martland and Robison (*Bioch. J.* **23**, 237 (1929)) gave a description of the preparation of the phosphatase of ossifying bone. The enzyme is best prepared from the bones of young rachitic rats by

extraction with chloroform water during 7—10 days, the bones first having been split longitudinally and the marrow removed. Evaporation of the filtered extracts over sulphuric acid in evacuated desiccators leaves a dry residue yielding 5—8 mgs. per c.c., no loss of activity occurring during the drying process. Purification can be effected in various ways; for instance, the active protein present in the dry residue may be precipitated at its isoelectric point (p_H 5·8); the enzyme may be precipitated with a mixture of alcohol and ether, or resort may be had to dialysis or ultrafiltration. No evidence of the existence of a co-enzyme for the bone phosphatase could be obtained.

To understand Robison's point of view, we can assume for the sake of argument that the serum and body fluids are saturated solutions of calcium phosphate and carbonate. There are definite conditions which must lead to deposition of these salts. One of these is a rise in concentration of phosphate or carbonate ions, and this will occur if there is an increase in the concentration of inorganic phosphate or a change of reaction towards the alkaline side.

The first of these conditions is set up if hydrolysis of a soluble hexose-phosphate takes place by the agency of the bone enzyme. The possibility of a change in the reaction of the tissue fluids occurring at centres of ossification must also be considered, since Robison has found that the enzyme shows its optimum activity between p_H 8·4—9·4, a reaction very much more alkaline than is associated with ordinary tissues. It is impossible yet to say whether *in vivo* the osteoblasts possess the power to raise the alkalinity of the blood in their immediate neighbourhood, and so enable the enzyme to work in the most efficient manner. Robison further adds that, as the equilibrium between HCO_3' and CO_2 will also be shifted by the change of reaction, there will tend to be deposition of calcium carbonate. On these grounds Robison explains the ten per cent or so of this substance which is normally found in bone.

Martland and Robison (*Bioch. J.* **18**, 740 (1924)) have shown by examining the cartilages and bones of human embryos and young infants, that although the ossified portions of young normal bones contain phosphatase in high degree, the non-ossifying cartilage is in all cases devoid of the enzyme. Fell and Robison (*Bioch. J.* **23**, 767 (1929)) have recently made an interesting investigation. The object of this has been to correlate the growth, the development, and the phosphatase activity of isolated early embryonic femora, and of undifferentiated limb-buds cultivated *in vitro*; and to compare these processes in the transplants with the corresponding process in the normal embryonic limb.

COMPOSITION OF BONE SUBSTANCE.

Taylor and Sheard (*J. Biol. Chem.* **81**, 479 (1929)) have investigated by X-ray diffraction patterns, and by an optical method, several types of calcified tissues, including normal bone, dental enamel, rachitic bone, bone low in phosphorus, and salivary and tubercular calculi, and find that the solid inorganic phase consists essentially of very small crystals of apatite minerals of the general formula $3\,Ca_3(PO_4)_2$, CaX_2, where X_2 ordinarily represents CO_3, F_2, $(OH)_2$, O, and SO_4; Ca can to some extent be replaced by Mg. The typical minerals of this formula are podilite, dahllite, and fluorapatite. No evidence of the presence of brushite, $CaHPO_4$, $2\,H_2O$ was obtained in either normal or pathological deposits. On the other hand, Shear, Washburn, and Kramer (*J. Biol. Chem.* **83**, 697 (1929)) still regard the composition of bone as an open question, and state that the presence of $CaHPO_4$ in bone generally and in primary calcification especially, should not be overlooked. Klement (*Zeit. physiol. Chem.* **184**, 132 (1929)) has published data which lead him to the conclusion that the inorganic portion of bone consists principally of a basic calcium phosphate of the composition $3\,Ca_3(PO_4)_2$, $Ca(OH)_2$, with inclusions of alkaline-earth and alkali bicarbonates. Lambie, Kermack, and Harvey (*Nature* **123**, 348 (1929)) state that the administration of parathyroid hormone to rats appears to cause a change in the form in which calcium exists in the bones. The author believes that the composition of bone is variable, and depends on the following factors (1) the concentration of the albuminous and other peptizing substances in the serum, (2) the p_H value of the serum and body fluids, and (3) the activity of the glands producing internal secretions like the parathyroid and the adrenals.

CHAPTER IX

Gout, Fever, and Use of Alkali, Phosphates, and Iron Preparations.

FORMATION OF DEPOSITS IN GOUT.

Gout has been defined "as a disorder of metabolism associated with retention of uric acid, and of other purin bodies in the system, characterized clinically by attacks of acute arthritis, the deposition of sodium biurate in and about the joints, and by the occurrence of irregular constitutional symptoms" (Osler: *Principles and Practice of Medicine*, 417 (1912)).

The veins around the painful joint first appear swollen, and shortly the whole joint becomes swollen, tense, shining, and of a deep red colour. The subcutaneous tissues are œdematous, and pit on pressure. It is believed that these joint affections are dependent on the precipitation and deposit of sodium biurate crystals in the cartilages, ligaments, synovial membranes, bursæ and other structures forming part of, or in proximity to, the articulation. In some of the most distressing forms of chronic gout the joints of the fingers, and less frequently those of the toes, become surrounded by massive deposits of sodium biurate, which sometimes ulcerate slowly through the skin. It has been observed that masses as large as horse beans are discharged. Small deposits are also often observed on the margins of the ears, and occasionally on the inside of the eyelids, on the arches of the palate and elsewhere. The most serious manifestations of gout are the cardiovascular and renal changes that are determined by the gouty state.

The crystalline urates precipitated in the cartilaginous and fibrous structures of the joints necessarily act as foreign bodies. They excite irritation, clog the lymph channels, exercise pressure on the tissue elements, and impede their nutritive operations; hence the inflammation, pain, swelling, and subsequent degenerative changes in the joints.

An attack of acute gout is commonly preceded by some troubles of digestion—acidity, flatulence, loss of appetite, constipation, and scanty but highly coloured urine. Before, and at the commencement of attack, the output of uric acid is generally low, but rises to normal or

above with full establishment of the arthritis. There is a remarkable parallelism in the output of uric acid and phosphoric acid both during and between the attacks.

According to newer views, gout is regarded as the disturbance of purin metabolism, or a perversion of the general metabolism of the tissues. The original crude experiments of Garrod in 1848, and the more recent investigations of Klemperer, Magnus-Levy, and Brugsch, have shown beyond doubt that the blood of the gouty contains an abnormal amount of uric acid. According to Brugsch and Schittenhelm (*Zeit. f. exper. Ther.* **4**, 438 (1907)); compare also Brugsch (*Berlin. Klin. Woch.* No. 34 (1912)); and Bloch (*Zeit. f. physiol. Chem.* **51**, 472 (1907)). This holds good in a remarkable manner for the gouty even on purin-free diet; from which, therefore, it may be inferred that the accumulation of uric acid in the blood also results from an abnormality in the usual course of the endogenous purins originating from tissue destruction, independently of the purin exchange. It was, however, early recognized that the increase of uric acid in the blood could not alone be regarded as the cause of the deposition of sodium biurate or uric acid in the tissues, precisely analogous increments being observed in leukæmia, and also in pneumonia. In the latter conditions no deposits are observed.

Uric acid increase in the blood may be due to

(1) increased formation of uric acid from the destruction of tissue nucleins,

(2) decrease of uric acid destruction by oxidation,

(3) decrease of uric acid excretion in the urine.

As regards (1), there is not much evidence for assuming that the primary factor in the pathology of gout is to be ascribed to cellular destruction; though at times there does occur an exaggerated destruction of tissue. With regard to destruction of uric acid by oxidation there was a vigorous discussion about twenty-five years ago. Burian and Schur's classic work may be looked upon as the central point of defence of those who believed that uric acid is oxidized in human tissues, whereas Wiechowski was one of the chief antagonists of this view. The first school, headed by Burian and Schur, considered the increase of uric acid in the blood of the gouty to be due to the lowered capacity of the gouty individual to destroy his uric acid by oxidation. The other school believed that the increase of uric acid in the blood was due to diminished excretion in the urine. There must exist some cause in gouty affections which makes uric acid excretion impossible, and this is

probably the retention affinity of the tissues, because of which uric acid is actually held within them. The idea of retention of uric acid in the tissues is important; if we are to reject the idea of retention in the tissues it will be difficult to understand why gouty patients do not simply expel by a compensatory hyperexcretion the uric acid which accumulates on account of failure of uricolysis, precisely as in leukæmia, where the patient compensates by an exaggerated excretion of the uric acid which accumulates in the body from excessive purin decomposition.

Recently Folin, Berglund, and Derick (*J. Biol. Chem.* **60**, 36 (1924)) have carried out experiments with men to determine whether uric acid is broken down in the human organism. They conclude that the human organs other than the kidneys are impermeable to uric arid. From 30—90 per cent is excreted by the kidneys, but evidence is presented to show that from 10 to 70 per cent is destroyed. Experiments with subjects prone to gout have led Folin and his collaborators to believe that in this disorder the organism possesses normal powers of destroying uric acid, but that the responsiveness of the kidneys to excretion is subnormal, and results in the maintenance of a high level of uric acid in the blood.

EXPLANATION OF THE ORIGIN OF DEPOSITS IN GOUT, ETC.

The author (Dhar. *J. Phys. Chem.* **30**, 277 (1926)) has accounted for the formation of deposits in gout in the following way:—From his researches on colloids, he is convinced that sparingly soluble substances like uric acid, calcium phosphate, calcium oxalate, etc. remain in the colloidal condition in the system owing to the presence of protective albuminous substances, which act as peptizing agents. When these colloidal substances are coagulated and crystallized in tissues we get diseases like gout, renal colic, oxaluria, lithiasis, gallstones, etc., which are diseases caused by the deposition of these sparingly soluble substances in various parts of the body.

How does this coagulation take place? The coagulation of peptized substances can take place from one or more of the following causes:—

(1) Increase of electrolytes in the system.
(2) Decrease of the peptizing influence either by loss of or dilution of the protecting agent.
(3) Decrease of temperature.
(4) Increase of concentration of the substance which is being peptized.
(5) Adsorption of peptized substance by some solid adsorbent.

Let us consider factors (2) and (4). The author has pointed out that an attack of gout is commonly preceded by some troubles of digestion, such as acidity, flatulence, constipation, scanty and highly coloured urine. When the system is not working properly, and the excretions are not normal, we get an extra amount of water in the system. The writer is of opinion that an important cause in the formation of deposits of uric acid, calcium oxalate etc. is the presence of the extra amount of water which in normal circumstances should have been eliminated from the system. This surplus water dilutes the peptizing agents (albuminous substances), and hence substances like uric acid, calcium oxalate, calcium phosphate, etc. which normally remain in the peptized condition in the system become coagulated. This view explains the prevalence of gout in cold and humid climates. The loss of water by perspiration, evaporation etc. is much less in humid and cold climates than in warm dry climates, and hence retention of water and consequent dilution of protective agents in the system is facilitated in humid and cold climates. Purgatives have been found to be effective in the treatment of gout; and according to the author's view, they should be effective because they help the elimination of large quantities of water, purin bodies, etc.

Moreover, the acidity which is usually associated with an attack of gout plays a very important part in the separation of uric acid. From the researches of Pauli and Samec we know that the solubility of sparingly soluble substances is increased by the presence of serum albumin and other protective agents, whilst the solubility of readily soluble substances is decreased, due to the presence of albuminous substances. Bechhold and Ziegler have shown that the solubility of uric acid is much greater in serum albumin than in water, whilst that of sodium urate is decreased, due to the presence of serum albumin. It seems certain that in gouty blood sodium urates should also be present along with uric acid. Now if the blood becomes acid, the sodium urate will be decomposed by the acids generated owing to insufficient oxidation, and uric acid will be set free, which being more sparingly soluble than sodium urate is likely to separate as a precipitate, due to the presence of the already existing urate ions. Therefore, it appears certain that the acidity caused by the lack of proper metabolism helps in the precipitation of uric acid in gout.

It is well known that the swelling of organic substances, like gelatin, albumin, etc. in water is accelerated by the presence of small quantities of free acid or alkali; thus the acidity produced in the system due to

want of proper metabolism helps the retention of water by the tissues, and thus facilitates the dilution of the protecting agent (albuminous matter etc.) and causes the separation of uric acid.

LOCALIZATION OF URIC ACID DEPOSITS.

As for the preferential formation of deposits in certain localities, the author suggests the following explanation: —

We have shown definitely that substances like barium sulphate, freshly precipitated ferric hydroxide, lead chromate, etc. can adsorb appreciable quantities of sols of various substances (compare Dhar and Chatterji, *J. Phys. Chem.* **28**, 41 (1924)). Hence we are convinced that the bone or cartilage of the system can adsorb the peptized uric acid, calcium oxalate, calcium phosphate, etc. existing in the system. Consequently the deposition of these substances round about the bones, cartilage, etc., is favoured. Many years ago Almagia (*Hofmeister's Beitr.*, **7**, 466 (1906)) showed that when thin sections of cartilage are left for some hours in a solution of sodium urate they take up uric acid. The concentration of the urate solution is diminished and direct examination of the cartilage reveals deposits of uric acid on it. The marked affinity of normal cartilage for uric acid is manifested by the fact that when large amounts are introduced into the peritoneal cavity of rabbits the acid may often be detected in the cartilages by the murexide reaction although not apparent in other tissues. The accumulation of uric acid in the cartilages when uric acid increases in gouty blood may be explained in the same way. Van Loghem (*Deut. Arch. f. Klin. Med.* **85**, 416 (1906); *Central. f. Stoffw.* 244 (1907)) has shown that the high predilection of cartilage and connective tissues for uric acid bears some relation to the large amount of sodium contained in these tissues. It has already been stated that the composition of bones is variable. Similarly, the composition of the deposits in gout is likely to vary according to the conditions of the body fluids and their p_H values. The author is of opinion that uric acid exists in the system as a negatively charged colloid peptized by the adsorption of albuminous matter, OH', and other negative ions. When uric acid coagulates in the system it will carry along with it different amounts of albuminous matter and electrolytes, and consequently the deposit in gout cannot have a definite composition. Similarly the deposits obtained in diseases like renal colic, oxaluria, lithiasis, phosphaturia, gallstones, etc. are variable in their composition.

ALKALI TREATMENT OF GOUT.

There seems to be a difference of opinion among physicians as regards the utility of alkali treatment in gout. Prof. Whitla (*Dictionary of Treatment* p. 306) says "Alkalis by forming soluble salts with uric acid, which salts, acting as diuretics, are freely washed out in the urine, cause marked elimination of uric acid, and are the most valuable of the gouty remedies". On the other hand, von Fürth (*Chemistry of Metabolism*, p. 182 (1916)) says that "the constant effort to favourably influence gout by alkalis is without the least theoretical foundation". Some workers even conclude that alkalis are not only useless in gout, but are actually harmful, and that it is possible to obtain beneficial results from long continued use of hydrochloric acid, which is supposed to alter the adsorption capacity of the blood and tissues for uric acid. (Compare Falkenstein, *Berl. Klin. Wochenschr.* 228 (1906), Schmidt, *Münch. med. Woch.* **83**, 1764 (1911)).

The author believes in the efficacy of alkali treatment; for alkalis not only help the elimination of uric acid, but they also increase the metabolism in the system. It has been found in the author's laboratories that oxidation of carbohydrates, fats, and nitrogenous substances by air at the ordinary temperature in the presence of reducing substances like ferrous hydroxide, cerous hydroxide, etc. is greatly increased by increase in the concentration of the alkalis. It is now well known that acidity in the system is associated with disturbance of digestion, and that acidosis is essentially connected with insufficient metabolism, and is mainly responsible for the causation of gout.

In this connection it is interesting to note that Palit and Dhar (*J. Phys. Chem.* **30**, 939 (1926); **32**, 1263, 1663 (1928); **34**, 710, 993, (1930)) have been able to oxidize solutions of uric acid and sodium urate by air at the ordinary temperature, in presence of sunlight or inductors like ferrous, cerous, manganous, and uranous hydroxide, sodium sulphite, and colloidal phosphorus. Photochemical and induced oxidations of other nitrogenous substances like egg-white, egg-yolk, hippuric acid, glycine, α-alanine, urea, etc. have also been carried out by the above authors by means of air at the ordinary temperature.

FEVER AS AN AUTOCATALYTIC REACTION.

It is well known that the action of metals on nitric acid becomes more and more rapid as the chemical change progresses, because the chemical change is largely accelerated by nitrous acid which is a product of the change. Similarly, the action of formic acid on iodic acid or

nitric acid is an autocatalytic change. In the author's view fever resembles these autocatalytic changes (compare Dhar, *Proc. K. Akad. Wetensch.* **23**, 44 (1920); *J. Phys. Chem.* **28**, 943 (1924)). He is of opinion that when a parasite enters the body it secretes a fluid which acts as a positive catalyst in the oxidation of foodstuffs by the inhaled oxygen. For example, when a malarial parasite enters the body, either the parasite itself or its secretion, which is taken up by the blood, promotes the oxidation of food materials by the oxygen of the blood; hence the amount of heat generated per unit time becomes greater, and we get the phenomenon of fever. This increased heat output, combined with the lowered capacity for heat elimination, brings about a rise in the body temperature. But a rise in temperature increases the velocity of any chemical reaction. Hence the rise in body temperature in febrile conditions brings about an increase in metabolism; the amount of oxidation of food-stuffs becomes still greater. This increased metabolism, resulting in increased heat production, in its turn brings about a further rise in the body temperature. This is the reason why the author regards fever as due to catalytic chemical change in the animal body.

SIGNIFICANCE OF THE RISE OF TEMPERATURE.

Since time immemorial physicians as well as patients have been thoroughly impressed with the idea that the rise of temperature is harmful, and measures for its suppression have always occupied a large place in therapy. We were disposed to look especially upon the parenchymatous and fatty degeneration of organs, a depression of the vasomotors and a weakening of the heart, as well as a loss of the hæmoglobin of the blood, as the immediately harmful effects of the heightened temperature itself. Modern studies, those of Naunyn in particular, as well as those of Rolly and Meltzer, have disproved this view, and have shown that these pathological features are to be ascribed primarily not to the influence of fever heat but rather to intoxication by products of pathogenic micro-organisms. Evidence is accumulating to show that the febrile temperature accession is a curative effort on the part of nature. Here may be mentioned the observations of Löwy and Richter upon pneumonia, diphtheria, chicken cholera, and swine erysipelas, and of Rolly and Meltzer upon infections with anthrax, streptococci, pneumococci and bacillus coli. From the standpoint of these workers, the elevated temperature can either act directly to influence harmfully the bacteria, or it may, on the other

hand, increase the bactericidal power of the blood, or the production of antibodies. Observations like those of Rolly and Meltzer, Lüdke, Fukuhara, Lissauer, and others upon the formation of agglutinins and hæmolysins in fever, make it very probable that we owe to the elevated temperature an important part in the production of antibodies. Modern pharmacologists, such as H. H. Meyer and Gottlieb (*Experimentelle Pharmakologie* p. 398, (1910)) also are taking the position that in application antipyretics serve far better if employed as fever narcotics; and that it is better to counteract certain associated features of the fever (as rapid cardiac action and respiration, restlessness, headache, loss of appetite, etc.) than to depress the high temperature itself.

Thus it seems probable that the marked rise of temperature in febrile conditions is advantageous to men and animals. When a toxin enters the system, the temperature of the body usually rises by two or three degrees and we get the phenomenon of fever, and the poison is destroyed about 10 or 20 times more rapidly at this fever temperature.

METABOLISM IN FEVER.

The author (*J. Phys. Chem.* **28**, 943 (1924)) has suggested that apparently there are two causes of increased metabolism in fever. One is the catalytic acceleration of oxidation of food materials due to the presence of parasites or their secretions, and the other is the rise in temperature of the body which causes an increase in oxidation. That there is an increase in total metabolism in fever seems to be generally recognized. Tice in his Practice of Medicine (vol. iv, p. 581 (1927)) says — "The total body metabolism in typhoid fever increases with the rise in temperature and decreases as the temperature falls. There is an average increase over that of the normal individual of about 40 per cent." Osler writes in his *Modern Medicine* (vol. I, p. 42 (1925)) thus — "Apart from the directly destructive changes, infection may bring about important alterations by stimulating katabolism, due either to a direct action of the infectious agents or their toxins on the tissues, or indirectly to nervous, circulatory, and respiratory changes. The increased katabolism manifests itself by the increased heat production, and by the appearance in the urine of increased amounts of all the metabolic end-products. Nitrogenous excretion may be increased to two or three times the normal" ..... "There is a rapid and ultimately an almost complete consumption of the body fats and carbohydrates, followed by a rapid consumption of the body proteins."

The experiments of Vaughan and his associates (*J. Amer. Med. Assoc.* **53**, 629 (1909); *Zeit. f. Immunitätsforschung* **9**, 458 (1911)) show that the breaking up of any foreign protein in the blood and tissues of the body may be associated with fever. These observers were able to induce a typhoid-like fever in rabbits by subcutaneous injection of egg-albumin. Furthermore, different types of fever, continuous or intermittent, may be produced at will by varying the quantities of the protein egg-white, and the frequency with which it is introduced. Schittenhelm, Weichhardt, and Hartmann (*Zeit. f. exper. Pathol.* **10**, 448 (1912)) contradict the results of Vaughan and collaborators. The introduction of body-foreign particles, e. g. the injection of fine suspensions of paraffin into the circulation, gives rise to fever. Introduction of caffeine, xanthine, or decoctions of coffee (Mandel. *Amer. J. Physiol.* **10**, 452 (1904); **20**, 439 (1909)) also produces rise of body temperature. Injection of many other foreign substances, like tetrahydronaphthylamine, is supposed to produce fever. These results are interesting but need confirmation.

USE OF ALKALI, PHOSPHATES, AND IRON PREPARATIONS.

Use of Alkali. — Alkali is usually administered in the form of bicarbonates of sodium and potassium, or alkali salts of citric and tartaric acids. Sodium tartrate and citrate not only function as mild alkalis, but also serve as food materials. Palit and Dhar (*J. Phys. Chem.* **34**, 710, 993 (1930)) have shown that tartrates and citrates can be oxidized to carbon dioxide by air in presence of sunlight, or inductors like $Fe(OH)_2$, $Mn(OH)_2$, etc. Alkalis are given in many diseases, e. g. diabetes, gout, lithiasis, renal colic, phosphaturia, oxaluria, etc. and in all troubles arising from stomachic disorder like acidity, flatulence, and dyspepsia. Most of these diseases result from improper metabolism of the food stuffs in the animal economy. When there is incomplete oxidation of carbohydrates, fats, and proteins, an accumulation of acid substances occurs; these acids neutralise the alkalis of the tissues and body fluids, giving rise to various disorders. According to the accepted views, alkalis neutralise the acids generated in the body due to improper metabolism, and thus keep up the p_H of the body fluids at its normal value. The author has suggested in several publications (Dhar, *J. Phys. Chem.* **30**, 277 (1926); **31**, 1259 (1927); *Chemie der Zelle und Gewebe*, **13**, 119, 209 (1926); *Zeit. anorg. u. allg. Chem.* **162**, 243 (1927); Palit and Dhar, *J. Phys. Chem.* **29**, 376, 799 (1925); **30**, 939 (1926); **32**, 1663 (1928)) that alkalis also serve a more important

function. They bring about an increase in the metabolism. Experiments *in vitro*, carried out in the author's laboratory on the induced oxidation of diverse food materials (fats, proteins, and carbohydrates) have definitely shown that slight alkalinity always increases the amount of oxidation. In this connection it is interesting to note that Rona and Wilenko (*Bioch. Zeit.* **59**, 173 (1913)) have shown that an increase in hydrogen ion concentration greatly reduces the utilization of glucose by an excised beating heart.

Use of Alkali in Diabetic Coma. It has been established by the researches of Naunyn, Magnus-Levy, and others that diabetic coma is caused by the output and accumulation of large quantities of acid in the diabetic. Magnus-Levy observes that the diabetic dies in coma not because of the neutralized acid, which is eliminated in the urine, but because of that retained in the body, which neutralizes the alkalis of the tissues and the body fluids. In the opinion of most physiologists, the lowering of the alkalinity of the blood and tissues should be anticipated by a prophylactic alkali treatment. Umber recommends the administration of sodium bicarbonate, even up to 200 grs., in those cases where the acetoacetic acid in the urine can be recognized by the ferric chloride reaction. Umber further adds "There is no question as to our ability to save severe cases of diabetes by alkali treatment in the face of an acidosis of a year's duration, although without this treatment this would be a matter of impossibility." C. von Noorden also believes strongly in the efficacy of alkali treatment, and is of opinion that there is no other effective remedy.

Apart from neutralizing the acids generated in the diabetic, alkali, according to the writer, has another and perhaps more important effect. From his experiments on the induced oxidation of food materials, the author concludes that alkalis facilitate the metabolism of glucose, which is in abeyance in the diabetic. This causes a concomitant decrease in acid production. Murlin and Kramer (*J. Biol. Chem.* **27**, 517 (1916)) have obtained results which indicate that if depancreatized dogs be given alkali they are able to oxidize some glucose.

RÔLE OF PHOSPHATES.

For the biochemist, phosphoric acid occupies a unique position by virtue of the fact that it occurs in the organism not only in the form of simple salts with alkali and alkaline-earth elements, but also in organic combination as compounds belonging to each of the three great classes of protoplasmic constituents, the proteins, fats, and carbohydrates.

Sörensen was the first to reveal the part played by the simple salts of phosphoric acid in the regulation of tissue neutrality, and since his time the progress of physical chemistry, and the employment of improved technique and more exact methods in biochemistry, have led to many important advances in our knowledge of the various phosphorus compounds found in animal and plant tissues, and their functions.

Phosphates in Sugar Metabolism. — The researches of Harden and Young have clearly demonstrated that in alcoholic fermentation by yeast, the sugars — glucose, fructose, and mannose — must pass through an intermediate stage of combination with phosphoric acid before they are broken down. These chemists have isolated two esters of the type of hexose monophosphate and hexose-diphosphate (compare Harden, *Nature* **125**, 277 (1930)). On the basis of some experiments, Embden was led, some years ago, to express the view that the precursor of lactic acid, the so-called lactacidogen, in muscle was of the nature of a hexosephosphate. He believed the complex to be a diphosphate of the type isolated by Harden from fermenting mixtures. The contraction and recovery phases in the muscle can be represented as follows: —

Contraction.

$$\text{Glycogen} \rightarrow C_6H_{12}O_6$$

$$C_6H_{12}O_6 + 2\,R_2HPO_4 \rightarrow C_6H_{10}O_4(R_2PO_4)_2 + 2\,H_2O$$

$$C_6H_{10}O_4(R_2PO_4)_2 + 2\,H_2O \rightarrow 2 \text{ lactic acid} + 2 \text{ phosphoric acid.}$$

Oxidative Recovery.

$$3 \text{ lactic acid} + 2 \text{ phosphoric acid} + 3\,O_2$$

$$= 3\,CO_2 + 5\,H_2O + \text{hexose diphosphoric acid.}$$

For some time the views of Embden remained unsupported by direct experimental evidence. Recently, however, Embden and Zimmermann (*Zeit. physiol. Chem.* **167**, 114 (1927)) have isolated a hexose monophosphate from rabbit's muscle, and this observation has been confirmed by Pryde and Waters (*J. Soc. Chem. Ind.* **46**, 1182 (1927)). Embden and Zimmermann state that the new hexose monophosphate differs from the two known hexose monophosphates (Robison's and Neuberg's). They have further shown that the new muscle monophosphoric acid is converted by muscle press juice into lactic acid. Therefore, they identify lactacidogen with the new hexose monophosphate. An important advance in this line is the isolation by Meyerhof (*Bioch. Zeit.* **178**, 395, 462 (1926)) of an active lactic acid-forming enzyme.

Two Viennese investigators, Audova and Wagner (*Klin. Woch.* **3**, No. 6 (1924)) reported that the injection of insulin resulted in large amounts of sugar being converted in the tissues into a complex resembling the hexosephosphate "lactacidogen". General confirmation of their work was provided by the work of Kay and Robison, who did not, however, believe that the proportion of sugar disappearing from the circulation and accountable for in this manner was quite as large as Audova and Wagner claimed, because a large amount of the sugar was also oxidized. Burn and Dale (*J. Physiol.* **59**, 164 (1924)) present further evidence to show that some sugar is synthesized to hexosephosphoric acid on injecting insulin. According to these workers formation of an intermediate phosphoric acid complex is necessary before sugar undergoes oxidation. But Best, Hoet, and Marks (*Proc. Roy. Soc. B*, **100**, 52 (1926)) report that no significant part of the sugar, which leaves the blood on insulin injection, is stored in the muscles as a phosphoric ester. Further work seems to be necessary in this line. It is interesting to note that Goodwin and Robison (*Bioch. J.* **18**, 1161 (1924); also Robison and Soames, ibid. 740; Kay and Robison, 755)) have shown that hexose-phosphoric esters exist in the blood.

Some physiologists suggest that by transition through the intermediate complex with phosphoric acid, the sugar of the blood is converted into a form that is more readily oxidized. In this connection it is interesting to note that Elias and St. Weiss (*Wiener Arch. inn. Med.* **4**, 29 (1922)) found that intravenous injections of sodium mono- and diphosphates may lower both alimentary and diabetic hyperglycæmia, but do not influence the level of blood sugar in normal subjects. It is suggested that in the former cases either the combustion of glucose is stimulated, or that storage of hexose as a phosphoric acid complex is facilitated. Embden, Grafe, and Schmitz (*Zeit. physiol. Chem.* **113**, 67 (1921)) record many experiments with soldiers and miners, in which administration of 7·5 grs. of sodium dihydrogen phosphate per day by mouth is said to have increased the capacity for muscular work. Embden and co-workers believe that in these cases the formation of lactacidogen, which plays such an important part in muscular work, is promoted. These results can, however, be explained on the basis that the administered phosphates increase the alkalinity of the blood, and thus promote metabolism of food materials, and this view has been emphasized by the author (Palit and Dhar, *J. Phys. Chem.* **29**, 799 (1925)).

It might be thought that experiments *in vitro* would provide a more definite solution of the problem. But even here the evidence is

conflicting. Löb and co-workers (*Bioch. Zeit.* **32**, 43 (1911); **46**, 288 (1912); **68**, 368 (1915)) believe that phosphates exert a specific accelerative effect on the oxidation of glucose by hydrogen peroxide. Their views are strongly supported by Witzemann (*J. Biol. Chem.* **45**, 1 (1920)) who believes that the effect is due to the intermediate formation of a hexose phosphate. On the other hand, Harden and Henley (*Bioch. J.* **16**, 143 (1922)) consider that the action of phosphates is not specific, but depends on their capacity to act as buffers. They find other buffer systems equally effective. Warburg and Yabusoe (*Bioch. Zeit.* **146**, 380 (1924)) have found that lævulose, but not dextrose, is oxidized in the presence of phosphates by atmospheric oxygen. They state that phosphates are specific in action and therefore cannot be replaced by other salts. In view of this disagreement further work seems to be necessary, particularly in the light of the observations of Meyerhof and Weber (*Bioch. Zeit.* **135**, 558 (1923)) that glucose is not appreciably oxidized at the surface of Warburg's active charcoal model (loc. cit), but that hexose phosphoric acid is broken down.

The rôle of phosphates in the calcification of cartilage has already been discussed in chapter VIII. Reference need only be made to the work of Lohmann (*Naturwiss.* **16**, 298 (1928); *Bioch. Zeit.* **202**, 466 (1928); *ibid.* **203**, 164 (1928); *ibid.* p. 172)) who finds that besides orthophosphate and its derivatives, pyrophosphate is also widely distributed in nature, being found in bacteria, yeast, pea seedlings, in the striated muscle of vertebrates and invertebrates, and in most of the organs of the vertebrates. All cells which utilize carbohydrate appear to contain pyrophosphate; yet the respiration and the degradation of glucose do not seem to depend on the pyrophosphate fraction.

Mention should also be made of the discovery by Fiske and Subba Rao (*Science* **65**, 401 (1927)) in America, of a labile compound of creatine and phosphoric acid (phosphagen). That most, or probably all, of the muscle creatine is present in the resting muscle in combination with phosphoric acid is suggested by the fact that creatine, like "phosphagen", is most abundant in voluntary muscle, less so in cardiac muscle, and present only in traces in involuntary muscle. This interesting discovery has led to numerous investigations, notably by Eggleton and Eggleton (*J. Soc. Chem. Ind.* **46**, 485 (1927); *J. Physiol.* **65**, 15 (1928)), Meyerhof and Lohmann (*Naturwiss.* **16**, 47 (1928); *Bioch. Zeit.* **195**, 22, 49 (1928); also *ibid.* p. 75)) and by Fiske and Subba Rao (*J. Biol. Chem.* **81**, 629 (1929)).

Thus phosphates seem to play an important rôle in life processes.

AN EXPLANATION OF THE USE OF IRON SALTS.

Iron in some form or other is essential to the life of many, perhaps all, forms of protoplasm. In the vertebrates this is obscured by the fact that most of the iron is contained in the hæmoglobin of the blood, and its importance in other tissues is ignored. In the invertebrates, however, in many of which no corresponding compound exists in the blood, considerable amounts of iron are found in the tissues. There is no question that, throughout the animal kingdom, iron is essential to living matter. It has been proved that it is also necessary for the development of lower vegetable forms, and it has been found that, in its absence, the higher plants fail to form chlorophyll. Iron seems to play an important part in oxidation reactions in plants and animals.

Iron has been long used in the treatment of anæmia, more especially of the form known as chlorosis, and it is tacitly assumed that the iron is readily absorbed from the alimentary tract. Nothing is known definitely regarding the changes which the iron preparations undergo in the stomach and in the intestines, or the form in which the iron is absorbed. The author is of opinion that at least a part of the iron added to the system is taken up by the hæmoglobin. Some of the iron may also exist in the colloidal condition (Compare Dhar, *J. Phys. Chem.* **28**, 943 (1924)).

Iron salts act as good catalysts in many oxidation reactions. It has been shown by the author (Dhar, *J. Chem. Soc.* **111**, 697 (1917)) that the oxidizing power of hydrogen peroxide is greatly accelerated by ferric and ferrous salts. Thus, if tartaric acid, starch, or sugar, and hydrogen peroxide be brought together at about the body temperature, hardly any chemical reaction takes place; but, as soon as a ferrous or ferric salt is added, oxidation of tartaric acid or starch takes place rapidly. Mathews and Walker (*J. Biol. Chem.* **6**, 289 (1909)) first drew attention to the great acceleration of the auto-oxidation of cysteine in presence of iron. Harrison (*Bioch. J.* **18**, 1009 (1924)) has shown that iron salts similarly catalyze the auto-oxidation of reduced glutathione. Warburg (*Naturwiss.* **11**, 862 (1923) has shown that iron exerts great catalytic action on the oxidation of amino-acids at the surface of charcoal. Robinson (*Bioch. J.* **18**, 255 (1924)) has also noted the catalytic effect of blood pigments and certain derivatives, in that hæmoglobin, methæmoglobin, and hæmin were found to exert a catalytic action on the auto-oxidation of linseed oil. The iron-free hæmatoporphyrin was inactive, so that the catalytic action of the other substances is ascribed to the iron present in the molecule. In

equal concentrations, the iron present as a blood pigment is more active than in the form of an inorganic salt. Spoehr (*J. Amer. Chem. Soc.* **40**, 1494 (1924)) was able to oxidize glucose by oxygen in the presence of phosphate and ferrous or ferric salt. Many such examples of the catalytic action of iron salts can be cited.

The author believes that iron salts catalyze the oxidation of food materials inside the body. When there is a deficiency of iron in the blood, the animal body suffers from anæmia because the amount of catalyst necessary for regular oxidation falls short. At this stage any iron salt taken into the system will make up the natural deficiency, and the necessary amount of oxidation will take place. This is the probable explanation of the use of iron salts in medicine. The author (*J. Phys. Chem.* **33**, 1897 (1929)) has advanced the opinion that many deficiency and metabolism diseases like beriberi, pellagra, scurvy, rickets, gout, diabetes, etc. should be amenable to treatment by iron preparations, because in the presence of iron the oxidation of food materials will be accelerated.

McCollum and Simmonds (*"Newer Knowledge of Nutrition"* (1925)) have shown that leafy vegetables are protective foods, because of their vitamin A content. The author is, however, of opinion that the efficacy of leafy vegetables is partly due to their iron content; for it is well known that iron is present in almost all green vegetables (compare Palit, Kaul, and Dhar, *J. Phys. Chem.* **34**, 737 (1930)).

CHAPTER X

Acclimatization and Physical Interpretation of Rubner's Law of Surface: The Ageing of Cells, Catalysts, and Body Colloids: The Problem of Old Age and Death.

ACCLIMATIZATION AND THE LAW OF SURFACE AREA.

The following views have been advanced by the author (Dhar, *J. Phys. Chem.* **30**, 480 (1926)) on the problem of Acclimatization and Rubner's Law of Surface:—

It is well-known that the temperature of a warm-blooded animal is maintained at the normal, though the temperature of the outside environments may vary from zero and lower to 30° or 35°. In cold-blooded animals, on the other hand, the temperature of the body is only slightly higher than that of the environment at the time. The metabolism of such animals varies with the temperature in such a manner that the respiratory exchange almost always rises with the increase in temperature, but generally irregularly, and to a very different degree in different animals. A frog in the mud during the winter at a temperature of 4° has quite a different metabolism from that which it enjoys during the summer sunshine, as it sits on the river bank and snaps at passing flies.

Röhrig and Zuntz (*Pflüger's Arch.* **4**, 57 (1871)) first showed that a curarized warm-blooded animal at ordinary room temperature lost the power of maintaining its body temperature, and that the intensity of metabolism decreased accordingly. Curare prevents the transmission of motor impulses to voluntary muscles. Krogh states that the curve of oxygen absorption as influenced by body temperature is the same in the anæsthetized frog and fish as in the curarized dog.

In warm-blooded animals the temperature is maintained at an almost constant level independent of the climatic conditions within certain limits, and this level is a favourable one for the activity of nerve and muscle. It would indeed be inconvenient were the active

life of a man dependent on the temperature of his environment. The essential mechanism for the regulation of body temperature is nervous.

In warm-blooded animals a fall in the surrounding temperature regularly causes not a decrease but an increase in the respiratory exchange, thanks to the mechanism of chemical heat regulation. A most elaborate study of the chemical heat regulation has been made by Rubner (1887, 1902), who obtained the following results in the case of a guinea pig:—

Temperature of air.	CO_2 in grm. per Kg. and Hour
0°	2·91
11°	2·15
21°	1·77
26°	1·54
30°	1·32
35°	1·27
40°	1·45

At 35° the regulation breaks down and the respiratory exchange rises with increase in the temperature of the body as seen in the last experiments of the above series.

In a paper (*Proc. k. Akad. Wet. Amsterdam* **23**, 44 (1920)) published from this laboratory we have shown that under standard conditions, where the effect of nervous influence is excluded, increase in temperature causes greater metabolism in both warm- and cold-blooded animals.

After studying a considerable range of animals, Rubner has found that all animals transform nearly the same total amount of energy per kilogram of body weight in the whole period from birth to natural death. The mean value of the constant Rubner finds to be 191,600 calories, the values for different species ranging from 141,090 and 265,500 calories. Small animals with an intensive metabolism live a relatively short time; large animals with more sluggish metabolism live a longer time. Rubner's view is that a definite sum of living action or energy transformation determines the physiological end of life. It is Rubner's law that the metabolism is proportional to the superficial area of an animal.

Erwin Voit (*Zeit. f. Biol.* **41**, 120 (1907)) has calculated the following general table showing the heat production in resting animals of various sizes at medium temperature of the environment.

Name of animal	Weight in Kilograms	Calories produced	
		Per Kilogram	Per square meter Surface
Horse	441	11·3	948
Pig	128	19·1	1078
Man	68·3	32·1	1042
Dog	15·2	51·5	1139
Rabbit	2·3	75·1	776
Goose	3·5	66·7	969
Fowl	2·0	71·0	943
Mouse	0·018	212·0	1188
Rabbit . . . . . (Without ears)	2·3	75·1	917

The above table supports approximately the generalization of Rubner.

Voit shows that the metabolism of the pigeon may be doubled after removing its feathers. From the experiments of Rubner it appears that the presence of adipose tissue acts in the same way as warm fur to extend the range of the physical regulation, and to delay the onset of chemical regulation for body temperature. That the range of physical regulation of temperature of a small dog was due to its long hair is shown by the change in its metabolism after shaving it. Rubner shows this in the following table:—

Temperature	Calories per Kilogramm	
	Normal coat of hair present	Shaved
20°	55·9	82·3
25°	54·2	61·2
30°	56·2	52·0

It is clearly seen that this dog lost its power of physical regulation between 20° and 30° as soon as it lost its covering of hair, and that its metabolism became like that of a guinea-pig, increasing with a reduction of temperature from 30° downwards; an illustration of chemical regulation.

To determine the influence of the protective layer of fat Rubner (Rubner, *Energiegesetze*, p. 137 (1902)) investigated the influence of temperature on the metabolism of a fasting, short haired dog at a time when it was emaciated, and compared it with the fasting metabolism after the same dog had been fattened.

Dog (thin)		Same dog (fat)	
Temperature	Calories per Kilogram	Temperature	Calories per Kilogram
5·1°	121·3	7·3°	120·5
14·4°	100·9	15·5°	83·5
23·3°	70·7	22·0°	67·0
30·6°	62·0	31·0°	64·5

It appears from the above that the metabolism of the dog was the same at a low temperature in both cases, but that the minimum metabolism was almost reached at a temperature of 22° when the dog had a protective covering of fat, which was not the case when it was thin.

The physical regulation may be increased by certain voluntary acts, such as are observed when a dog or a man exposed to cold lies down and curls himself up in such a way as to offer as small an exposed surface as possible. The contrast to this is offered when on a hot day the dog lies on its back and extends its limbs so as to promote loss of heat.

Voit (*Zeit. f. Biol.* **14**, 80 (1878)) gives the following results concerning the effect of temperature on the metabolism of a fasting man.

Temperature	Carbon dioxide excreted in grms.
4·4°	210·7
6·5°	206·0
9·0°	192·0
14·3°	155·1
16·2°	158·3
23·7°	164·8
24·2°	166·5
26·7°	160·0
30·0°	170·6

Voit believed the increase in metabolism to be a reflex stimulus of cold on the skin which raised the power of the muscle cells to metabolize.

Another factor in the heat regulation of man is clothes; certain savage races living in cold climates do without clothes; for example, the aborigines of Tierra del Fuego, who, according to the reports of travellers, substitute a covering of oil for clothes. In such races the process of "hardening" or the development of physical regulation must be carried to a "maximum". In civilized countries man endeav-

ours to remove all the influence of chemical regulation by keeping his skin covered. Only about 20 per cent of his body surface is exposed to air. The most important constituent of clothes is air, which is a worse conductor of heat than fibre. Two experiments cited by Rubner (*Energiegesetze*, p. 225 (1902)) indicate the effect of clothes on metabolism. An individual was kept at a temperature of 11° and 12° and wore different clothes at different times. His CO_2 and water excretion were as follows:—

Influence of clothes on metabolism in man at a temperature of 11° to 12°.

Clothes	CO_2 in grm. per hour	Remarks
Summer clothes	28·4	Cold, occasional shivering
Summer clothes and winter overcoat	26·9	Chilly part of the time
Summer clothes and fur coat	23·6	Comfortably warm

When the man was comfortable the chemical regulation was eliminated.

Fat persons have been directly observed to have a smaller respiratory action than lean ones. Benedict and Smith have shown, — by comparing a number of athletes with normal subjects of similar heights and weights, — that the metabolism of athletes is on the average distinctly greater than that of non-athletes.

While it had often been observed that smaller animals had per unit weight a greater respiratory exchange than larger ones, a quantitative study of the influence of size upon metabolism was first made by Rubner on grown up dogs weighing from 3·4 to 30·4 kgms. Rubner found that the metabolism calculated per kilogram increased regularly with decreasing size. When, however, the surface of the animal was taken into account a practically constant metabolism per square metre of surface was found for all.

Kettner (*Arch. f. Physiol.* 447 (1909)), from his experiments on guinea-pigs of different ages and weights, finds that the metabolism per kilogram an hour decreases fairly regularly with increasing weight, whilst the differences in the results per square metre are independent of size. On the other hand, in a recent discussion, Benedict (*J. Biol. Chem.* **20**, 263 (1915)) denies that there is any close relationship between size and metabolism, and deprecates especially the use of the surface as a basis for comparison. His own figures and charts show,

however, that such relationships exist, and that the metabolism per kilogram of the body weight decreases fairly regularly with increasing weight.

The surface S of an animal is approximately proportional to the square of a linear dimension e.g. length of the body, while the weight is proportional to the third power of a linear dimension. We have therefore $S = CW^{\frac{2}{3}}$. The constant C has been worked out for different species. It does not vary much even in forms of very different shapes. For man and also for a dog we have $C = 12{\cdot}3$; for the rabbit $12{\cdot}9$; horse $9{\cdot}0$; rat $9{\cdot}1$ and guinea-pig $8{\cdot}9$.

It is quite possible that the surface as at present defined $(CW^{\frac{2}{3}})$ does not give the very best result in comparisons of different individuals. The main point is that metabolism in warm-blooded animals is not proportional to the weight W but to W^n, where n is certainly not far from $\frac{2}{3}$.

On the whole, looking at the problem from a broad point of view, it seems pretty certain that the surface law of Rubner is generally proved as far as the metabolism of warm-blooded animals is concerned.

DERIVATION OF RUBNER'S LAW FROM LAWS OF RADIATION.

In the following pages, an attempt will be made to find the physical significance of Rubner's generalization and other facts regarding the influence of temperature on both warm and cold-blooded animals.

We can look at this problem of the metabolism of various warm-blooded animals with the following considerations in view:—

(1) The body temperature of warm-blooded animals is normally much higher than the surrounding air. In the case of some birds, sparrow, hen etc., the body temperature is about 42°, with the rabbit it si 39·6° and with the dog 39·2°.

(2) Experimental results have shown that radiation is the most important factor in the loss of heat from the animal body. Let us assume that a metallic ball of radius r and density ϱ is placed in air at say T_0° and that we are supplying heat to the ball so that its temperature may be kept constant at T° where T° is greater than T_0°. Now in order to maintain this constant temperature a supply of heat has to be given, otherwise the body by losing heat will cool down to the temperature of its surroundings. From Stefan's law of radiation, we know that the loss of energy from the surface is equal to $4\pi r^2 \sigma (T^4 - T_0^4)$, where σ is Stefan's constant and $4\pi r^2$ is the surface of the body in question.

Therefore the rate of supply of heat to the body per unit mass necessary to keep the body temperature constant at $T°$ is equal to

$$\frac{4\pi r^2 \sigma (T^4 - T_0^4)}{\frac{4}{3}\pi r^3 \varrho} = \frac{3\sigma}{r\varrho}(T^4 - T_0^4).$$

From the foregoing relation it will be seen that the rate of supply of heat per unit mass varies inversely as the radius of the body in question. In other words, a small ball requires a much larger quantity of heat per unit mass than a large ball of the same material. Let us apply these considerations to the question of metabolism in animals. Ordinarily warm-blooded animals are surrounded by air of a much lower temperature than the temperature of their bodies. In other words, the animal is constantly giving out heat to the outside surroundings, mainly by radiation; and in order that this phenomenon may take place the metabolism of the system must increase to keep the body temperature constant. From the foregoing considerations it is evident that the amount of heat lost by the animal per unit weight of body through radiation, and the amount of metabolism required to compensate this loss, is greater the smaller the animal. This conclusion is actually corroborated by experiments. Thus from physical principles it follows that the gain of heat per unit weight of the body required to make up for this loss of heat is greater the smaller the size of the animal.

From the relation obtained it is seen that the rate of supply of heat per unit mass is dependent upon the difference in temperature between the body and the surrounding air; in other words, the greater the difference in temperature the greater is the rate of supply of energy per unit mass of substance. Consequently when an warm-blooded animal is surrounded by air which is colder than the air with which it is normally surrounded, its rate of supply of energy and consequent metabolism should also increase; and that is the reason why metabolism in the case of warm-blooded animals increases with the fall of surrounding temperature.

We have already shown that the loss of energy from the surface $= 4\pi r^2\sigma(T^4 - T_0^4)$. Now if we express this loss per unit surface, the expression becomes $\sigma(T^4 - T_0^4)$; in other words, the question of the radius or size of the body does not come into consideration, and the loss of energy per unit surface comes to depend only on the difference of temperature between the body and the surrounding air. This has been experimentally proved by Rubner.

Rubner has obtained the following result with a guinea-pig:—

Temperature	Carbon dioxide
0°	2·91
11°	2·15
21°	1·77

If we calculate the metabolism according to the relation $\sigma(T^4 - T_0^4)$, we find that the ratio of the metabolisms at 0° and 11° is about 1·2, whilst the observed ratio of the metabolisms is about 1·3. The calculated value between 21° and 26° is 1·38 and the observed value is 1·2, taking the average temperature of the guinea-pig as 38·2°. Hence we get a physical meaning for Rubner's law.

From the foregoing pages it is evident that Rubner's generalization is applicable mainly to warm-blooded animals, because usually they maintain a higher body temperature irrespective of the temperature of the surroundings; and the laws of radiation would be applicable only to such cases.

In the case of cold-blooded animals the body temperature is only slightly higher than the temperature of the surroundings, and the foregoing considerations are not applicable in these cases, so that Rubner's generalization is not valid for cold-blooded animals.

We have already suggested that the life principle depends essentially on the activity of the catalyst or enzymes existing in the body. In the foregoing pages, we have observed that usually smaller animals have more metabolism per unit weight of the body than larger animals; in other words, weight for weight the catalyst or the enzymes in smaller animals are more reactive than the catalyst in the larger animals. It sounds strange to say that the activity of the enzymes present in the system of a dog is much greater than the activity of those present in that of a man; the alternative is to assume that the amount of the catalyst per unit weight of the body is much greater in small than in large animals. It will be seen in the subsequent discussion that the former proposition is more reasonable than the latter. In other words, we are led to the conclusion that the physical activity and the amount of oxidation per unit weight of the body is much greater in the case of a dog than in that of a man. Even a most casual observation of the domesticated animals shows that as a rule small animals do not live so long as large ones. As a general rule it may be said that a large animal takes more time than a small one to reach maturity, and it has

been inferred from this that the length of the period of growth is in proportion to longevity.

Hence small animals with an intensive metabolism live a relatively short time, whilst large animals with a more sluggish metabolism live a longer time. We have already mentioned Rubner's view that a definite sum of living action or energy transformation determines the physiological end of life.

There are chemical analogies to these biological facts. Sabatier and his colleagues have shown that when metallic nickel, which is used as a catalyst in the hydrogenation processes, is prepared under suitable conditions at as low a temperature as possible, the activity of the catalyst is extremely great, but that it loses its activity very readily. From experience of other catalysts we know that an extremely active catalytic surface also deteriorates very readily. In other words, an extremely active catalytic surface is more liable to be poisoned or undergo other changes which would affect its activity than the surface of a moderately active catalyst.

Consequently, it seems probable that in biological processes of metabolism extremely active catalysts will lose their activity more readily than moderately active catalysts. In other words, the catalysts which accelerate metabolism or oxidation in the case of dogs induce in unit time more oxidation than the less active catalysts present in the human system; but the more active catalysts present in smaller animals are more liable to lose their activities by poisoning or other alterations than the less active catalysts present in the human body. That is the reason why death occurs sooner with animals having very active catalysts than with animals having moderately active ones.

ACCLIMATIZATION OF COLD-BLOODED AND WARM-BLOODED ANIMALS.

We shall now offer an explanation of the possibility of acclimatization of warm-blooded animals from this point of view. As we have already mentioned, when there is a fall in the surrounding temperature the metabolism of warm-blooded animals increases; in other words, when a warm-blooded animal is brought from a warmer climate to a cooler climate its metabolism and the catalytic activity of its body enzymes and cells are increased, so that there is a strain on the system. In the case of human beings this relation should also be valid. We have already mentioned that usually 20 per cent of the body surface is exposed to air in the case of human beings, and we have to consider

only the exposed portion. Now even for this comparatively small exposed portion, the metabolism of the body should increase on lowering the temperature of the surroundings. Consequently the catalyst in the body would be activated; but, as Rubner has shown, the standard of metabolism cannot undergo rapid changes, as the oxidative energy of the cells is adapted to the usual conditions regarding the loss of heat, and is altered very gradually with those conditions; hence the system of a human being or an animal brought from a warmer to a cooler climate will be in a state of strain.

It is evident that the metabolism of cold-blooded animals is much slower than that of warm-blooded ones. Hence the catalytic activity of the enzymes and cells present in cold-blooded animals is not so great as that in warm-blooded animals of the same size. So that the duration of life of a cold-blooded animal is usually greater than that of a warm-blooded animal of the same size. This is corroborated by evidence from biology, because experience shows that cold-blooded animals live much longer than warm-blooded ones of the same size.

When warm-blooded animals are transported from a warmer to a cooler climate, metabolism is increased. The effect of this is that the catalytic activity of the enzymes and cells has to increase in order to produce greater combustion in an unit of time.

It has already been pointed out that when the catalyst is made to work at a greater speed than the normal one its life period is decreased. Consequently one effect of the transportation of a warm-blooded animal from a warmer climate to a cooler climate will be to activate the enzymes and cells in the body and lead to shortening of the life period.

The temperature of a warm-blooded animal remains nearly constant whatever may be the temperature of the surroundings. Consequently the catalyst has to work at the same temperature irrespective of the temperature of the outside surroundings. Thus in the case of warm-blooded animals the question of the ageing of the catalyst at a greater rate due to the increase in temperature does not arise, because the catalyst works at a constant temperature, which is the body temperature of the animal in question, provided the external temperature is less than the body temperature. So the main effect of transporting a warm-blooded animal from a warmer country to a colder country is to increase the activity of the body enzymes and cells and metabolism, and thus to shorten the life period of the animal in question. Now if the enzymes and cells which were used to generate smaller quantities of heat in a warmer climate are required to produce

greater quantities of heat in a cooler climate, they will sooner be tired out. In course of time the individual or animal in question will feel the strain, and it seems possible that as years go by he will feel this more and more. Thus it is probable that a human being transported from a warmer to a cooler climate will feel the cold more and more as years go by.

On the other hand, if a warm-blooded animal is transported from a cooler to a warmer climate, let us see what will be the effect of this transportation on his system. As soon as he is surrounded by a warmer atmosphere, the amount of metabolism which he was used to produce in colder surroundings becomes less; consequently the catalyst in the body has to work less in a warmer than in a colder climate. Hence the life period of the individual in question is increased when he is transported from a colder to a warmer climate, provided that the external temperature is not greater than his body temperature.

It appears, therefore, that it is more advantageous for a man living in a colder climate to go to a warmer climate than the reverse.

When a warm-blooded animal has to live in a country where the outside temperature is greater than the body temperature, then the animal will age, grow old, and die more quickly than an animal living in a cold country, because at the higher temperature the body catalysts will age more quickly. Thus this experience of a warm-blooded animal will be allied to that of a cold-blooded animal.

Recently, Dhar and Satya Prakash (*J. Phys. Chem.* **34** (1930)) have shown that sols of ferric hydroxide, stannic hydroxide, zirconium hydroxide, aluminium hydroxide, chromium hydroxide, cerium hydroxide, and cupric ferrocyanide, require smaller quantities of electrolytes for coagulation at higher temperatures than at 30°. In other words, the above sols become more aged and unstable when kept at higher temperatures. From our experiments on the coagulation of sols, it will be observed that the stability of several colloids decreases considerably on increasing the temperature of the colloid, so that the colloids age more rapidly. Consequently the colloids present in cold-blooded animals will have a longer duration of healthy life, and this will lead to the greater longevity of the animals themselves.

The body temperature of warm-blooded animals is normally much higher than that of the surrounding air. In the case of some birds, sparrow, hen, etc., the body temperature is about 42°, in the case of a rabbit 39·6° and in the case of a dog 39·2°. It is clear, therefore, that the body colloids, cells, and enzymes present in these animals will age readily and become unstable at these moderately high temperatures.

Moreover, these cells and catalysts have to work at a high speed in order to make up for heat lost by radiation, and in other ways. Consequently the longevity of these warm-blooded animals cannot be as high as that of cold-blooded animals.

Voit gives the following results on the influence of temperature on the metabolism of a fasting man:

Temperature	4·4°	6·5°	9°	14·3°	16·2°	24·2°	26·7°	30°
CO_2 expired in grms. .	201·7	206·0	192	155·1	158·3	166·5	160	170·6

It appears, from the above results, that when the outside temperature is about 15° the metabolism of the animal body is at the minimum. Similar results, showing that the metabolism is at a minimum when the external temperature is about 15°, have been obtained with other warm-blooded animals. It is probable that the longevity of an animal is increased if the body cells and enzymes are made to work at a minimum speed. Moreover, at the temperature of 15° the body colloids do not age rapidly; hence this temperature is the most suitable one for the healthy life of warm-blooded animals which maintain a higher body temperature than the surrounding air. People living in a country under suitable hygienic conditions should have the maximum longevity, if the average temperature is about 15°.

In this discussion we have neglected the consideration of humidity and its influence on human beings and animals.

Another factor is that of the colour of the skin surface; for animals with a deeper colour are likely to radiate heat more readily than animals with a fair complexion.

It has been pointed out that the metabolism of cold-blooded animals is much less than that of warm-blooded animals under the same conditions; in other words, the enzymes and cells present in cold-blooded animals are not as active as those present in warm-blooded animals. We have also observed that the body temperature of a cold-blooded animal is usually slightly higher than the temperature of the surrounding air, and that metabolism in the case of a cold-blooded animal goes on increasing as the surrounding temperature is increased.

Let us see what takes place when a cold-blooded animal living in a warmer country is taken to a colder country:—The metabolism in the system will decrease, and the animal will have to live a life of less intensity, and possibly with a less sense and feeling of well-being. The enzymes and cells have to generate lesser intensity of heat in the cool atmosphere, and consequently their period of life will be increased, and

the animal may expect to live longer in the cooler surroundings. Moreover, the body catalysts will not age as rapidly in the cooler surroundings as in a warmer country. Consequently, these two factors will both lead to a greater longevity of the cold-blooded animal in question, when it is transported from a warmer to a cooler country. Moreover, in a warm country the body catalyst is likely to age more rapidly than in a cold country. Consequently, the effect of both these factors will be that old age and death will follow more rapidly in a cold-blooded animal transported from a colder to a warmer place.

On the other hand, when a cold-blooded animal habituated to a cooler locality is transported to a warmer country, his metabolism in a unit of time will be increased, and the catalysts in the body have to perform more work. Consequently, the period of activity of the catalysts will be decreased, and the life of the animal is likely to be shortened, though the animal will enjoy a more intense and active life in warmer surroundings.

THE AGEING OF ANIMAL CELLS, CATALYSTS, AND BODY COLLOIDS.

In publications (*Zeit. anorg. u. allg. Chem.* **162**, 237; **164**, 63 (1927); *Kolloid. Zeit.* **42**, 120 (1927)); see also Dhar (*J. Phys. Chem.* **34**, 549 (1930)) from these laboratories we have shown that the activity, adsorptive power, stability, and viscosity of hydrophobe colloids decrease with time; but that the viscosity and the amount of hydration of hydrophile colloids increase with time up to a limiting value.

In a recent communication we have shown that the viscosity of a sol of ceric hydroxide, prepared in the cold, increases with time up to a limit, and the sol then sets to a stiff jelly; but that if the jelly is kept in a stoppered bottle the viscosity decreases and the sol becomes mobile again. The electric conductivity of the sol decreases down to a minimum value and then increases. Exactly similar results are obtainable with a sol of vanadium pentoxide. In this case we prepared a concentrated sol and measured the conductivity and viscosity day by day, and found that the viscosity increased up to a limiting value and then decreased. The electrical conductivity, on the other hand, decreases to a minimum and then increases again. These two sols behave as typical hydrophile colloids. With silicic acid some interesting results have been obtained. The sol prepared both in the cold and hot conditions shows increased viscosity with time, and on keeping the silicic

acid sets to a jelly. In course of time the jelly is broken and some of the liquid extruded.

Exactly similar behaviour is observable with animal cells and protoplasm. From ultramicroscopic observations it can be definitely concluded that amœbæ consist of colloidal particles. Similar conclusions are drawn by Mott (*The Brain and the Voice in Speech and Song*, 112 (1912)) from observations on living nerve cells by the same method. As a general rule, the small granules often seen in living cells do not show active Brownian movement, indicating that protoplasm is often of considerable viscosity. This is in harmony with the fact that it can often be drawn into long sticky threads.

The degenerative changes which precede the death of a cell are accompanied by a liquefaction of the protoplasm and by the appearance of small granules in active Brownian movement. These granules become larger in course of time, whilst the Brownian movement ceases, and this indicates that the protoplasm has irreversibly coagulated.

The protoplasm of young cells is often homogeneous, whilst that of the mature cell may show a definite structure. Thus, immature eggs of echinoderms are quite clear and transparent, whilst in the mature eggs, Chambers (*J. Exp. Zool.* **23**, 483 (1917)) distinguishes two kinds of particles visible in the ordinary microscope—very small ones and larger ones (macrosomes). The former are stable and the latter very sensitive to injury. Mitochondria are granules apparently composed of albumin and lecithin, which stain with dyes containing di-ethyl safranin. They are present in the living cell and modify the activities of the cell. It appears, therefore, that the protoplasm of young cells behaves like a freshly prepared colloidal solution, and in the mature egg partial separation of the solid and increased opacity are observable, due to ageing. This behaviour of protoplasm is similar to that observed with a sol of silicic acid. Fischer has shown that the same type of change takes place with proteins. Gelatine jelly, as well as other protein media, e. g. the familiar blood serum of the bacteriologists, all extrude fluid on standing. The more highly hydrated the protein gel or jelly, the more liquid is extruded. Hence it appears that in course of time the body proteins have a tendency to lose their adsorptive power and activity. This is one of the important factors that lead to old age and death. The cells of the tissues are, like other living beings, dependent for their life and activity upon a constant and abundant supply of food and oxygen, and an equally adequate removal of their waste products. Now the utilization of food and oxygen is possible because of the existence of catalysts in the body. In course of time the

activity of the enzymes and catalysts, as well as that of the cells, becomes less, and hence the metabolism is decreased.

In the case of vanadium pentoxide, the sol shows increased viscosity and decreased conductivity with time up to a limiting value. This is because vanadium pentoxide has a great affinity for water, and will combine with more and more water, hence the viscosity will increase, and along with it the electrical conductivity will decrease to a limiting value. This hydration tendency of substances like vanadium pentoxide, ceric hydroxide, silicic acid, etc., depends upon the affinity of the substances for water, and is possibly controlled by those forces which make substances dissolve in water. Now when the chemical affinity of the particles of vanadium pentoxide, ceric hydroxide, etc., for water is satisfied, and the maximum hydration and viscosity are reached, the particles on further ageing begin to agglomerate and partially lose their adsorptive power, stability, and hydration tendency. Our experimental results on the viscosity of gelatine show that this viscosity goes on increasing with time up to a limiting value, and then decreases. Hence with gelatine the viscosity and the hydration tendency increase up to a maximum, and then fall off due to ageing.

Typical animal products like albumin, gelatine, protoplasm, cells, etc. also behave in a similar manner, as has already been mentioned. From the above results it will be clear that there is no essential difference between the behaviour of inorganic hydrophile colloids and typical animal products like albumin, protoplasm, etc. The ageing phenomenon is similar in both these groups of substances.

It is well known that Dony-Hénault prepared artificial laccase, which is an oxidizing enzyme, by alcoholic precipitation of a solution containing gum arabic, manganese formate, and sodium bicarbonate. This precipitate can be dissolved in water and reprecipitated by alcohol. It is undoubtedly an adsorption compound of gum and colloidal manganese hydroxide. This artificial enzyme will age in course of time and partly lose its activity.

In publications from these laboratories we have shown that iron salts and colloidal ferric hydroxide can act as active catalytic agents in oxidation reactions. Thus the oxidation of tartaric acid, starch, etc. by hydrogen peroxide can be largely increased by ferric or ferrous salts, or colloidal ferric hydroxide. In this way we have explained the internal use of iron in medicine. Now it is well known that animal blood contains iron, and that a good deal of oxidation of food material takes place in the blood. It seems likely that in course of time the iron com-

pound present in adult animal blood will lose a part of its catalytic influence, and the amount of oxidation in the animal body is therefore likely to decrease with age.

Moreover, the inorganic salts which are present in the body in consequence of adsorption by the protein matter are likely to be partly given up due to ageing of the protein particles. The body cells, enzymes, hormones, internal secretions, etc., are likely to act with the help of the adsorbed inorganic and organic substances; but in course of time the activity of the cells, the enzymes etc. is likely to decrease owing to the loss of these adsorbed substances. The cartilages, connective tissues, bone cells, etc., which consist mainly of calcium carbonate and phosphate, will also age in course of time, and partially lose their activity and adsorptive power. The author has suggested (*Z. anorg. u. allg. Chem.* **162**, 243 (1927)) that bone formation takes place by adsorption by the solid or semi-solid cartilages, bones, etc., of the calcium phosphate and calcium carbonate existing in the colloidal state in the body. In the course of time these adsorbents will partly lose their adsorptive power and be unable to adsorb the requisite amounts of sols of calcium phosphate and carbonate from the body; hence bone formation will be difficult in old age.

Whenever an electric stimulus is sent through an amœba, the organism contracts, so that its surface may be the least possible; in fact it becomes more or less spherical. It has been proved mathematically that when the charge on colloid particles is increased, these particles tend to become more and more spherical. In this respect an amœba behaves exactly like a sol. With the establishment of the cellular nature of the tissues it may well be that the ultimate unit of life has been reached, and that no minuter element need be considered. Indeed our doctrine of pathology is essentially a cellular one, although we realize that the cell in all cases is a vastly complicated structure, within which there are elaborate mechanisms developed in almost infinite variety; and within which, too, we can discern evidence of the accomplishment of chemical processes, which in complexity and ingenuity of combination surpass anything that can be achieved in a laboratory. From the foregoing pages, it will be clear that the cells of the animal body lose their adsorptive power, hydration tendency, and activity with time. It has already been mentioned that the protoplasm in the mature egg consists of two kinds of particles, small and large. The former are stable, and the latter very sensitive to injury. It appears, therefore, that on ageing the cells in the animal body become comparatively powerless, and sensitive to injury and bacteria.

OLD AGE AND DEATH FROM A CHEMICAL POINT OF VIEW.

In publications from these laboratories (*Z. anorg. Chem.* **162**, 237; **164**, 63 (1927); *Kolloid Z.* **42**, 120 (1927)) we have discussed the question of ageing of sols and gels. As life processes are mainly due to catalysts and colloids, the author (Dhar, *J. Phys. Chem.* **30**, 378, 480 (1926); **34**, 549 (1930)) has endeavoured to explain the phenomena of old age and death, which have been described by Metchnikoff as the most mysterious of natural phenomena, from a purely chemical point of view.

When a lump of sugar is left in air at the ordinary temperature, no oxidation of the sugar takes place; but when the same lump is taken into the animal body it is burnt readily with evolution of carbon dioxide and water, thus supplying heat to the animal body. We now know definitely that the oxidation of sugar in the animal body is accelerated by the enzymes and cells which are always present in the animal system, and whose activity really controls the velocity of oxidation of food materials in the animal body.

We have repeatedly observed that the chemical reactivity of a freshly precipitated substance is much greater than that of a substance which has been long prepared. Thus when ferric hydroxide, chromic hydroxide, etc., are freshly precipitated, they dissolve very readily even in dilute HCl; whilst the same substances, prepared in a similar manner, and placed at the ordinary temperature in a flask even in contact with water, lose much of their chemical reactivity and do not dissolve in dilute hydrochloric acid; even concentrated hydrochloric acid dissolves these old samples of hydroxides with difficulty. The adsorptive power of these substances decreases considerably on ageing. Moreover, we have shown that the catalytic decomposition of hydrogen peroxide due to the presence of manganese dioxide, as measured by evolved oxygen, is much greater when the dioxide has been freshly precipitated than when the same weight of an old sample is used.

It is well known that the percentage of water which enters into the composition of the animal body decreases with the age of the animal, as will be shown in what follows:—

A three-months' human fœtus contains 94 per cent of water; at birth the water content is from 69 to 66 per cent; in adult life 58 per cent. It is held that in old age the water content decreases; turgescence in general, and of the skin in particular, is obviously lost. With ageing there occurs shrinking.

From our experience on gels and sols we know that the ageing of a sol is always associated with its dehydration.

We shall now utilize these results to explain old age and death from a purely chemical point of view. We have already mentioned that the speed of oxidation of food materials is essentially dependent on the activity of the catalysts and cells inside the animal body, provided a sufficient supply of food and air are available for that body. Now just as freshly precipitated manganese dioxide is more reactive as a catalyst in the decomposition of hydrogen peroxide, so enzymes, cells, or other catalysts in the body of a child or a young man are likely to be more reactive than the enzymes, cells, or catalysts in the system of an old man. Consequently, the amount of oxidation per unit weight of the body of a young person should be much greater than that obtaining in the case of an old person. As the animal or individual grows older, the amount of oxidation per unit weight of its body grows less and less, as the enzymes, cells, and activators in its body become more and more aged and inactive. It is well known that a certain minimum quantity of heat is necessary for the maintenance of the body temperature of about 37·4°. When the system cannot generate this minimum quantity of heat it breaks down, and death follows. As time goes on the activity of the enzymes, cells, and other catalysts in the body becomes less and less, and after a time a certain stage arrives in which the catalyst is unable to accelerate the oxidation of food materials by the oxygen of the air to a sufficient extent for the minimum quantity of heat required for the maintenance of the body temperature to be obtainable. At this stage death is likely to occur.

It also follows from a general dynamical principle that the free energy of a system tends to decrease. We have repeatedly observed that a freshly precipitated sample of $Fe(OH)_3$, $Cr(OH)_3$, hydrated manganese dioxide, etc., loses its chemical reactivity more readily when kept at a higher temperature, even in contact with water, than when kept at a low temperature under the same conditions. Thus we have observed that the amount of adsorption of arsenious acid by a definite weight of ferric hydroxide kept at a temperature of 50°, even in contact with water, is much less than the adsorption of the same substance by the same weight of ferric hydroxide kept in contact with water at 25°; in other words, the decrease in the chemical reactivity of these substances, or of their ageing, is more marked and rapid at higher temperatures. It follows that freshly precipitated manganese dioxide will lose its catalytic activity towards the decomposition of hydrogen peroxide more readily at a higher temperature than at ordinary temperature; and this is corroborated by experiments. It seems probable, therefore, that the life period of the enzymes, cells, and other catalysts in the human or

animal body will decrease more readily if the human being or the animal has to live in surroundings at a higher temperature than his body temperature, although the mechanism of chemical heat regulation and perspiration tends to nullify the influence of temperature higher than the body temperature. The experiments of Rubner on a guinea pig show that at 35° the chemical heat regulation breaks down, and the respiratory exchange rises with increase in the temperature of the body. Similarly, the experiments of Voit on the metabolism of a fasting man show that his metabolism goes on decreasing up to the limiting temperature of about 15°; after this, the metabolism increases as the surrounding temperature is raised. It follows, therefore, that the fall in the velocity and amount of oxidation of food materials in the animal body will be more marked with time when the animal lives in a warmer than in a colder climate, because the catalysts lose their activity more readily at higher temperatures, though the amount of oxidation in a warm country will be larger at first than in a cooler country. Moreover, as the catalyst has to oxidize a greater quantity of food material at a higher temperature in unit time, its activity should decrease more rapidly. Consequently, it appears that old age and death will occur more quickly in warmer than in colder climates under otherwise identical conditions, especially with cold-blooded animals. Krehl and Soetbeer (*Pflüger's Archiv* **77**, 611 (1899)) have compared the heat production of animals from temperate climates (Lacerta, Rana) with that of tropical animals (Alligator, Uromastix), and found higher figures for the former than for the latter at identical temperatures.

Heat Production of Different Animals.

	Weight in grms.	Cal per Kg. and hour at 25·3°	Cal. per Kg. and hour at 37°
Lacerta	110	0·8	1·5
Rana	600	0·5	0·95
Alligator . . .	1380	0·3	0·47
Uromastix . . .	1250	0·26	0·4

Statistics show that the average longevity of human beings in tropical countries is less than in colder countries, as in tropical countries for part of the year the outside temperature is higher than the body temperature.

The work of Loeb and Northrop (*J. Biol. Chem.* **32**, 103 (1917)) affords direct experimental evidence of this, as will be seen from the following table.

Effect of Temperature on Duration of Life of Drosophila (fly)—a Cold-blooded Insect.

Temperature in °C	Total Duration of Life from egg to death days
10	177·5
15	123·9
20	54·3
25	38·5
30	21·15

From this table it is seen that at the lowest temperature the duration of life is longest, and at the highest temperature shortest. Cold slows down the rate of living for the fly, heat hastens it. One gathers from the account which Loeb and Northrop give of their work, that at low temperature flies are sluggish and inactive, and perhaps live a long time because they live slowly. At high temperatures, on the other hand, a fly is very active and lives its life through quickly at a pace that kills. This relation is more easily applicable to cold-blooded animals, because with warm-blooded animals the relation is more complicated on account of the so-called chemical heat regulation.

The activity of the catalysts of the body cells is usually associated with their surface energy. In course of time the surface energy of all systems tends to decrease. Consequently the activity of the cells or enzymes is likely to decrease because of the fall of their surface energy with time; hence their catalytic activity is likely to decrease with time. It is quite possible for the membranes or tissues in the body, which consist mainly of cells or protoplasm, to become hardened in course of time due to their partial coagulation and dehydration. It should be emphasized that dehydration and decrease of surface energy play a very important rôle in the catalytic activity of tissues, membranes, enzymes, and cells.

There are several biological facts in support of the above views. In investigations made upon isolated organs it has been shown that a certain minimum metabolism involving respiratory exchange is inseparable from the life of every organ, and will persist when the organ is doing no work whatever, so long as the ability to do work remains.

Comparative experiments on the standard metabolism at different ages have been made on man by Magnus-Levy and Falk (1899). The older experiments of Tigerstedt and Sondén (1895) were also made on man, but the standard conditions were not rigorously observed. The general results of Tigerstedt and Sondén's experiments were, however, very similar to those of Magnus-Levy and Falk.

Calculated per unit weight (kilogram) the metabolism of growing individuals is much larger than that of adults, and in old age there is a distinct decrease. Adult individuals belonging to the same species, but of different size, have a larger gas-exchange per kilogram the smaller their size; while it has been shown that when the metabolism is calculated per unit surface (square metre), approximately the same figure is obtained for all sizes. A comparison between young and adult human beings must, therefore, be instituted on the basis of surface instead of weight, as there are large differences in size. When this is done, Magnus-Levy and Falk find that the differences, though of course diminished, are not abolished, as the table given below shows:—

Standard Metabolism of Males of Different Ages.

Age, years	Weight, Kg.	Surface, Sq. metre	Per square metre and minute	
			Oxygen c.c.	CO_2 c.c.
2·5—11	11·5—26·5	0·627—1·094	175—154	501—122
10—16	30·6—57·5	1·205—1·834	159—132	133—109
22—56	43·2—88·3	1·516—2·411	129—111	105—82
64—78	47·8—69·3	1·622—2·077	103—87	86—63

Magnus-Levy and Falk have also made experiments on three subjects of approximately the same weight and height (and consequently the same surface) but very different age. They obtained the results given in the following table.

Standard Metabolism of Persons with Same Surface.

Age, years	Weight, Kg.	O_2 per minute c.c.	O_2 per Kg. relative figures
15	43·7	217	110
24	43·2	196	100
71	47·8	163	75

Individuals of the same age and stature may also have very different standards of metabolism. Consequently, these experimental results support the view that old age is associated with marked decrease of the catalytic activity of the body enzymes and cells, so that in old age there is a marked decrease of metabolism. Death, according to this point of view, follows when the amount of oxidation in the body falls just below the minimum necessary to maintain the body temperature.

CHAPTER XI

Coagulation of Blood, Serum, and Milk.

VIEWS ON BLOOD CLOTTING.

A. Schmidt (*Pflüger's Arch.* **6**, 413, 491 (1873); **11**, 515 (1876)) was the first to explain the mechanism of blood clotting on the assumption of the transformation of a soluble protein, fibrinogen, to the insoluble fibrin under the influence of a hydrolytic enzyme, now called thrombin. Schmidt observed the following facts regarding blood clotting:—(1) The amount of fibrin formed is practically independent of the quantity of the enzyme. (2) The activity of the enzyme increases with temperature, reaching a maximum at the body temperature, and is completely destroyed at 100°. (3) The clear liquid, obtained after the separation of the fibrin, can coagulate a fluid containing fibrinogen and fibrined plastic substance. Schmidt attempted to strengthen his hypothesis by microscopical observations on clotting, and stated that the fibrin ferment did not pre-exist in the blood, but was caused by the colourless blood corpuscles.

Later on, Wooldridge (*Proc. Roy. Soc.* **36**, 417 (1885); **38**, 69 (1885)) and Bürker (*Pflüger's Arch.* **102**, 36 (1904)) advanced the view that the fibrin ferment is not generated by blood corpuscles, as assumed by Schmidt, but that the origin of this ferment is the blood platelet.

On the other hand, Halliburton (*Proc. Roy. Soc.* **44**, 255 (1888)) ascribed the clotting of blood primarily to the disintegration of the white blood corpuscles liberating cell-globulin, which converts fibrinogen into fibrin.

Morawitz (*Beitr. Chem. Physiol. Path.* **4**, 381 (1903); **5**, 133 (1904)) believed that the clotting of blood was more complicated than was hitherto assumed, and stated that fibrin ferment consisted of two substances, α- and β-thrombin, each having its percursor α- and β-prothrombin (compare Fuld and Spiro *ibid.* **5**, 171 (1904)). Nolf (*Bull. Acad. roy. Belg.* 71 (1906)) assumed that thrombin contained leucothrombin, secreted by leucocytes, and hepato-thrombin, secreted by liver. Nolf's hepato-thrombin appears to be identical with the anti-thrombin recognized by Howell (*Amer. J. Physiol.* **26**, 453 (1910)) and se-

veral other physiologists. In blood, Howell assumes the existence of another substance, prothrombin, which is converted into thrombin by the action of calcium salts. According to Howell, blood remains in the fluid condition in the animal body owing to the presence of antithrombin, which is assumed to prevent the calcium from activating prothrombin to thrombin. In shed blood, the tissue elements furnish thromboplastin, which nullifies the effect of antithrombin, so that clotting takes place.

It appears that the views of Howell favourably impressed many physiologists, because Barratt (*Bioch. J.* **9**, 511 (1915)), Dale, and Walpole (*ibid.* **10**, 331 (1916)), Resch (*Bioch. Zeit.* **78**, 297 (1917)), and Gasser (*Amer. J. Physiol.* **42**, 378 (1917)) are in substantial agreement with his views.

The theories so far discussed assume the existence of different ferments, but no conclusive evidence has been advanced to elucidate their true nature. It is quite possible that some of the ferments may assist the process of clotting, but their existence in animal blood inside the body and yet without causing clotting appears doubtful. The numerous assumptions regarding the existence and functions of antithrombin, prothrombin, leuco- and hepatothrombin, thrombokinase, thromboplastin, and various other hypothetical substances, do not appear to elucidate the nature of blood clotting. In this connection, the following remarks of McDonagh (*The Nature of Disease*, Part I, 243 (1924)) are of interest "The existence of thromboplastic bodies is doubtful, and in my opinion there are no such substances as ferments. There seems no reason to suppose that there is such a substance as thrombin. If this is the case then there is no need for the suggested existence of the mystic body, antithrombin. Blood may clot in the living vessels, and it may be made to do so by administration of suitable conductors (electrolytes). The logical sequence of this is that even the presence of calcium is not necessary".

THEORIES OF BLOOD CLOTTING FROM THE VIEW POINT OF COLLOIDS.

The clotting of blood from the viewpoint of colloid chemistry appears to have been considered first by Hekma (*Proc. K. Akad. Wetensch. Amsterdam* **16**, 172 (1913)), who observed that coagulation of blood could also be effected in solutions which had been boiled; and from this he concluded that ferments play no part in the process of clotting. Hekma realized that fibrinogen is simply an alkali hydro-

sol of fibrin, and that its conversion into the gel-fibrin is merely due to the withdrawal of hydroxyl ions from the adsorbed compounds. He (*Bioch. Zeit.* **77**, 273 (1916)) has also applied von Nägeli's micellar theory to the structure of fibrin gel.

Regarding the fibrinogen sol, Howell (*Amer. J. Physiol.* **40**, 526 (1916)) states that it may have either a positive or negative electrical charge according to the reaction of the medium. From his ultramicroscopic observations, he regards the structure of fibrin gel to be reticular or sponge-like, and not like honeycomb. Recently Stuber and co-workers (*Bioch. Zeit.* **134**, 239, 250, 260 (1922); **140**, 42 (1923); **150**, 542 (1924); **155**, 477 (1925)) appear to have studied the same problem from the colloidal point of view.

From the foregoing introduction it appears that very little is known definitely regarding blood clotting.

The same problem has been investigated in detail in the author's laboratories (compare Satya Prakash, Ghosh, and Dhar. *J. Indian Chem. Soc.* **5**, 313 (1928); Dhar and Satya Prakash, *J. Phys. Chem.* **33**, 459 (1929); **34** (1930); *J. Indian Chem. Soc.* **7**, 723, (1930)); the coagulation of serum under various conditions, and of milk has also been studied.

In communications (*Z. anorg. u. allg. Chem.* **152**, 399 (1926); **164**, **63** (1927); **168**, 214 (1927); *J. Indian Chem. Soc.* **6**, 391, 587 (1929); **7**, 367, 417 (1930)) from these laboratories, we have pointed out the conditions of the formation of jellies of different substances. We have proved that jellies can be divided into three groups. The first group consists of gelatine, starch, agar, silicic acid, soaps, manganese arsenate, zinc arsenate, vanadium pentoxide, ceric hydroxide, etc. In this group, the particles forming the jellies consist of some kind of network. These substances produce most stable jellies which are formed very readily. Jellies of the second group are obtained by the slow coagulation of their sols throughout the whole mass. Possibly hydroxides of iron, aluminium, and chromium form typical members of this group, whose particles need not consist of a network. The stability of this group is less than that of the first group; but both have marked affinity for water. The third group forms the von Weimarn jellies, which are the least stable ones. These jellies consist of finely divided substances which are precipitated very suddenly.

We are of the opinion that clotted blood is nothing but a jelly, which is similar to that obtained in the first group of substances, consisting of particles forming a network. We have proved that the stable jellies of ceric hydroxide, vanadium pentoxide, and silicic acid

undergo syneresis in course of time due to the decrease of the hydration tendency of the particles forming the jelly.

It has been shown in the author's laboratory, that sols of vanadium pentoxide, ceric hydroxide, silicic acid, several borates, vanadates, arsenates, molybdates, tungstates, etc. spontaneously set to a jelly on keeping, even without the addition of electrolytes. Moreover, Dhar and Gore (*J. Indian Chem. Soc.* **6**, 31, 641 (1929)) have shown that highly purified sols of ferric, chromic, aluminium, zirconium, and thorium hydroxides also spontaneously set to jellies on keeping. Hence the author is of the opinion that the spontaneous coagulation of blood is not an unusual phenomenon in colloid chemistry, because numerous organic and inorganic colloids show the same behaviour.

It seems likely that blood as a whole is an unstable colloid like the above mentioned sols, which set to a jelly very readily. In many respects blood is something like ceric hydroxide sol. On keeping, blood forms a gel very readily; in this respect it is certainly less stable than ceric hydroxide sol prepared in the cold. When blood sets it undergoes syneresis very readily. The syneresis in case of blood is a much quicker process than in the case of other inorganic and organic jellies. We have advanced the view that blood forms an unstable negatively charged suspension colloid. Its coagulation in the body is prevented by its circulatory motion in capillary vessels. The rapid and continual pumping of blood in veins and arteries does not allow the suspended particles to agglomerate. It appears that the forces of surface tension also play an important part in maintaining the fluidity of blood, the blood vessels being capillary. It is an interesting fact that the coagulation of blood and milk can be effected very readily by acids, and in this respect these substances resemble mastic, gamboge, and other readily hydrolyzable sols.

We have shown that when a jelly is formed readily from a sol the particles must have a tendency to form a network or mesh; and we are convinced that blood consists of particles which form a network or mesh very readily. Consequently, blood belongs to the group of sols containing those of vanadium pentoxide, ceric hydroxide, gelatine, albumin, starch, etc. When viewed with a powerful microscope the fibrin appears as woolly, finely divided crystals. We have observed that when a jelly, like that of zinc arsenate or manganese arsenate, is broken up, it cannot re-form because the network which was formed from the particles is destroyed. Now with blood an exactly similar behaviour is observed. If a big blood clot is taken out and churned, the clot is broken up into a mobile fluid which does not

re-set readily. Similarly, starch jelly and gelatine jelly, when broken, do not re-set readily. Consequently, in many respects blood and possibly milk resemble a sol of vanadium pentoxide, ceric hydroxide, or manganese arsenate, though blood is far less stable than these other sols. The important question which now arises and remains unanswered is—why do not blood and milk coagulate in the animal system? In the case of blood, it seems likely that the clotting is prevented in the system by the motion of the fluid. When the motion is disturbed by some means or other, clots or thrombi are formed.

Moreover, by some recent experiments it has been proved that the clotting tendency is most marked near the neutral point of blood. On the acid side and alkaline side the charge on the blood is increased, and hence the hydration tendency is less, and clotting happens with greater difficulty in alkaline or acid solutions of blood.

If blood received in a vessel is not stirred the clot forms uniformly throughout the whole quantity of blood, converting it to a solid, rather dry, firm mass. Such a clot is of a uniform dark red colour. If it be squeezed, a dark red fluid is forced out which is identical with defibrinated blood. If the fresh blood be kept very cold, or if it be received in an oiled dish, it will not clot so quickly; and, since the red corpuscles are heavier than the plasma, there may be time for them to sink to the bottom in a very thick layer. The leucocytes are lighter, and rest in a layer on top of the red corpuscles, whilst above them there is some plasma almost free from cells. By this time clotting has occurred throughout, the clot differing from the uniform red one formed by rapid coagulation by the presence of a greyish yellow upper layer which contains most of the leucocytes and platelets. This slow clotting, showing the effect of gravity, is seen very commonly in the heart at autopsy, for the intact endothelial lining of the heart keeps its contents a long time without clotting. Then the deep red portion of the clot is in the lower part, while the tough, elastic, translucent yellowish substance occupies the uppermost part of the heart.

The foregoing observations can be explained by the view that blood consists of a mixture of colloids having different densities. If the clotting is very rapid, as usually happens when blood is shed, network or meshes are formed, the whole of the liquid is adsorbed in the network, and a uniform jelly is obtained, there being no time for the separation of the colloids according to their different densities. When the clotting is retarded, there is time for different colloids to separate in different layers, and we get clotting in different layers. We are of the opinion, therefore, that blood consists of a mixture of

colloids, forms a very unstable system, and has a great tendency to clot. This clotting tendency is probably caused by the same forces which cause the gelation of the sols of silicic acid, vanadium pentoxide, ceric hydroxide, starch, gelatine, etc., and seems to be due to the increase in the hydration tendency of the particles which form a network.

It is well-known that the action of copper on nitric acid is an autocatalytic process, because the product nitrous acid markedly accelerates the reaction. It seems likely that the clotting of blood is also autocatalytic in its nature, because the liquid given out immediately after clotting accelerates the clotting process. Hence it appears that the clotting of blood is a process which takes place by itself due to the extremely unstable nature of the fluid, and that it is not initiated by thrombin or any other substance. It seems likely, however, that thrombin and other products which appear after clotting markedly accelerate the clotting process. The syneresis that is observed is really an ageing phenomenon, which is observed with most other gels, and is due to the decrease in the free surface, activity, and hydration tendency of the particles forming the jelly. We have repeatedly observed that sols of silicic acid, vanadium pentoxide, ceric hydroxide, etc., form transparent jellies when merely kept in stoppered bottles at the ordinary temperature. In course of time these stable jellies undergo syneresis. Exactly similar behaviour is observable with blood, the only difference being that blood is far less stable than the inorganic sols; and so clotting and syneresis are quicker processes in blood than the gelation and syneresis observed with inorganic sols. Hence the author (Dhar and Satya Prakash, *J. Phys. Chem.* **33**, 459 (1929)) has advanced the view that the clotting of blood is guided by the same laws as the formation of jellies of vanadium pentoxide, ceric hydroxide, silicic acid, and various other organic and inorganic colloids. The only difference seems to be that blood is far less stable than the inorganic sols; so that clotting and syneresis occur more quickly. The relation between fibrinogen and fibrin is the same as that between the disperse phase and the coagulum.

INFLUENCE OF HYDROGEN IONS ON BLOOD CLOTTING.

Fibrin exists as a colloidal suspension in blood in a highly buffered system, and, as de Waele (*Ann. physiol. physicochim. biol.* **3**, 94 (1927)) has shown, the hydrogen ion concentration of the system is subject to variations. His experiments show that fibrinogen is precipitated at p_H 5—6, forms a gel at p_H 7—9, and remains dissolved at p_H 10.

The significance of hydrogen ion concentration in blood was also shown by Michaelis (*Chem. Zentr.* (i) 1004 (1915)).

Rabinovitch (*Anal. Asoc. Quim. Argentina* **14**, 139 (1926)) has observed that the coagulation of oxalated plasma is effected by hydrogen ion concentration, the maximum coagulability being at p_H 6·3—6·8. Both plasma and fibrinogen are uncoagulable by p_H 5·0, and above p_H 8·0. The transformation of fibrinogen into fibrin can take place in both acid and alkaline solutions.

In a publication (*J. Phys. Chem.* **33**, 459 (1929)) Dhar and Satya Prakash have stated that the clotting tendency of blood is most marked near the neutral point of blood. On the acid side and the alkaline side the charge on blood is increased, and it becomes more stable, hence clotting is retarded.

It has been found by Anson and Mirsky (*J. Physiol.* **60**, 50, 1161 (1925)) that the blood pigment can exist as such only in the neighbourhood of neutrality, for in definitely acid and alkaline solutions it is converted into hæmochromogen. Both hæmochromogen and its oxide hæmatin are conjugated proteins containing globin. Anson and Mirsky have suggested the name "hæm" for the non-protein part of the molecule containing pyrrole nuclei and iron. An investigation of the influence of globin on the properties of hæmoglobin has been carried out by comparing "hæm" in a series of hæmochromogens and hæmoglobins, in respect to their combination with carbon monoxide under different conditions. This investigation supports the view that the blood pigment is a most highly evolved substance.

COAGULATION OF BLOOD AND MILK BY MIXTURES OF ELECTROLYTES, AND THEIR STABILIZATION BY ADSORPTION OF SIMILARLY CHARGED IONS

The stabilization of blood and milk by the addition of small quantities of oxalate, citrate, fluoride, tartrate, and sulphate ions is certainly due to the adsorption of the negative ions from various electrolytes, and the consequent increase of the negative charge on blood and milk. We are of the opinion that blood and milk will show marked ionic antagonism when coagulated by ions of varying valencies.

The following experimental results were obtained in the coagulation of hæmolyzed goat's blood by ammonium nitrate and by cupric sulphate in presence of small quantities of sodium citrate, sodium acetate, potassium fluoride, sodium tartrate, and potassium hydroxide.

Table I.
2 c.cs. of blood made up to 10 c.cs.

Time = 1 hour

	Amount of electrolyte added in mols M	Concentration of ammonium nitrate necessary for coagulation M
No salt added		4·482
Sodium citrate	0·022	5·258
Potassium fluoride	0·114	4·741
Sodium acetate	0·095	4·913
Potassium hydroxide	0·002	4·999

Table II.
2 c cs of dilute blood made up to 10 c.cs.

Time = 1 hour.

	Amount of electrolyte added in mols M	Concentration of copper sulphate necessary for coagulation M
No salt added		0·000025
Sodium acetate	0·0966	0·000175
Sodium tartrate	0·0178	0·000950
Sodium citrate	0·0022	0·000200
Potassium hydroxide	0·0020	0·000700

The following results were obtained in the coagulation of dilute milk by cupric sulphate in presence of sodium acetate, sodium tartrate, sodium citrate, and potassium hydroxide.

Table III.
2 c.cs. of dilute milk made up to 10 c.cs.

Time = 1 hour.

	Electrolyte added in mols M	Concentration of copper sulphate necessary for coagulation M
No salt added		0·0011
Sodium acetate	0·00946	0·0015
Sodium tartrate	0·00356	0·0015
Sodium citrate	0·00220	0·0027
Potassium hydroxide	0·00200	0·0017

The experimental results given in tables I—III prove conclusively that blood and milk show ionic antagonism markedly when coagu-

lated by ammonium nitrate or copper sulphate in presence of small quantities of sodium citrate, potassium fluoride, sodium acetate, and potassium hydroxide. In presence of the above substances the particles of blood and milk are stabilized by the adsorption of OH′ and other negative ions from the above electrolytes. This stabilization is due to the increase in the amount of negative charge on the particles, and is exactly identical with the behaviour of sols of arsenious sulphide, antimony sulphide, prussian blue, etc., already investigated.

The above results show that the explanation of the stability of blood and milk due to the presence of small amounts of citrate, tartrate, oxalate, fluoride, etc., based on the view of the removal of the precipitating calcium ions by these negative ions, is incorrect. The real explanation of the stability of both blood and milk in the presence of sodium or potassium salts of the above acids is the increase in the electric charge on their particles due to the adsorption of the negative ions, and of OH′ ions derived from the hydrolysis of the sodium or potassium salts of the weak acids already mentioned.

Blood and milk are negatively charged colloids having a well marked tendency to adsorb similarly charged ions, whereby they are appreciably stabilized due to increase in the charge on their particles.

From our experiments on the coagulation of blood and milk, it is clear that in presence of small quantities of sodium citrate, potassium fluoride, sodium acetate, sodium tartrate, or caustic potash, blood and milk are stabilized by the adsorption of OH′ or other negative ions.

It has long been known that if blood is received into a solution of sodium or ammonium oxalate, fluoride, or citrate, it will remain in the liquid condition indefinitely. If, however, sufficient calcium chloride is added to such oxalated or citrated blood, clotting occurs normally. Fluoride blood does not clot spontaneously on the addition of an excess of calcium chloride. Since these substances, except citrate, have the power of precipitating calcium salts at the ordinary temperature, it has been concluded by several investigators that the presence of calcium salts is necessary for blood clotting. While citrate does not precipitate calcium in the cold, it is believed to unite with calcium and hold it in a non-ionised form; so that the action of citrate ion is also supposed to be that of a decalcifier.

While Stuber holds that calcium is not essential for the clotting of blood, he (Bioch. Z. **134**, 260; **140**, 42 (1923)) ascribes the stabilization of blood by oxalates, citrates, cholates, glycocholate, tauro-

cholate, magnesium sulphate, and sodium chloride to the formation of fibrinogen-salt complexes, and the clotting by an excess of calcium or strontium salts to a reversal of the ionization of the complex.

We are of the opinion that the assumption of complex salt formation has no substantial evidence in its support. The stabilization of blood and milk by different salts is due to the adsorption of negative ions from the salt, while the adsorption of positively charged calcium ions again coagulates the oxalated or citrated plasma.

It is well known that sodium citrate is added in small amounts to milk in infant and invalid feeding. The function of citrate is as follows:—Milk is a negatively charged colloid, and in presence of sodium citrate it adsorbs the citrate ion; hence its stability is increased and its tendency to coagulation markedly decreased. Moreover, the OH' ions present in sodium citrate due to its hydrolysis increase the stability of milk. Consequently, addition of citrate prevents the formation of large lumps of coagulated milk inside the body, and thus facilitates digestion. Moreover, the increase in alkalinity arising from the addition of citrate will lead to increased oxidation within the body of the protein, fat, and milk sugar contained in milk. This view has already been emphasized in the foregoing pages.

SENSITIZATION OF BLOOD BY GELATINE AND SAPONIN.

We have also studied the coagulation of blood in presence of small quantities of gelatine and saponin. Saponin and gelatine render blood unstable towards salts; with acids, gelatine does not markedly sensitize blood. Thus it seems that the sensitizing influence of gelatine is due to $H^{\cdot}$ ions present in it.

We have also stated that the hæmolytic action of saponin on blood is due to its sensitizing influence, and its rendering blood unstable towards electrolytes.

EXPLANATION OF THE USE OF CONCENTRATED SOLUTIONS OF SODIUM CITRATE IN THE TREATMENT OF TUBERCULOSIS.

Recently, medical men have used solutions of sodium citrate with great success for stopping the outflow of blood with sputum in cases of tuberculosis. Concentrated solutions of sodium citrate are intraveneously injected, and the outflow of blood is stopped in a short time. From our experimental results (*J. Phys. Chem.* **33**, 459 (1929)),

it is clear that the stopping of the outflow of blood is due to its coagulation by concentrated solutions of sodium citrate. These results are interesting in view of the well known fact that dilute solutions of sodium citrate hinder the coagulation of blood. Our results explain satisfactorily the peculiar behaviour of blood, that it is stabilized and its clotting hindered in presence of dilute solutions of sodium citrate, oxalate, fluoride, etc., but that the outflow of blood is stopped by concentrated solutions of the above electrolytes.

GELATION OF BLOOD AND ITS SYNERESIS.

In numerous publications from the author's laboratory on the formation of inorganic jellies, it has been shown that the formation of a jelly depends on the balance of the hydration and agglomeration tendencies of the colloid particles; and again, that this hydration is a function of the charge on the colloid. Under similar conditions, uncharged particles of both hydrophilic and hydrophobic sols are more hydrated than the charged ones.

The charge on a particular particle depends upon the nature of the ions adsorbed by it. Whenever similarly charged ions are adsorbed the electric charge increases, the system becomes less viscous and less hydrated, but more stable. When oppositely charged ions are adsorbed the charge is diminished, and the particles develop more hydration tendency and viscosity. Whenever the hydration tendency is very great, and preponderates over the agglomeration tendency of the particles, jellies or gelatinous precipitates are to be expected.

The clotting of blood depends on the characteristic unstable nature of fibrin suspension, its concentration, and the nature and concentration of the electrolytes present; and all these have been so regulated in blood that as soon as the capillary action of the blood vessels and circulatory motion are stopped jelly-forming forces begin to assert themselves, and a solid clot is formed in a few minutes.

The existence of different electrolytes in the original blood leads to the agglomeration of the coagulated fibrin particles, and the contraction of the blood clot with time, and thus the synerized serum is squeezed out from the network forming the clot. Such syneresis has been observed with various inorganic jellies, such as those of vanadium pentoxide, ceric hydroxide, ferric arsenate and borate, and various zirconium jellies. These jellies, on ageing, markedly lose their hydration capacity, and on account of the agglomeration of

the particles forming the jelly the adsorbed liquid medium is squeezed out.

It has already been mentioned that the process of jelly formation is controlled by the agglomeration and hydration tendencies of the colloid particles. In presence of coagulating electrolytes the charge on a jelly-forming sol decreases, and the amount of hydration increases up to a limiting value, and a jelly may be formed. When the concentration of the electrolyte is increased, agglomeration of the particles begins, with the result that the jelly contracts and undergoes syneresis.

The same behaviour is observed with blood. Under the action of coagulating ions blood forms a clot, and due to the presence of an excess of the same ions its particles agglomerate, the clot undergoes contraction, and finally serum is squeezed out.

We have repeatedly observed that the jellies obtained by the spontaneous gelation of sols of ceric hydroxide, vanadium pentoxide, silicic acid, several borates, tungstates, molybdates, etc., undergo syneresis on ageing even in the absence of electrolytes; because on ageing the hydration tendency of the particles forming the jelly is decreased. Exactly similar behaviour is observed with blood.

From our experiments on syneresis of blood and of various inorganic jellies, it is clear that the extent of syneresis is regulated by the amount of the electrolytes present. In fact, the syneresis is brought about by the presence of an excess of coagulating electrolytes. The action of the stabilizing ions on the syneresis is just the reverse of that of the coagulating ions. As blood has a great tendency to adsorb similarly charged ions, the following stages will be observed with respect to the concentration of electrolytes on the syneresis of blood:—

(a) in presence of small concentrations the amount of syneresis will not be much affected;

(b) with increase in the concentration the amount of syneresis will decrease;

(c) with higher concentrations the time of clotting will be delayed, and the syneresis will markedly decrease; whilst at some concentrations no syneresis will be observed within a day or two;

(d) at still higher concentrations of electrolytes the blood will be so much stabilized that it will not clot at all.

We have actually observed all these stages in our experiments on the syneresis of blood in presence of such electrolytes as sodium tartrate and citrate, potassium oxalate, potassium fluoride, potassium chloride, sodium hydroxide, etc.

In the following table we indicate the approximate concentrations of various electrolytes necessary for the stabilization of blood to such an extent that no marked syneresis occurs even 12 hours after the formation of blood clot:—

Electrolyte	Concentration necessary to stop syneresis
Potassium chloride . . .	0·12 N
Calcium chloride	0·084 N
Ammonium sulphate . . .	0·176 N
Potassium oxalate	0·017 N
Sodium citrate	0·016 N
Potassium fluoride . . .	0·049 N
Sodium hydroxide	0·039 N

From the above table it will be seen that the stabilizing influence of these salts is in the following decreasing order:—sodium citrate > potassium oxalate > sodium hydroxide > potassium fluoride > calcium chloride > potassium chloride > ammonium sulphate. Hence it appears that the stabilizing influence of electrolytes depends on the valency of the ions as well as the alkalinity of the medium.

Our results on the influence of calcium chloride on the syneresis of blood clot show that, even in presence of calcium ions, chloride ions are preferentially adsorbed by the particles of blood. We have shown that by increasing the concentration of calcium chloride blood is stabilized, and the amount of syneresis is markedly decreased, whilst with high concentrations of calcium chloride blood does not clot at all. This is because particles of blood have a marked tendency to adsorb the similarly charged chloride ions, whereby the negative charge on blood is increased, and it becomes more stable. Similar results are obtained with potassium chloride. These results explain the erratic behaviour of calcium chloride when it is injected into the body for stopping the outflow of blood. Medical men have reported that when the concentration of calcium chloride is high the outflow of blood is not stopped by injecting calcium chloride. Their observations are explained by our experimental results.

Hence it is clear that the clotting of blood is similar to the formation of such inorganic jellies as vanadium pentoxide, ceric hydroxide, silicic acid, various arsenate, phosphate, molybdate, tungstate, and borate jellies; the only difference being that the clotting and syneresis are more rapid with blood than in other cases, and this is due to the complex and unstable nature of the fibrin suspension in blood.

CHARACTERISTICS OF SERUM AS A COLLOID.

We (*J. Indian Chem. Soc.* **5**, 313 (1928); **7**, 723 (1930)) have carried out detailed investigations on the coagulation of serum. We have observed that polyvalent cations and acids possess high coagulating powers towards sheep or goat serum. The stability of a diluted serum towards its coagulation by univalent ions of salts like sodium acetate, potassium fluoride, potassium oxalate, etc., is far greater than that of the concentrated serum. On the other hand, small amounts of acids and polyvalent cations more readily coagulate a dilute serum than a concentrated one.

Serum also exhibits marked ionic antagonism when coagulated by mixtures of cations of varying valencies, or an acid and salt.

Several workers, notably Freundlich (*Z. phys. Chem.* **44**, 143 (1903)), Weiser (*J. Phys. Chem.* **23**, 399 (1921)), and Ghosh and Dhar (*ibid.* **29**, 659 (1925); **31**, 649 (1927); *Kolloid. Z.* **37**, 141 (1926)), have shown that the amount of electrolyte necessary for coagulation of some colloids is greater when the addition of the electrolyte to the sol is slow and spread over a long time, than when it is rapid and the coagulation is finished quickly. Ghosh and Dhar have termed this phenomenon, which is frequently observed with many sols, "positive acclimatization".

The phenomenon of positive acclimatization is observed when serum is coagulated by either dilute acids or salts, and it is more marked with salts than with dilute acids. With potassium fluoride and potassium oxalate no positive acclimatization is observed, because of the formation of calcium fluoride and oxalate from the calcium ions present in the serum. The phenomenon of acclimatization is more marked for the dilute serum than the concentrated one when coagulated by salts. With acids, the phenomenon is less developed with dilute serum.

Serum, therefore, behaves in certain respects like sols of mastic, gamboge, arsenious sulphide, etc., and is capable of adsorbing similarly charged ions from coagulating electrolytes. It is, however, not hydrolyzed to the same extent as the gums, prussian blue, and cupric ferrocyanide, and possibly does not adsorb the coagulating ion very highly.

From our experiments on coagulation of serum by acids, we have observed the interesting fact that there are two points of coagulation in serum, one with dilute acids and the other with concentrated acids. Serum behaves normally towards dilution when coagulated by

dilute acids, but owing to the adsorption of similarly charged ions, dilute serum requires more concentrated acid to coagulate it at the second point than concentrated serum.

We are of the opinion that it is chiefly the globulin portion of serum which is precipitated by the dilute acids at the first point of coagulation. Near the second point of coagulation serum albumin is also thrown down.

We believe, also, that a reversal in charge of serum takes place when we pass from the first point of coagulation by dilute acids to the second point of coagulation by concentrated acids. This view is confirmed by the fact that serum is easily coagulated by ammonium sulphate in presence of small quantities of acids. This may also be partly due to the fact that the hydrogen ions markedly retard the hydrolysis of serum, and make it unstable like the sols of arsenious sulphide, gamboge, mastic, prussian blue, etc. (Compare, *J. Phys. Chem.* **30**, 830 (1926); *Kolloid. Z.* **29**, 346 (1926)).

Coagulation of serum has been tried with hydrochloric, nitric, sulphuric, oxalic, tartaric, citric, acetic, monochloracetic, trichloracetic, hypophosphorous, phosphoric, and formic acids, and it is found that the reversal of charge of serum always takes place at almost the same hydrogen ion concentrations (p_H 5·3—5).

The coagulating power of all the dilute acids at the first coagulation point is of the same order, since all the acids are fairly completely dissociated in the dilute solutions investigated. In the case of the second point, the coagulating powers of different acids follow the order of their dissociation constants.

As we have stated, near the second point of coagulation serum albumin is also thrown down. We believe that the particles of serum-albumin are capable of developing more hydration than those of serum globulin.

We have been able to gelatinize serum at the second point of coagulation by strong acids. Transparent and stable jellies have been obtained by the use of glacial acetic acid, concentrated formic, monochloracetic, and hypophosphorous acids. Solid oxalic acid also gives stable jellies. Strong acids like hydrochloric, sulphuric, nitric, and trichloracetic yield opaque jellies. No jellies have yet been obtained with weak acids like tartaric, citric, and phosphoric; because it is not possible for solutions of these acids to be as concentrated as those of formic acid or glacial acetic acid. The concentrations of these acids necessary for the formation of jellies follow the order of their dissociation constants.

As the albuminous particles of serum have the tendency to adsorb similarly charged ions, the behaviour of serum towards dilution with concentrated acids is abnormal. Similarly, our results on gelation of serum with concentrated acids show that more acid is required to gelatinize a dilute serum in a definite time than a concentrated one.

All the jellies obtained from serum are very stable, and do not undergo any marked syneresis.

From the foregoing discussion on the stability of blood, serum, and milk, it is evident that these have a marked tendency to increased stabilization by the adsorption of hydroxyl and other negative ions. Hence life processes are possible by the maintenance of these complex and unstable fluids in the liquid condition inside the animal body. Moreover, these fluids are rather subtle, inasmuch as they can take up either a positive or a negative electric charge, according to the reaction of the medium.

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