Oxidations and reductions in the animal body / by H.D. Dakin.

Monographs on Biochemistry

OXIDATIONS AND REDUCTIONS IN THE ANIMAL BODY

BY
H. D. DAKIN, D.Sc., F.I.C.

P. P. Laidlaw

MONOGRAPHS ON BIOCHEMISTRY

EDITED BY

R. H. A. PLIMMER, D.Sc.

AND

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OXIDATIONS AND REDUCTIONS

IN

THE ANIMAL BODY

BY

H. D. DAKIN, D.Sc., F.I.C.

THE HERTER LABORATORY, NEW YORK

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TO

CHRISTIAN A. HERTER

1865-1910

GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single text-book upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult, in the case of the larger text-books, to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason, an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

It has been decided as a general scheme that the volumes first issued shall deal with the pure chemistry of physiological products and with certain general aspects of the subject. Subsequent monographs will be devoted to such questions as the chemistry of special tissues and particular aspects of metabolism. So the series, if continued, will proceed from physiological chemistry to what may be now more properly termed chemical physiology. This will depend upon the success which the first series achieves, and upon the divisions of the subject which may be of interest at the time.

R. H. A. P.
F. G. H.

PREFACE.

THIS small volume aims to give an account of the principal chemical reactions involving oxidation, or reduction, which are known to take place in the animal body. The subject is treated simply from the standpoint of the structure of the substances undergoing change.

The statements that fats and sugars are oxidized in the body to carbon dioxide and water, while proteins yield urea in addition, are no longer considered all-sufficient explanations of the chemical rôle of these substances in the animal economy. The study of chemical structure is rapidly changing the whole aspect of biological science, and we may confidently look forward to the time when the orderly succession of chemical reactions constituting the activities of the living cell will be resolved into their individual phases.

It is only within the last six or seven years that substantial progress has been made in unravelling, at least in part, the details of some of the simpler oxidation and reduction processes occurring in the animal body. But enough has been done to show that the problem is capable of successful attack by our present limited experimental methods. The significance of these investigations for the biological sciences, including medicine, hardly requires emphasis.

With the development of modern organic chemistry, the realization of the inadequacy of the common representation of chemical reactions by means of the usual formulæ becomes increasingly evident. The study of valence which is attracting investigators from all sides is likely to prove a most helpful aid in the adequate comprehension of reactions both *in vitro* and in the living organism.

H. D. D.

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CHAPTER I.

I. INTRODUCTION.

THE evolution of heat as the result of vital activities and its cessation with death must have been obvious to man in the earliest stages of his development. It was recognized long ago that the phenomena of production of animal heat and the combustion of carbonaceous materials outside the body had much in common. The discovery of oxygen by Priestley and by Scheele (1771) first made it possible to form a rational conception of the chemical changes involved in combustion, but it was left for Lavoisier to clearly state the fundamental facts in a relatively modern form. The chemistry of the oxidations taking place in the animal body may fairly be said to date from the publication in 1780 of Lavoisier's "Experiences sur la respiration des animaux et sur les changements qui arrivent à l'air en passant par leur poumon". It was clearly shown by Lavoisier that oxygen was taken up by the lungs and in part converted into carbon dioxide, the carbon in Lavoisier's opinion being derived from the blood. The whole process was recognized as one of combustion and was believed to constitute the natural mechanism for the supply of animal heat. The necessity of furnishing the body with combustible material in the form of food in order to avoid injurious loss of body substance was clearly understood as was also the increased output of carbon dioxide following muscular activity.

Perhaps one of the most interesting results of Lavoisier's investigations is the fact that they led him to the conviction that vital processes are made up of a series of chemical reactions (1792). Unfortunately this idea was not revived until a much later date, and not until Liebig emphasized the importance for biology of the newly developing organic chemistry were material advances made. By degrees a clearer knowledge was obtained of the chemical nature of the fats, carbohydrates and proteins and of the laws governing their mutual interconversions in the body. One of the most important methods devised for the investigation of the metabolic activities of living organisms consists in the study of so-called "balances" in which the intake and output of carbonaceous and nitrogenous substances are compared. Methods for

the estimation of the final products of animal catabolism—carbon dioxide, water, urea, etc.—as well as of heat production have attained a remarkable degree of accuracy. But it is clear that such methods of study leave untouched the infinitely intricate problems of intermediary metabolism. It is relatively easy to obtain a balance sheet representing the intake and output of substances in the animal body, but what is fundamentally necessary for the proper appreciation of this balance sheet is a knowledge of the various chemical transactions which (to continue the simile) should be comprised in a trading account. For it is by the proper adjustment and regulation of these transactions that the energy represented by food and tissue substance are economically utilized according to the varying needs of the body. The rapidly developing appreciation of the fact that different proteins, fats and sugars are not physiologically equivalent but that certain definite chemical groups subserve special functions in the animal organism emphasizes the necessity of the study of intermediary metabolism.

A molecule of stearic acid taken into the body in the form of fat is known to undergo combustion so that eventually each of its eighteen carbon atoms will be converted into carbon dioxide. But no one imagines that such a change is immediate or direct—that every carbon atom simultaneously parts with its attached hydrogen atoms and by combining with oxygen yields carbon dioxide and water. No, the resolution of the fatty acid molecule is undoubtedly affected by a complicated series of chemical reactions following upon each other in definite sequence, and it is likely that most of these reactions are of a reversible character. A true knowledge of metabolic processes can only be obtained by the tedious unravelling of the complex system of biochemical changes into individual chemical reactions. At the present time only a few of these simple reactions have been recognized and studied, but even now it requires little imagination to realize that in the future it will be possible to construct an accurately itemized account of the animal body's chemical transactions both anabolic and catabolic. The value of such knowledge for the advancement of biology and medicine is sufficiently obvious.

Questions of chemical structure are paramount in these investigations, and the object of the following monograph is to give an account of some of the results which have been obtained by considering the oxidation and reduction processes of the animal body from the standpoint of the structure of the substances undergoing change. The purely biological aspects and also the thermodynamics of the problems of oxidation and reduction have been necessarily omitted as outside the

scope of the work. References to the many enzymes, oxygenases, peroxidases, etc., which, so far as is known, are without action upon the principal groups of substances which furnish energy to the organism have also been omitted.

Oxidation of saturated substances either in the laboratory or in the living organism usually consists in the replacement of hydrogen atoms by hydroxyl groups with formation of new substances which in many cases are capable of undergoing further decomposition. In the case of oxidations carried out in the animal body the primary products are so seldom capable of resisting further changes that it is commonly impossible to recognize the intermediate stages leading to the end-products of oxidation. The possibility of actually isolating intermediate products of oxidation from animal tissues and excretions is of course in large measure dependent upon whether the rate of their formation is great compared with the rate of decomposition. But in many cases in which it is impossible to detect intermediate products of oxidation under normal conditions, clues may be obtained as to their formation by indirect methods.

A consideration of the phenomena presented by the combustion of hydrocarbons is of interest in this connexion. Ordinarily a hydrocarbon burned with an adequate supply of oxygen yields nothing but carbon dioxide and water, without any indication of the formation of intermediate products of oxidation. But Bone and others have shown, by suitably modifying the conditions, that the oxidation of hydrocarbons by both slow and explosive combustion must be regarded as a process involving the initial formation of unstable hydroxylated molecules which subsequently undergo decomposition into simpler products. For example, formaldehyde and steam may be identified at an early stage in the combustion of methane, the formaldehyde decomposing at the high temperature into carbonic oxide and hydrogen, which are in turn oxidized to carbon dioxide and water. The changes are believed to take place as follows:—

$$CH_4 \rightarrow CH_3(OH) \rightarrow CH_2(OH)_2 \rightarrow CH_2O \rightarrow H_2 + CO \rightarrow H_2O + CO_2$$

The definite detection of these intermediate products of a reaction which usually results simply in the production of carbon dioxide and water is of great significance, for their formation is essentially analogous to what is believed to occur in the animal body. In the following pages will be found a large number of cases of biochemical oxidations involving the similar formation of hydroxylated intermediate products. Thus butyric acid may yield β-hydroxybutyric acid and acetoacetic

acid, the latter substance being formed from the hypothetical β-dihydroxybutyric acid by loss of water :—

$$\underset{\text{(Butyric acid)}}{CH_3.CH_2.CH_2.COOH} \rightarrow \underset{\text{(β-Hydroxybutyric acid)}}{CH_3.CH(OH)CH_2.COOH} \rightarrow \underset{\text{(β-Dihydroxybutyric acid)}}{CH_3.C(OH)_2.CH_2.COOH}$$

$$\downarrow$$

$$\underset{\text{(Acetoacetic acid)}}{CH_3.CO.CH_2.COOH}$$

Similarly *a*-hydroxy and *a*-amino acids yield *a*-ketonic acids, while hypoxanthine (6-hydroxy-purine) gives on oxidation in the body successively xanthine (2·6-dihydroxy-purine) and uric acid (2·6·8-trihydroxy-purine).

It will be seen later that most of the striking biochemical oxidations of the living cell may be imitated more or less satisfactorily by experiments *in vitro*, and there is no evidence that suggests that the oxidative processes of the living organism differ in any fundamental way from chemical oxidations known to take place in inanimate nature.

II. THE NATURE OF THE OXIDIZING AND REDUCING AGENTS OF THE ANIMAL BODY.

The substances undergoing active metabolism in the animal body, comprising the proteins, carbohydrates, fats, and their derivatives, are practically entirely resistant to oxidation by oxygen under ordinary conditions; yet in the animal body the carbon of all these types of compounds is readily oxidized to carbon dioxide. It is generally conceded that some process of activation of the atmospheric oxygen must take place in the body in order to account for the observed chemical changes. Oxygen loosely bound in the form of oxy-hæmoglobin is an ineffective oxidizing agent, so that the main function of hæmoglobin appears to be that of a more or less indifferent oxygen transport agent.

Many theories have been propounded from time to time to account for the activation of oxygen, not only in biochemical oxidation but in other reactions as well. A detailed discussion of this question lies outside the scope of this monograph, but brief reference may be made to a few of the more important suggestions.[1]

As a result of his studies upon ozone Schonbein was inclined to seek the cause of the activation of oxygen in its polymerization, while many writers in more recent times have put forward the hypothesis that the activation of molecular oxygen is due to a separation of the oxygen molecule into atoms or ions (Clausius, Van t'Hoff, and others). Hoppe-Seyler, and later Baumann, on the other hand, were inclined to ascribe the activation of oxygen in living cells to the resolution of the oxygen molecule by means of nascent hydrogen or other reducing agent, with formation of water and an atom of active oxygen. Hoppe-Seyler arrived at this hypothesis largely from a study of the changes involved in anærobic fermentation in which the formation of active hydrogen capable of effecting reduction is commonly

[1] For information upon these matters reference may be made to "Kritische Studien über die Vorgänge der Autoxydation," by C. Engler and J. Weissberg, Vieweg and Sohn, 1904, and to an excellent monograph by J. H. Kastle, "The Oxidases and other Oxygen-catalysts concerned in Biochemical Oxidations," Bulletin No. 59, Hygienic Laboratory, Public Health and Marine Hospital Service of the United States. Also G. Bodländer, "Ueber langsame Verbrennung," Ahrens' Vorträge, 1899, 385 (Enke, Stuttgart).

observed. In support of his theory he showed how hydrogen absorbed by palladium in the presence of water and oxygen might effect many interesting oxidations as well as reductions. Examples of these oxidations are the oxidation of benzene to phenol and of toluene to benzoic acid—both of these reactions occurring in the animal body. There are, however, many difficulties in the application of Hoppe-Seyler's theory to the reactions occurring in animal cells.

The most generally accepted views upon auto-oxidation in general are based upon modifications of Moritz Traube's peroxide theory of oxidations. Traube introduced the idea of "oxygen carriers"—substances capable of uniting with molecular oxygen with formation of superoxides. In contrast with most other theories, no decomposition of molecular oxygen into the atomic or ionic condition is believed to occur. According to Traube the presence of water is an essential condition for the auto-oxidation of all substances. At first it was believed that hydrogen peroxide was the essential oxidizing agent in every case, but later, especially owing to the work of Engler and Wild and of Bach, it became generally recognized that a great variety of superoxides might be formed as intermediate stages in auto-oxidations. According to Engler and Weissberg, two main types of auto-oxidation may be recognized—the simplest of these is presented by the case of substances which at the same time induce the oxidative process (autoxidator) and are themselves oxidized. The second type of auto-oxidation is presented by reactions in which a third substance, not ordinarily capable of undergoing oxidation, is oxidized through the agency of a superoxide formed by the union of molecular oxygen with a reactive substance capable of undergoing auto-oxidation. A striking example of this type of change is presented by the oxidation of indigo-blue or similar substances by means of benzaldehyde and oxygen. Under ordinary circumstances indigo solutions are completely unaffected by oxygen, that is to say, no auto-oxidation occurs. When, however, benzaldehyde is present half of the oxygen taken up by the aldehyde while undergoing auto-oxidation with formation of benzoic acid is utilized in effecting the oxidation of indigo. The precise mechanism of the reaction has been experimentally determined by Baeyer and Villiger, and found to accord with the previously formulated theory of Bodländer. Benzaldehyde when undergoing auto-oxidation unites with a molecule of oxygen to form benzoylhydrogen peroxide $C_6H_5 . CO . O . OH$ (1), a substance possessing powerful oxidizing properties. In the absence of other oxidizable substances, this peroxide reacts with a second molecule of benzaldehyde forming two

molecules of benzoic acid (2). In the presence of an oxidizable substance such as indigo, however, one atom of oxygen is appropriated for oxidizing the indigo, one molecule of benzoic acid being formed (3):—

(1) $C_6H_5 . CHO + O_2 = C_6H_5 . CO . O . OH$ (benzoylhydrogen peroxide).
(2) $C_6H_5 . CO . O . OH + C_6H_5 . CHO = 2C_6H_5 . COOH$.
(3) $C_6H_5 . CO . O . OH$ + Indigo = $C_6H_5 . COOH$ + oxidation products of indigo.

These reactions concerning the auto-oxidation of benzaldehyde are reproduced for the reason that they appear to present certain analogies with changes concerned with biochemical oxidations and reductions. It is generally believed that living cells contain labile substances capable of taking up molecular oxygen from the oxyhæmoglobin of the blood with the formation of unstable peroxides possessing marked oxidizing properties. Schönbein, and later Bach, have shown that a large number of substances of the most diverse kinds, when undergoing slow oxidation yield substances giving the reactions of hydrogen peroxide (cp. also Radziszewski). These peroxide-yielding substances include representatives of the following classes: elementary metals and non-metals such as hydrogen, phosphorus, zinc, etc., hydrocarbons, terpenes, alcohols, aldehydes, acids, carbohydrates, ethers, phenols, and aromatic bases and alkaloids. In addition, Baeyer and others have actually isolated a number of superoxides and substituted hydrogen peroxides derived from many different types of aldehydes and ketones. It certainly appears likely that substances of this type are concerned with the oxidations of substances in living tissues, and indeed such knowledge as has been derived from a study of the various oxidations effected by enzymes found in the living cells strongly supports such a supposition. The occurrence of certain metallic salts, especially those of iron and manganese, in conjunction with certain vegetable oxidases, and the extraordinary influence they have upon the ferment activity, is paralleled by the catalytic action of these same salts in accelerating oxidations *in vitro* by means of hydrogen peroxide (Bertrand, Fenton, Wolff, and others).

Largely owing to the work of Bach and Chodat it has been commonly assumed that the oxidases concerned with biochemical oxidations represent systems composed of a superoxide together with a catalyst (peroxidase) capable of acting upon it with liberation of active oxygen. The marked individuality of the animal as well as some of the vegetable oxidases makes it appear likely that both the peroxide and peroxidase must bear a special relation to the substance under-

going change. The specific character of animal oxidations is most remarkable, especially when phenomena such as those presented by diabetes and alcaptonuria are concerned. In these conditions oxidation of a single readily oxidizable product of metabolism (glucose, homogentisic acid) may be completely restrained without in the least impairing the capacity of the body for effecting the oxidation of other substances.

Within the last few years other evidence has been secured in favour of the belief of the formation of unstable superoxides as the active oxidizing reagents of the body. If the hypothesis of superoxide formation is correct, one would expect a certain similarity between the oxidations effected in the body and those brought about by the simplest superoxide, namely hydrogen peroxide. As a matter of fact, an extraordinarily close similarity as regards the types of reactions exists between the two sets of phenomena. Thus the normal saturated fatty acids in the body undergo oxidation in the β-position, butyric acid yielding acetoacetic acid—a truly remarkable change.

Hydrogen peroxide alone of all the various chemical oxidizing agents brings about precisely the same reaction (p. 20):—

$$\underset{\text{(Butyric acid)}}{CH_3 . CH_2 . CH_2 . COOH} \rightarrow \underset{\text{(Acetoacetic acid)}}{CH_3 . CO . CH_2 . COOH}$$

Hydroxy acids, such as lactic acid and β-hydroxybutyric acid, are oxidized to ketonic acids, both in the body and by hydrogen peroxide (p. 56):—

$$\underset{\text{(β-Hydroxybutyric acid)}}{CH_3 . CHOH . CH_2 . COOH} \rightarrow \underset{\text{(Acetoacetic acid)}}{CH_3 . CO . CH_2 . COOH}$$

Amino acids and ketonic acids, such as leucine and phenylpyruvic acid, are oxidized to lower fatty acids with liberation of carbon dioxide and either ammonia or water (p. 49):—

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!\rangle CH_2 . CH_2 . \underset{\text{(Leucine)}}{CH(NH_2) . COOH} \rightarrow \begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!\rangle \underset{\text{(Isovaleric acid)}}{CH_2 . CH_2 . COOH} + NH_3 + H_2O$$

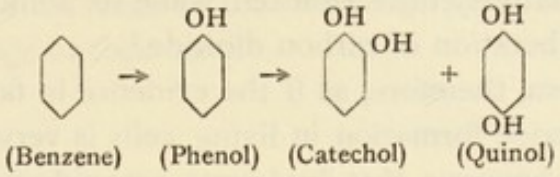
Glycerol may be oxidized by hydrogen peroxide to glyceric aldehyde or dihydroxyacetone. The same reaction is believed to occur in the body (p. 91):—

$$\underset{\text{(Glycerol)}}{CH_2(OH) . CH(OH) . CH_2(OH)} \rightarrow \underset{\text{(Glyceric aldehyde)}}{CH_2(OH) . CH(OH) . CHO}$$

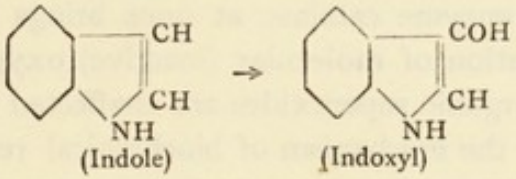
Glucose may be oxidized in the body to glucuronic acid, while hydrogen peroxide is the only reagent capable of effecting this change outside the body (p. 84):—

$$\underset{\text{(Glucose)}}{CH_2OH . (CHOH)_4 . CHO} \rightarrow \underset{\text{(Glucuronic acid)}}{COOH . (CHOH)_4 . CHO}$$

Benzene is oxidized in the body to phenol, catechol and quinol, and precisely the same change is brought about by hydrogen peroxide, but by scarcely any other reagent (p. 102):—



Indole is oxidized to indoxyl in the body while no reagent other than hydrogen peroxide has been observed to bring about this change *in vitro* (p. 108):—



A great many other biochemical reactions of less strikingly characteristic types such as the oxidation of simple alcohols, aldehydes, acids, etc., may be reproduced *in vitro* by means of hydrogen peroxide.

An additional point of similarity between the types of oxidation effected by the animal tissues and by hydrogen peroxide is seen in the behaviour of the saturated and unsaturated acids. In the animal body the unsaturated acids, e.g. oleic acid, occur along with the corresponding saturated acids, and there does not appear to be a very profound difference in the ease with which the two types of acids undergo oxidation in the living organization. Indeed, in the case of some of the simpler acids the saturated acids appear to be more readily oxidized in the body. When these saturated and unsaturated acids are subjected to oxidation with most of the common laboratory reagents, the unsaturated acids are, of course, infinitely more readily oxidized than are the saturated acids, which appear remarkably stable. But if hydrogen peroxide be chosen as oxidizing agent, and is allowed to act on the neutral salts of the saturated and unsaturated acids under comparable conditions, it is found that they both undergo oxidation to not widely different extents.[1]

The behaviour of some of the dibasic acids is also instructive. Oxalic acid, the simplest member of the group, although very readily oxidized by many laboratory reagents, is oxidized with great difficulty in the animal body. Malonic, succinic, and glutaric acids, on the other

[1] Unpublished observations.

hand, are readily oxidized in the body although more stable than oxalic acid to many oxidizing reagents. On oxidizing the neutral salts of these acids with hydrogen peroxide, it is found that as in the body, oxalic acid is relatively little attacked, while its homologues are readily oxidized with liberation of carbon dioxide.[1]

It would seem, therefore, as if the evidence in favour of the hypothesis of superoxide formation in living cells is very strong. It must not be inferred, however, that hydrogen peroxide is the active agent; indeed there is distinct evidence against such an assumption. It is very likely that hydrogen peroxide may be formed in small amounts during the processes of auto-oxidation occurring in the body, but the widely distributed enzyme catalase at once brings about its decomposition with liberation of molecular (inactive) oxygen. So far as is known, the other organic superoxides are unaffected by catalase.

With regard to the mechanism of biochemical reductions but little can be said. It appears that in some cases at any rate, the reduction of one molecule of substance takes place with simultaneous oxidation of a second molecule, so that the net result of the whole may be an exothermic hydrolysis. The Cannizzaro reaction (p. 105) which takes place in the body is an example of such a change. Two molecules of an aldehyde undergo rearrangement with formation of one molecule of an alcohol (reduction) and one molecule of an acid (oxidation) (p. 106) :—

$$2R.CHO + H_2O = R.CH_2OH + R.COOH$$

The types of reduction which are known to occur in the body are mostly of a kind which may be readily reproduced *in vitro*. The reduction of α- and β-ketonic acids and of ketones to the corresponding hydroxy-compounds and the reduction of the ammonium salts of α-ketonic acids to α-amino acids are well-established biochemical reactions. But the mechanism of these reductions by the organism is not understood. It is possible that two molecules of the ketonic compound undergo simultaneous reduction and oxidation, or it may be that the reduction of the ketonic compound is brought about by the oxidation of some other labile easily oxidized substance. The striking investigations of Ciamician and Silber have shown that the reduction of many types of compounds when dissolved in alcohol and exposed to sunlight is effected with the simultaneous oxidation of a portion of the alcohol to acetaldehyde. In addition, Bach has recently shown how acetaldehyde may assist in the catalytic reduction of nitrates to nitrites

[1] Unpublished observations.

by certain enzymes. Schardinger's observations on the reduction of methylene-blue in the presence of aldehydes may also be recalled. It is possible that similar easily oxidized substances occur in living cells and are responsible for some of the observed reductions, but we have as yet no knowledge of their nature.

III. METHODS OF INVESTIGATION.

The methods employed for characterizing the oxidations and reductions which various substances undergo in the animal body are relatively few and simple. In the case of oxidations the first step in the investigation consists in determining the *end-product* of the reaction. This is usually a simple problem and may generally be determined by administering small quantities of the substance under investigation to an animal, preferably by subcutaneous injection.[1] The urine excreted during the period following the administration is specially examined for possible derivatives of the substance and also for the unchanged compound. Negative results from these experiments are usually indicative of the complete oxidation of the substance. In such cases it may be necessary to make special metabolism experiments in which the carbon and nitrogen balances are determined. When an organic substance has undergone complete oxidation in the animal organism, the carbon and nitrogen are approximately quantitatively converted into carbon dioxide and urea respectively.

The second step in the investigation aims at the determination of the intermediate steps in the process by which the substance has been converted into its end-products of oxidation. This is a very much more difficult problem than the first. By utilizing a number of methods it is usually possible to definitely determine the occurrence of certain reactions, to recognize the possible or probable occurrence of others and finally to definitely exclude a number of other reactions which theoretically might be concerned in the sequence of changes.

The first method commonly made use of may be termed a "method of exclusion" and depends upon the testing of provisional hypotheses constructed by analogy with chemical experiments *in vitro*. An example will render this clear. Acetic acid is oxidized in the body with great ease, yielding as end-products carbon dioxide and water. Now

[1] Subcutaneous injection is usually preferable to administration by mouth owing to the avoidance of possible bacterial decomposition in the alimentary tract prior to absorption. The method may present difficulties in the case of sparingly soluble substances. The use of dilute alcohol or sterile olive oil as solvents, or the conversion of the substances into soluble derivatives, salts, etc., may obviate the difficulty. Intraperitoneal injection is occasionally useful, but in some cases absorption is slow.

it is known that in the laboratory acetic acid may be oxidized to oxalic acid by a variety of oxidizing reagents (e.g. alkaline permanganate) and that by other reagents (e.g. permanganic acid) the oxalic acid thus formed may be oxidized to carbon dioxide and water. The question arises: Is oxalic acid an intermediate product in the oxidation of acetic acid in the animal body? The answer appears to be in the negative since on administering oxalic acid to an animal under conditions similar to those prevailing in the experiments with acetic acid, it is found that not only is the oxalic acid much more toxic than acetic acid but undergoes oxidation in the body with great difficulty compared with acetic acid. Oxalic acid is therefore probably *not* an intermediate product of the oxidation of acetic acid in the animal organism. On the other hand, acetic acid may be oxidized to formic acid by purely chemical means (hydrogen peroxide). Can formic acid be regarded as a possible stage in the biochemical oxidation of acetic acid? On testing the behaviour of formic acid in the animal body, it is found to undergo oxidation yielding the same end-product as acetic acid, namely carbon dioxide and water, and also to resemble acetic acid reasonably closely as regards toxicity and ease of oxidation. The inference to be drawn therefore is that formic acid *may be* an intermediate product of the oxidation of acetic acid in the animal organism.

It is clear that this "method of exclusion" is beset with great limitations since it is perfectly possible for substances of high toxicity to be constantly produced and rapidly transformed into other bodies so that the relative concentration of the toxic substance is always low. The production of formaldehyde in the photosynthetical processes occurring in green leaves is a case in point. Moreover, the behaviour of a substance when gradually produced, at low concentration and rapidly undergoing further change, may be very different from that of the same substance when rapidly injected in relatively high concentration into the tissues of an animal. Indeed, in the latter case it is always uncertain whether the substance ever really reaches the sphere of action in the cells normally concerned with its metabolism.

The second method is intimately bound up with the first and essentially based upon experience gained by a study of the behaviour of substances of biological importance under a variety of conditions. An attempt is then made to apply the knowledge thus gained to the elucidation of biochemical transformations. An example will illustrate the method. It had long been known that administration of butyric acid to a diabetic animal was followed by the excretion of acetoacetic acid. For a long time the reaction was regarded as an indirect one—

it was believed that the butyric acid in some way was broken down into smaller molecules which under suitable conditions might undergo synthesis with formation of acetoacetic acid. No chemical analogy was known for the conversion of butyric acid into acetoacetic acid by direct oxidation. It was subsequently found, however, that by a suitable choice of reagent, namely hydrogen peroxide, this type of reaction could be readily brought about *in vitro* and it is now generally conceded that the same reaction takes place in the animal body.

Many additional examples of the results of this type of investigation will be found in succeeding chapters.

A third method of obtaining information about intermediate products of biochemical oxidation is based upon the following consideration. For any particular substance under investigation it is possible to determine by trial the amount which on administration to an animal undergoes practically complete oxidation to its end-products. If in a second experiment a quantity of substance considerably larger than that which can be completely oxidized is administered to the animal, it will be found that in some cases the urine, in addition to unchanged substance, will contain compounds which appear to be intermediate products of oxidation. The possibility of isolating intermediate products by this direct method obviously depends upon the relative rates of oxidation of the parent substance and the intermediate products under the particular conditions of the experiment. The necessity of employing large quantities of substance make the conditions of experiment somewhat abnormal and the results must be accepted with some caution.

As examples of the method, reference may be made to the formation of intermediate products from β-phenylpropionic acid (p. 23) to the excretion of oxalic acid by rabbits following large doses of glucose (p. 84) and the isolation of uric acid as an intermediate product in the oxidation of xanthine to allantoine (p. 96).

A fourth method of investigation is based upon the study of the action upon oxidizable substances of isolated surviving organs, or of the crushed tissue pulp or cell juices or aqueous extracts of various organs. The investigation of the oxidations which may be effected by the surviving liver when perfused with oxygenated blood has proved specially valuable. Embden's work on acetoacetic acid formation from fatty acids (p. 21) and Wiechowski's demonstration of the oxidation of uric acid to allantoine (p. 95) are excellent examples of this method. By the use of isolated organs or organ extracts the tendency of the intact animal to effect complete oxidation of the substance is

often avoided and the chance of detecting intermediate products is consequently increased.

A constant difficulty in determining the formation of intermediate products is the ease with which these substances undergo further change and so avoid detection. Knoop conceived the idea of introducing into the substance under investigation a resistant radical which would therefore be excreted in combination with some part of the molecule of the original substance. He studied the fate in the animal organism of fatty acids in which a phenyl group had been introduced in the position furthest removed from the carboxyl group, e.g. phenylacetic acid, β-phenylpropionic acid, γ-phenylbutyric acid and δ-phenylvaleric acid. Knoop found that the aliphatic side-chain underwent oxidation in the body in such a way that either benzoic or phenylacetic acid was excreted in combination with glycine. These important results will be referred to later.

The study of the fate of various substances in the animal organism under pathological conditions, especially human or experimental diabetes, has proved very fruitful. Experiments of this kind have brought to light many interesting relationships. Thus it has been found that many amino acids derived from proteins are converted into glucose in the diabetic organism while others yield the so-called "acetone bodies" β-hydroxybutyric acid, acetoacetic acid, and acetone (p. 58).

Investigation of the fate of various amino acids in cases of cystinuria, alcaptonuria (p. 63), and melanuria (p. 74), conditions which are associated with peculiar metabolic abnormalities, has also given valuable results. It is always difficult, however, to decide how closely reactions observed under pathological or abnormal conditions resemble those occurring in the normal organism.

The administration to animals of certain foreign substances often results in the excretion of these substances in combination with a second substance derived from the tissues of the organism. The reaction is frequently of the nature of a protective mechanism, the compound excreted being commonly less toxic than the original drug. In some cases the second substance derived from the tissues appears to be an intermediate product of normal metabolism. Examples of the use of this pharmacological method are found in the excretion of glucuronic acid derivatives when many aromatic alcohols and ketones are administered to animals (p. 83). The glucuronic acid is undoubtedly derived from glucose and is an intermediate product of oxidation. A similar example is furnished by the behaviour of bromobenzene when administered to dogs. The substance is excreted in

the form of bromophenylmercapturic acid, the sulphur-containing group being derived from the protein derivative, cysteine. The limitations of this method are of course great and the correlation of the results with the processes of oxidation in the normal organism in the absence of foreign substances is always difficult.

CHAPTER II.

I. THE OXIDATION OF FATTY ACIDS.

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 varies from four (butyric acid) to twenty-four (carnaubic acid) or more. Palmitic, stearic, and oleic acids with sixteen and eighteen carbon atoms are quantitatively predominant. Formic acid and acetic acid and possibly propionic acid also occur in the body but not in the form of fats. All of these fatty acids undergo complete oxidation in the animal body with formation of carbon dioxide and water (Wöhler, Buchheim, Schotten, Pohl, Bergell and others). Schotten gave from 10 to 20 grms. of the fatty acids from formic to caproic acid to dogs by mouth in the form of sodium salts. The caproic, valeric, and butyric acids were practically completely oxidized, while about 10 per cent. of the acetate and 25 per cent. of the formate was excreted unchanged. Large quantities of sodium carbonate were present in all the urines. It has since been shown that when smaller amounts of acetates and formates are administered they are more completely oxidized in the body[1] (Pohl, Mallèvre, Pellacani). With the exception of formic acid, all of these saturated fatty acids are very resistant to the action of the ordinary chemical reagents, yet in the animal body they undergo oxidation with extraordinary ease. It appears that the liver is the main seat of at any rate the initial reactions involved in the oxidation of the higher fatty acids. What is the mechanism by which these fatty acids are oxidized? The complete oxidation of the molecule of a higher fatty acid to carbon dioxide and water must necessarily involve the successive formation of a series of intermediate substances. The detection and identification of these substances is an extremely difficult problem owing to the ease with which they undergo further change.

It is only since 1904, the year when Knoop published his

[1] It is often stated that formates and acetates are less readily oxidized than the higher acids. In the absence of information as to the relative rate of excretion of these salts by the kidneys, this conclusion is unwarranted.

important paper upon "Der Abbau aromatischer Fettsäuren im Tierkörper," that any material success has been obtained in the experimental investigation of fatty acid catabolism.

Knoop's Theory of β-Oxidation.—The ease with which the intermediary products of fatty acid catabolism undergo complete oxidation and so escape detection led Knoop to study the fate of fatty acids in which a radical had been introduced which was resistant to oxidation in the body. He chose among other substances the phenyl derivatives of acetic, propionic, butyric, and valeric acids. Phenylacetic acid does not undergo oxidation in the animal body but combines with glycine to yield phenaceturic acid,[1] $C_6H_5 . CH_2 . CO . NH . CH_2 . COOH$. β-Phenylpropionic acid, on the other hand, is oxidized to benzoic acid, which unites with glycine to form hippuric acid (E. & H. Salkowski). Knoop concluded that the oxidation of the side-chain of three carbon atoms in phenylpropionic acid did not take place with intermediate formation of a two-carbon side-chain (phenylacetic acid) as in this case the excretion of phenaceturic acid would have followed. Two carbon atoms appeared to have been removed from the phenylpropionic acid side-chain at one time.

γ-Phenylbutyric acid, on the other hand, was converted into phenaceturic acid, two carbon atoms being again removed from the fatty acid side-chain. δ-Phenylvaleric acid gave hippuric acid with loss of four carbon atoms and Dakin subsequently proved that this took place in two stages in each of which two carbon atoms were removed. The results may be represented as follows:—

Acid Fed.	Oxidation Product.	Excreted as
Benzoic acid, $C_6H_5 . COOH$	Not oxidized	Hippuric acid
Phenylacetic acid, $C_6H_5 . CH_2 . COOH$	Not oxidized	Phenaceturic acid
Phenylpropionic acid, $C_6H_5 . CH_2 . CH_2 . COOH$	$C_6H_5 . COOH$	Hippuric acid
Phenylbutyric acid, $C_6H_5 . CH_2 . CH_2 . CH_2 . COOH$	$C_6H_5 . CH_2 . COOH$	Phenaceturic acid
Phenylvaleric acid, $C_6H_5 . CH_2 . CH_2 . CH . CH_2 . COOH$	$C_6H_5 . COOH$	Hippuric acid

On the basis of these experiments Knoop founded his theory of β-oxidation. According to this theory oxidation of the side-chains of these normal phenyl-fatty acids takes place in such a manner that the hydrogen attached to the β-carbon atom is selected for oxidation. In this way the side-chains are reduced by the loss of two or some multiple of two carbon atoms at each successive step:—

[1] The failure of phenylacetic acid to undergo oxidation in the body was ascribed by Baumann to the protection afforded to the CH_2 group by two non-oxidizable groups, C_6H_5 and COOH. It is more likely, however, that its ready condensation with glycine to form phenaceturic acid is in part responsible, since the glycine derivatives of phenyl-fatty acids are very stable substances.

$$C_6H_5 . CH_2 . CH_2 . CH_2 \vdots CH_2 . COOH \rightarrow C_6H_5 . CH_2 \vdots CH_2 . COOH \rightarrow C_6H_5 . COOH$$

(Phenylvaleric acid) (Phenylpropionic acid) (Benzoic acid)

Knoop indicated the probability of similar changes playing a part in the oxidations of the physiologically important normal fatty acids present in the animal body. Attention was drawn to the formation and excretion of β-hydroxybutyric acid and acetoacetic acid by diabetics, especially when large amounts of fats were being consumed (Geelmuyden, Magnus Levy, Waldvogel, Schwarz, and many others). Furthermore, the fact was recalled that normal men when on a diet containing little or no carbohydrate excreted the same acids, especially when the consumption of fats was large. It appeared not unlikely, therefore, that β-hydroxybutyric acid and acetoacetic acid were intermediate products in the β-oxidation of butyric acid or of higher fatty acids which might yield butyric acid on oxidation. Moreover, direct experiments upon normal dogs and upon human diabetics showed that an increased excretion of β-hydroxybutyric acid and acetoacetic acid (and acetone) may follow administration of salts of butyric acid (Blum, Schwarz):—

$$CH_3 . CH_2 . CH_2 . COOH \rightarrow CH_3 . CHOH . CH_2 . COOH \rightarrow CH_3 . CO . CH_2 . COOH$$

(Butyric acid) (β-Hydroxybutyric acid) (Acetoacetic acid)

The theory of the degradation of fatty acids by loss of two carbon atoms at a time by oxidation in the β-position bore an interesting relation to the fact that fatty acids are contained in milk fat having 18, 16, 14, 12, 10, 8, 6, and 4 carbon atoms, thus indicating the possibility of a progressive β-oxidation from higher fatty acid to lower Fatty acids with an uneven number of carbon atoms are absent from most typical animal fats.

Notwithstanding the foregoing evidence pointing to the probability of the occurrence of β-oxidation, there was much opposition to the acceptance of this theory. The formation of β-hydroxybutyric acid and acetoacetic acid from fatty acids was ascribed by many to *synthesis* from simpler substances instead of to the β-oxidation of butyric acid. The objection was put forward that there was no purely chemical analogy for β-oxidation of saturated fatty acids. Friedmann wrote " From the standpoint of pure chemistry, the assumption of oxidation at the β-position is opposed to the facts that are known about the oxidation, substitution and condensation of fatty acids, since only the hydrogen atoms attached to the *a*-carbon atom have been found to be capable of reaction and no observation is known showing that the

hydrogen atoms attached to the β-carbon atoms are capable of undergoing reaction". Shortly before this Dakin was able to show that as a matter of fact an extraordinarily close pure chemical analogy did exist for Knoop's theory of biochemical oxidation.

While it is true that most of the laboratory oxidizing agents only act on fatty acids at high temperatures and that the products formed by these violent reactions have no biological significance [1] it was found that by suitable choice of an oxidizing agent entirely different results might be obtained. Thus it was found that butyric acid when neutralized and digested at 37° with hydrogen peroxide gave acetoacetic acid, acetone and other products, principally lower fatty acids and carbon dioxide. When the reaction was carried out at higher temperatures, the acetoacetic acid is converted into acetone with loss of carbon dioxide, according to the general reaction of β-ketonic acids. The yield of acetone may amount to as much as fifty per cent of the theoretical amount:—

$$\underset{\text{(Butyric acid)}}{CH_3 . CH_2 . CH_2 . COOH} \rightarrow \underset{\text{(Acetoacetic acid)}}{CH_3 . CO . CH_2 . COOH} \rightarrow \underset{\text{(Acetone)}}{CH_3 . CO . CH_3}$$

This reaction demonstrated the occurrence of β-oxidation *in vitro* in the clearest fashion. It was found impossible to detect β-hydroxybutyric acid as an intermediate product of oxidation, although this substance is readily oxidized by hydrogen peroxide to acetoacetic acid, acetone, etc. Acetoacetic acid may be regarded as derived from the hypothetical di-hydroxybutyric acid by loss of a molecule of water.

This reaction with butyric acid was extended to higher fatty acids and it was found that every normal higher fatty acid when neutralized and warmed with hydrogen peroxide, gave the corresponding ketone containing one less carbon atom. The ketone must be assumed to be derived from a β-ketonic acid by loss of carbon dioxide. Stearic acid, for example, gave quindecyl-methyl-ketone: $CH_3 . (CH_2)_{14} . CO . CH_3$. Lower fatty acids are formed simultaneously by oxidation.

Phenylpropionic acid which, according to Knoop, underwent β-oxidation in the body, with formation of benzoic acid, when oxidized with hydrogen peroxide gave benzoylacetic acid, acetophenone, and

[1] In general, the oxidation of the higher fatty acids with the usual chemical reagents results principally in the production of dibasic acids. For example, myristic acid ($C_{14}H_{28}O_2$) on long-continued boiling with nitric acid (sp. gr. 1·3) yields varying proportions of suberic, pimelic, adipic, glutaric, succinic, and oxalic acids. Palmitic acid on oxidation with alkaline permanganate yields adipic, succinic, and oxalic acids together with caproic, butyric, and acetic acids. There is no reason to believe that the higher dibasic acids are intermediary products in the metabolism of fats.

benzoic acid. Benzoylacetic acid and acetophenone were subsequently found to be intermediate products of the biochemical oxidation of phenylpropionic acid (Dakin):—

$$\underset{\text{(Phenylpropionic acid)}}{C_6H_5 . CH_2 . CH_2 . COOH} \rightarrow \underset{\text{(Benzoylacetic acid)}}{C_6H_5 . CO . CH_2 . COOH} \rightarrow \underset{\text{(Acetophenone)}}{C_6H_5 . CO . CH_3}$$

It was clear therefore that exception to Knoop's theory of β-oxidation could not be taken on the ground of lack of chemical analogy.

Formation in the Liver of Acetoacetic Acid from Higher Fatty Acids.—In the meantime Knoop's theory had led Embden and his co-workers to make experiments upon the catabolism of fatty acids which furnished striking confirmation of the theory of β-oxidation. They found that the small quantity of acetone normally formed during the perfusion of a freshly excised liver with defibrinated blood was greatly increased when certain amino acids or fatty acids were added to the blood prior to perfusion. The systematic examination of the fatty acids led to the following remarkable result. Of the normal fatty acids from butyric to decoic acid all those and only those with an even number of carbon atoms gave rise to a marked increase in acetone formation. Subsequent experiments showed that the acetone was derived from the decomposition of acetoacetic acid.[1]

Normal Fatty Acid.		Formation of Acetoacetic Acid
Acetic acid[2]	$CH_3 . COOH$	−
Propionic acid[3]	$CH_3 . CH_2 . COOH$	−
Butyric acid	$CH_3 . CH_2 . CH_2 . COOH$	+
Valeric acid	$CH_3 . CH_2 . CH_2 . CH_2 . COOH$	−
Caproic acid	$CH_3 . CH_2 . CH_2 . CH_2 . CH_2 . COOH$	+
Heptylic acid	$CH_3 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . COOH$	−
Octoic acid	$CH_3 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . COOH$	+
Nonoic acid	$CH_3 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . COOH$	−
Decoic acid	$CH_3 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . CH_2 . COOH$	+

The assumption that the normal fatty acids undergo oxidation with loss of two, or some multiple of two, carbon atoms at each successive step, furnishes a very satisfactory explanation of the widely different behaviour of the fatty acids with an even and an uneven number of carbon atoms, as regards their ability to form acetoacetic acid. The oxidation of a normal acid with an even number of carbon atoms would eventually lead to a straight chain of four carbon atoms from which acetoacetic acid would be formed, whereas the acids with

[1] The later experiments of Blum, Dakin, Friedmann and Maase and Neubauer make it appear most probable that part of the acetoacetic acid was asymmetrically reduced to β-hydroxybutyric acid. Thus all of the so-called "acetone bodies" are undoubtedly formed from the catabolism of fatty acids in the liver.

[2] Friedmann's experiments. [3] Unpublished experiments.

an uneven number of carbon atoms would yield chains of either three or five carbon atoms which obviously for structural reasons would not yield acetoacetic acid.[1]

Acetoacetic acid is of course not to be regarded as an end-product of fatty acid catabolism. It is itself an intermediate product and is normally further oxidized to carbon dioxide and water. Fortunately for the success of the experimental method, acetoacetic acid appears to be relatively more stable than most other intermediate substances formed in fatty acid catabolism and hence lends itself more readily to isolation. Its ready conversion on heating into acetone which may be distilled off and easily estimated quantitatively are also important practical points.

Formation of Acetoacetic Acid from Fatty Acids in Diabetic Organisms.—The study of the fate of fatty acids, especially as regards their ability to yield β-hydroxybutyric acid, acetoacetic acid and acetone when fed to cases of human or experimental diabetes, has also given valuable results. Baer and Blum have been chiefly responsible for this work, which supplements and corroborates the results obtained by Embden's method. Thus administration of the salts of butyric acid and isovaleric acid led to an increased excretion of β-hydroxybutyric acid, acetoacetic acid and acetone while propionic acid and normal valeric acid did not. Baer and Blum in addition experimented with a number of acids with branched chains and also with amino acids. These results will be referred to later.

More recently Blum has shown that the administration of very large quantities of salts of butyric, caproic, or isovaleric acid to *normal* dogs is followed by the excretion of β-hydroxybutyric acid, acetoacetic acid, and acetone in the urine. Propionic and normal valeric acid, as expected, fail to yield these substances.

From the evidence presented it is inferred that normal saturated fatty acids and their phenyl derivatives at least in part undergo oxidation in such a fashion that they lose two terminal carbon atoms (or some multiple of two carbon atoms) at each successive step in their decomposition.

[1] Embden and Wirth have recently shown that some of the acids which do not yield acetoacetic acid, e.g. normal valeric acid, when added to blood actually inhibit the formation of acetoacetic acid from substances which otherwise would yield it. The degree of inhibition appears to depend in part upon the ease of oxidation in the liver of the added substance.

The Mechanism of β-Oxidation.

For many reasons it is unlikely that a single reaction is involved in the shortening of a fatty acid chain by two carbon atoms through a process of β-oxidation :—

$$R.CH_2. \vdots CH_2.COOH \rightarrow R.COOH$$

A complex oxidation of this character would necessitate a number of successive reactions, and it is obviously of great importance that this type of change should be accurately resolved into its simplest phases.

The excretion of *l*-β-hydroxybutyric acid following the administration of butyric acid to animals under certain conditions and the simultaneous presence of acetoacetic acid and acetone in the urine led to the belief that β-hydroxybutyric acid was the initial product of the oxidation of butyric acid and acetoacetic acid the second :—

$$CH_3.CH_2.CH_2.COOH \rightarrow CH_3.CHOH.CH_2.COOH \rightarrow CH_3.CO.CH_2.COOH$$

Embden's demonstration of the oxidation to acetoacetic acid of both butyric and β-hydroxybutyric acid when perfused through a surviving liver harmonized with this hypothesis.[1]

A case analogous to the formation of β-hydroxybutyric acid was furnished by Dakin's observation of the excretion of *l*-β-hydroxyphenylpropionic acid on administering phenylpropionic acid to cats. In addition to the hydroxy acid the corresponding ketonic acid, benzoylacetic acid, and acetophenone were also detected and the fact was further determined by Knoop and by Nencki that each of these intermediate substances when administered to an animal underwent oxidation with production of benzoic acid, i.e. the same end-product of oxidation as that obtained from phenylpropionic acid. The oxidation of phenylpropionic acid was represented as follows :—

(Phenylpropionic acid) $C_6H_5.CH_2.CH_2.COOH$ ↓

(Phenyl-β-hydroxypropionic acid) $C_6H_5.CHOH.CH_2.COOH$ ↓

(Benzoylacetic acid) $C_6H_5.CO.CH_2.COOH \rightarrow C_6H_5.CO.CH_3$ (Acetophenone) ↓

(Benzoic acid) $C_6H_5.COOH$

From these results the inference was drawn that normal saturated fatty acids in general might undergo β-oxidation according to the following scheme :—

[1] The oxidation of β-hydroxybutyric acid to acetoacetic acid was shown by Dakin and Wakeman to be due to an enzyme which could be roughly separated from liver tissue. The action of the enzyme was not very vigorous but was markedly increased by the presence of oxyhæmoglobin. Oxyhæmoglobin alone was entirely without action.

$$\begin{array}{c} R.CH_2.CH_2.COOH \\ \downarrow \\ R.CHOH.CH_2.COOH \\ \downarrow \\ R.CO.CH_2.COOH \\ \downarrow \\ R.COOH \end{array}$$

There are, however, certain difficulties in the assumption of a β-hydroxy acid as the first step in the normal catabolism of fatty acids and their aromatic derivatives, which call for consideration. Perhaps the least serious objection to the theory is the fact that the hydroxy acids in general appear to be much less readily oxidized in the body than the corresponding saturated or unsaturated acids.[1] From the purely chemical point of view it is certainly remarkable to find that in the animal body butyric acid is more readily oxidized than β-hydroxybutyric acid. With the usual laboratory oxidizing agents the hydroxy acids are infinitely more readily oxidized than the unsubstituted acids.

But it may be argued with some considerable justice that the results of the sudden administration of very large quantities of a substance may be entirely different from the results obtained when the substance is slowly formed in cells provided with a mechanism for its immediate transformation into other compounds. When large doses of substances are rapidly administered to an animal one has no proof that the bulk of the substance ever reaches the sphere normally concerned with its metabolism.

That β-hydroxybutyric acid certainly does not originate *exclusively* by the direct hydroxylation of butyric acid was shown almost simultaneously by Blum, Dakin, Friedmann and Maase, and by Neubauer. It was found that the liver was able to effect the asymmetric *reduction* of acetoacetic acid to *l*-β-hydroxybutyric acid.[2] This reaction is the exact reverse of the oxidation of β-hydroxybutyric acid by the enzyme previously referred to. The liver is thus provided with a mechanism, dependent upon the antagonistic action of two ferments, by which the mutual interconversion of β-hydroxybutyric acid and acetoacetic acid may be effected. The one ferment action is an oxidation dependent upon the presence of free oxygen or oxyhæmoglobin, while the other ferment action is a reduction. The source of the two hydrogen atoms necessary for this reduction is unknown.[3]

[1] On administering 3·0 grms. β-phenyl-β-hydroxypropionic acid as sodium salt to a 3 kg. cat, 2·35 grms. were recovered unchanged. No unoxidized β-phenylpropionic acid could be found after injection of an equal quantity of this acid.

[2] Benzoylacetic acid is similarly reduced to *l*-β-phenyl β-hydroxypropionic acid (Friedmann, Dakin).

[3] It is possible that a β-ketonic acid might undergo a type of Cannizzaro reaction by

$$CH_3 . CHOH . CH_2 . COOH \underset{\text{oxidation}}{\overset{\text{reduction}}{\rightleftharpoons}} CH_3 . CO . CH_2 . COOH$$

Little is known of the conditions determining whether oxidation or reduction shall predominate and doubtless we have here to deal with a delicately adjusted equilibrium. Under ordinary conditions a normal minced dog's liver is more active in reducing acetoacetic acid than in oxidizing β-hydroxybutyric acid, but these observations are of little value in judging of the reactions during life. It is probable that the presence of readily oxidizable substances (e.g. carbohydrates) in the liver would influence the balance markedly, and in this connexion the influence of carbohydrates in diminishing the acidosis of diabetes may be recalled (Hirschfeld, Rosenfeld).

There is still another way by which β-hydroxy acids may arise in the body apart from the direct hydroxylation of fatty acids or the reduction of ketonic acids. It has been found that the unsaturated acids may take up the elements of water with formation of β-hydroxy acids. Thus, cinnamic acid may yield phenyl-β-hydroxypropionic acid (Dakin). The reaction is a reversible one :—

$$\underset{\text{(Cinnamic acid)}}{C_6H_5 . CH = CH . COOH} \rightleftharpoons \underset{\text{(Phenyl-β-hydroxypropionic acid)}}{C_6H_5 . CHOH . CH_2 . COOH}$$

It is manifest therefore that *the detection of a β-hydroxy acid as an intermediate product of the oxidation of a normal fatty acid cannot in itself be regarded as convincing proof of its formation by direct oxidation of the fatty acid in a single step. On the other hand, the possibility of the direct oxidation of saturated fatty acids with formation of β-hydroxy acids has not been disproved.*

Blum has advanced the view that the normal path for the catabolism of butyric acid is by way of acetoacetic acid without intermediate formation of β-hydroxybutyric acid. The latter substance when formed is assumed to be derived exclusively from the reduction of acetoacetic acid and not by the direct oxidation of butyric acid. Blum assumes, moreover, that β-hydroxybutyric acid does not normally undergo oxidation in the body with formation of acetoacetic acid but is decomposed in some other way. The actual demonstration of the formation of acetoacetic acid from β-hydroxybutyric acid by Embden and by Wakeman and Dakin is referred by Blum to a pathological condition of the liver cells. The evidence adduced by Blum in support of his contention that β-hydroxybutyric acid is not an intermediate step

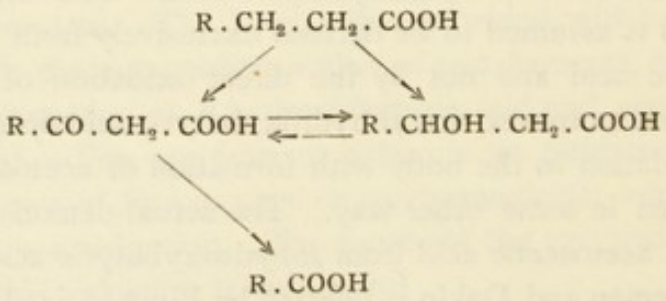
which one molecule of the ketonic acid would be reduced to the β-hydroxy acid, while a second would undergo further oxidation. Thus, the body possesses catalysts capable of converting benzaldehyde (2 mols.) into benzyl alcohol (1 mol.) and benzoic acid (1 mol.).

in the oxidation of butyric acid to acetoacetic acid is mainly based upon the fact that he failed to produce a marked acetonuria by the subcutaneous administration of sodium β-hydroxybutyrate to a dog which under similar conditions developed acetonuria when sodium butyrate or sodium acetoacetate was administered.

It may be fairly questioned whether these results really justify the interpretation placed upon them by Blum. When it is realized that the increase in the amount of acetone in the urine following the administration of 17 grms. of sodium butyrate only corresponds to about one-half per cent. of the butyric acid while the acetone found following the giving of 10 grms. of sodium acetoacetate itself only corresponds to a little over 3 per cent of the acetoacetic acid, it is obvious that much acetoacetic acid might have been formed from the β-hydroxybutyric acid without materially increasing the acetoacetic acid and acetone of the urine.[1]

Moreover, other workers have apparently demonstrated the formation of acetoacetic acid from β-hydroxybutyric acid in the intact animal. Thus McKenzie found acetoacetic acid and acetone in the urines of dogs that had received injections of inactive sodium β-hydroxybutyrate. Minkowski gave 10 grms. of sodium *l*-β-hydroxybutyrate to a depancreatized dog and observed the excretion of acetoacetic acid and acetone, while Magnus Levy obtained similar results with a dog rendered diabetic by phlorizin.

From the results of experiments with butyric acid and phenylpropionic acid it can hardly be doubted that the oxidation, at least in the case of these acids, takes place with formation of a β-ketonic acid, and that the β-hydroxy acids are in part at least secondary products of the reduction of the ketonic acid. The results may be represented as follows:—



[1] It is frequently very difficult to produce an excretion of acetoacetic acid by the intact animal through the administration of substances which are believed to yield acetoacetic acid as products of their catabolism. The writer has frequently given phenylalanine and tyrosine to animals so that much appeared unchanged in the urine, without detecting acetoacetic acid in the urine. On perfusing the surviving liver with these amino acids, acetoacetic acid is formed in abundance.

The question arises as to whether the foregoing scheme represents the mechanism of β-oxidation not only of the simple acids above referred to but also in the case of the higher fatty acids. Judging simply by chemical analogy it would be expected that a fatty acid such as stearic acid might undergo the same type of oxidation as butyric acid. But it must be admitted that no β-ketonic acids other than acetoacetic and benzoylacetic acid have been detected among the products of fatty acid catabolism in the animal body.[1] On the other hand unsaturated hydroxy acids of the type of ricinoleic acid are known and these acids are isomeric with the ketonic acids. Moreover, normal ketonic fatty acids have been found in vegetable organisms, and it is not unlikely that the naturally occurring aliphatic ketones found in essential oils such as methyl-n-nonyl ketone, methyl-n-heptyl ketone and methyl-n-amyl ketone are formed from the β-ketonic acids derived from lauric, capric, and caprylic acids (Dakin).

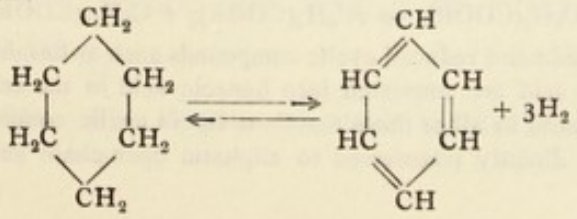
It would appear, therefore, that β-oxidation of normal saturated acids with intermediate formation of β-ketonic acids does occur in the oxidation of butyric and phenylpropionic acids (i.e. the simplest acids of the type $R.CH_2.CH_2.COOH$ *in which such a reaction is possible), but the question whether β-ketonic acids are formed as initial stages in the oxidation of the higher fatty acids is at present an open one.* It may at least be said that there is no satisfactory evidence to negative such a hypothesis.

There is another group of substances, other than the β-hydroxy and β-ketonic acids, which must be considered as possible oxidation products of the fatty acids, namely the unsaturated acids. From the purely chemical point of view such an assumption appears almost incredible, mainly because no chemical analogy for such a change is known,[2] and indeed is not likely ever to be encountered in reactions in

[1] Blum and Koppel have very recently described the formation of methyl-propyl-ketone from the oxidation of diethylacetic acid in the animal body. The ketone is doubtless derived from a β-ketonic acid :—

$$CH_3.CH_2.\underset{\displaystyle C_2H_5}{\underset{|}{CH}}.COOH \rightarrow CH_3.CO.\underset{\displaystyle C_2H_5}{\underset{|}{CH}}.COOH \rightarrow CH_3.CO.CH_2.C_2H_5.$$

[2] The closest approximation to such an analogy is furnished by the "dehydrogenization" of reduced aromatic compounds to benzene derivatives. Thus, Zelinsky has shown that cyclohexane when passed over reduced palladium at 200-300° is rapidly converted into benzene and hydrogen. At lower temperatures, 100-110°, the reaction proceeds readily in the opposite direction :—



which the commoner chemical oxidizing agents are used. The reason for this is that the unsaturated acids under these conditions are so much more readily oxidized than the saturated acids. This difference is not nearly so marked in the case of the animal organism where unsaturated acids are abundantly formed (cf. p. 36).

There are at least two cases in which the formation of an unsaturated acid by the oxidation of a saturated acid has been demonstrated. Dakin found cinnamoylglycine in the urines of cats which had received injections of the salts of phenylpropionic acid and phenylvaleric acid and other related substances, and Sasaki found the glycine derivative of furfuracrylic acid in the urine of animals which had been given furfurpropionic acid :—

$$R.CH_2.CH_2.COOH \rightarrow R.CH = CH.COOH.$$

Leathes and Meyer Wedell have shown that on feeding animals with oils or fats containing unsaturated acids, fats accumulate in the liver, the fatty acids of which, judged by their iodine values, are even more unsaturated than those contained in the food. But there are two possible explanations of this phenomenon. Either new double linkages are introduced into the fatty acid molecule by oxidation, or structurally isomeric unsaturated acids which absorb iodine more readily are formed by intramolecular rearrangement.

It is certain that unsaturated fatty acids are formed in the body by the oxidation of saturated acids, but we have not at present any means of knowing whether this change may be *directly* affected by a single process of oxidation. Thus, although cinnamic acid is unquestionably formed from phenylpropionic acid in the animal body, it may originate indirectly from phenyl-β-hydroxypropionic acid or benzoylacetic acid, both of which substances are intermediary products of the catabolism of phenylpropionic acid (Dakin, cf. diagram, p. 29).

Sabatier and Senderens have observed similar changes when reduced nickel is substituted for palladium.

Other related types of reaction are furnished by the oxidation of reduced cyclic compounds such as the hydrobenzoic acids, hydrophthalic acids, piperidine, etc., to benzoic acid, phthalic acid and pyridine. The reagents most commonly used for these oxidations are the halogens, potassium ferricyanide and permanganate, mercury and silver salts.

An interesting cause of auto-oxidation was observed by Knoevenagel and Bergdolt. They found that dihydroterephthalic ester (3 mols.) was slowly converted into terephthalic ester (2 mols.) by oxidation and hexahydroterephthalic ester (1 mol.) by reduction.

$$3C_6H_8(COOR)_2 \rightarrow 2C_6H_4(COOR)_2 + C_6H_{10}(COOR)_2$$

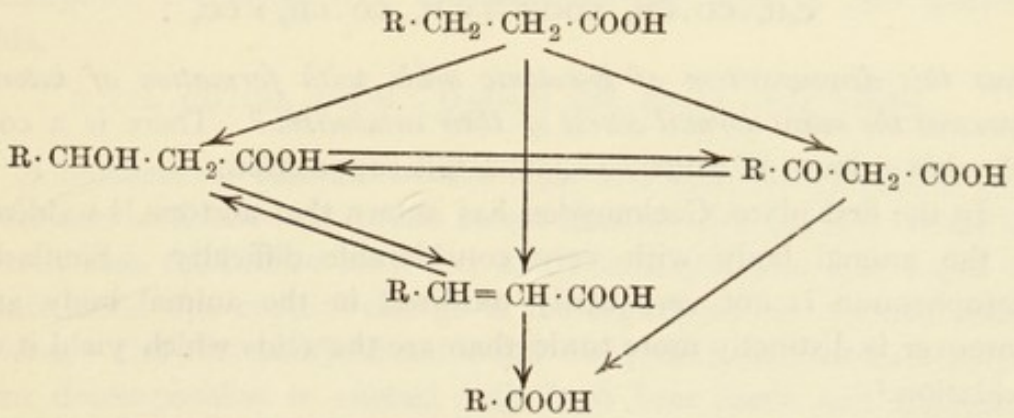
According to Friedmann reduced cyclic compounds such as hexahydrobenzoic acid and hexahydroanthranilic acid are converted into benzoic acid in the animal body. But the double linkages produced in all of these reactions are in cyclic compounds and the results obviously cannot be directly transferred to aliphatic open chain substances such as the fatty acids.

In general it may be said that unsaturated acids are formed in the animal body during the catabolism of saturated fatty acids; that a, β unsaturated acids may be formed indirectly from saturated acids through the intermediate formation of β-hydroxy and β-ketonic acids; and that the direct oxidation of saturated to unsaturated acids without intermediate formation of substitution derivatives is possible but not yet demonstrated. (See following diagram.)

Assuming that the *a*, *β* unsaturated acids, however formed, do represent an intermediate stage in the *β*-oxidation of saturated fatty acids, their further oxidation with loss of two carbon atoms is readily understood:—

$$R.CH=CH.COOH \rightarrow R.COOH$$

Chemical analogies for such an oxidation abound, but in the case of the biochemical oxidation it appears that the reaction is not always as simple as might appear. It will be shown later (p. 37) that the unsaturated acids may by reversible reactions take up water and pass over into *β*-hydroxy acids and in turn yield *β*-ketonic acids, so that it is not certain that the oxidation of unsaturated acids to saturated acids with two fewer carbon atoms takes place *directly* without intermediate formation of *β*-substitution derivatives. The various changes which are believed to be mainly concerned with *β*-oxidation, including oxidation and reduction, hydration and dehydration, most of them reversible reactions, are shown diagrammatically as follows:—



It will be noted that no reference has as yet been made to the fate of the two carbon atoms removed from saturated fatty acids at each successive *β*-oxidation of the higher normal fatty acids. It might be imagined that formic acid could be an intermediate step in their conversion into carbon dioxide, but there is no satisfactory evidence in favour of this belief.

$$R.CH_2 \vdots CH_2.COOH \rightarrow R.COOH + HCOOH + CO_2$$

Attempts that have been made to demonstrate the production of formic acid as the result of the oxidation of higher fatty acids have not given decisive results. But Dakin and Wakeman have recently found that there is no increase of formic acid in the blood used for perfusing livers in which the oxidation of higher fatty acids has been in progress (unpublished results). Further information as to the fate of the carbon groupings set free from fatty acids by biochemical oxidation is much needed.

Ketone Formation from β-Ketonic Acids.—Acetoacetic acid is found in the urine of animals in an advanced stage of diabetes; in normal animals after administering very large amounts of substances which yield acetoacetic acid on oxidation; and also in man when carbohydrates are omitted from the diet and fat catabolism is much increased. The acetoacetic acid is commonly accompanied by more or less acetone. Until the recent discovery of good methods for the separate determination of acetoacetic acid and acetone (Folin, Embden) small amounts of acetoacetic acid were commonly mistaken for acetone into which it passed with ease. Acetone was therefore regarded as one of the first steps in the decomposition of acetoacetic acid in the body. Similarly acetophenone was found to accompany benzoylacetic acid when the latter substance was detected in the urine of animals receiving aromatic acids such as phenylpropionic acid and phenylvaleric acid:—

$$CH_3 . CO . CH_2 . COOH \rightarrow CH_3 . CO . CH_3 + CO_2$$
$$C_6H_5 . CO . CH_2 . COOH \rightarrow C_6H_5 . CO . CH_3 + CO_2$$

Does this decomposition of β-ketonic acids with formation of ketones represent the main normal course of their catabolism? There is a considerable amount of evidence against this supposition.

In the first place, Geelmuyden has shown that acetone is oxidized in the animal body with very considerable difficulty. Similarly, acetophenone is not very easily oxidized in the animal body and moreover is distinctly more toxic than are the acids which yield it on oxidation.[1]

Experiments made upon the oxidation of phenylbutyric acid and phenylvaleric acid have a direct bearing upon the question of ketone formation and prove that at least in the case of these acids, ketones, derived from the corresponding β-ketonic acids, are *not* formed. The evidence for this is as follows: phenylbutyric acid on oxidation in the

[1] Benzoic acid is the end-product of the oxidation of acetophenone in the body, but under certain conditions part of the acetophenone may undergo reduction to phenyl-methyl-carbinol, which is excreted in combination with glucuronic acid.

body gives phenaceturic acid and no hippuric acid (Knoop); phenylacetone, the corresponding ketone, on the other hand, gives hippuric acid but no phenaceturic acid (Dakin); phenylvaleric acid gives on oxidation in the body hippuric acid (Knoop); while benzylacetone, the corresponding ketone, gives phenaceturic acid (Dakin).

It appears legitimate to transfer the results obtained with phenylvaleric acid to the higher fatty acids and to conclude that *if the higher fatty acids in undergoing β-oxidation do yield β-ketonic acids, these acids are not converted into the corresponding ketones by loss of carbon dioxide.*

	Substance.	Oxidation Product Excreted in Combination with Glycine.
Acid . . .	$C_6H_5 . CH_2 . CH_2 . CH_2 . COOH$	$C_6H_5 . CH_2 . COOH$
Ketone . . .	$C_6H_5 . CH_2 . CO . CH_3$	$C_6H_5 . COOH$
Acid . . .	$C_6H_5 . CH_2 . CH_2 . CH_2 . CH_2 . COOH$	$C_6H_5 . COOH$
Ketone . . .	$C_6H_5 . CH_2 . CH_2 . CO . CH_3$	$C_6H_5 . CH_2 . COOH$

It is probably safe to assume that acetone and acetophenone are not normal steps in the catabolism of butyric and phenylpropionic acids, but that, under certain conditions, the acetoacetic acid and benzoylacetic acid formed from these acids escape complete oxidation and may slowly pass over into the ketones.

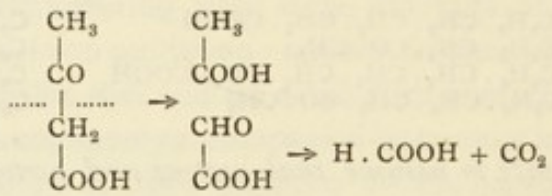
Hydrolysis and Oxidation of β-Ketonic Acids.—In searching for an alternative scheme for the decomposition of β-ketonic acids in the animal body, the possibility of "acid hydrolysis" must be considered. As is well known, strong alkali acting on a β-ketonic acid, or ester, decomposes the latter with formation of the salts of two simpler acids.

$$R . CH_2 . CO . CH_2 . COOH + H_2O = R . CH_2 . COOH + CH_3 . COOH$$

A biochemical reaction of this type involving the formation of a lower acid with two less carbon atoms than the ketonic acid would fit in well with the known facts and many attempts have been made to demonstrate this type of change in the organism, but without much success. Embden and Michaud found that sodium acetoacetate underwent decomposition in contact with fresh liver tissue and suggested the occurrence of acid hydrolysis. They were unable, however, to demonstrate the formation of acetic acid, or other volatile acid, and the later experiments of Blum, Dakin, Friedmann and Maase and of Neubauer have shown that the reaction taking place under these conditions was in reality one of reduction to *l*-hydroxybutyric acid.

There still remains the possibility of the decomposition of β-ketonic acids by oxidation. Very little work has been done upon the chemical oxidation of β-ketonic acids. Emmerling and Oppenheim oxidized

acetoacetic ester with potassium permanganate and obtained acetic and oxalic acids, but this result is of limited biochemical significance. The writer has carried out a number of experiments upon the oxidation of acetoacetic acid and its derivatives with hydrogen peroxide.[1] This oxidizing agent was chosen since in many cases it closely simulates biochemical reactions. It was found that acetoacetic acid or its ethyl ester, in neutral or alkaline solution readily underwent oxidation with formation of lower acids. The principal products were acetic and formic acids, carbon dioxide and some glyoxylic acid. The changes may possibly be represented as follows:—



It will be noted from these experiments that acetoacetic acid is readily oxidized at low temperatures in neutral solution, giving products which are themselves readily oxidized in the animal body to carbon dioxide and water. It is conceivable that a somewhat analogous oxidation of β-ketonic acids takes place in the living organism.

Propionic, Acetic and Formic Acids.—In the introduction to this chapter reference has been made to the ease with which salts of these acids undergo practically complete oxidation in the animal body. Almost nothing is known of the mechanism of this process of oxidation. Acetic and formic acids obviously cannot undergo β-oxidation as the higher fatty acids do, and there is no good evidence to prove that propionic acid undergoes this type of change. On the other hand, it is improbable that propionic acid undergoes α-oxidation in the animal body since one would then expect either lactic or pyruvic acid to be formed as intermediate products. Since these two last acids readily yield glucose in the diabetic organism, whereas propionic acid does not, it may be inferred that oxidation of the hydrogen attached to the α-carbon atom is probably not the first step in the oxidation.

The chemistry of the oxidation of acetic and formic acids is of special importance since these acids are constantly excreted in small amounts in the urine and it appears not improbable that these acids may be formed in the body as intermediate products of oxidation in large amounts, of which only a very small proportion escapes complete oxidation and is excreted. As to the mechanism of the oxidation of

[1] Unpublished results.

these acids, there is not much to be said. Formic acid is presumably directly oxidized to carbon dioxide,[1] but in the case of acetic acid the question of intermediate products arises. Moderate amounts of acetic acid are completely burned in the body. Oxalic acid is not found in the urine following acetate administration, so that this substance, which is relatively resistant to further oxidation in the body, is not an intermediate step.[2] It would seem more probable that glyoxylic and formic acids might be intermediate products in the change. This reaction is readily brought about outside the body by oxidation with hydrogen peroxide (Hopkins). The only experimental evidence in support of this hypothesis is the fact that, under certain conditions, Wakeman and Dakin found an increased formic acid excretion to follow intravenous injection of sodium acetate. The increase, however, is very small and the results are not constant. Further investigation is much needed.

Other Types of Oxidation.—Satisfactory evidence has been adduced showing that the higher normal fatty acids undergo β-oxidation and that, in the case of the oxidation of butyric acid and phenylpropionic acid, the intermediate formation of β-hydroxy and β-ketonic acids has been determined. We have now to consider the question whether oxidation of saturated normal fatty acids may not take place in other ways. Although it is by no means improbable that other types of reaction than that of β-oxidation will be found to occur, so far no other has been observed.

Oxidation of normal saturated fatty acids in the α-position (except in the case of acetic acid) is excluded for many reasons: (1) Propionic acid does not yield lactic acid, or substances derived from lactic acid, e.g. glucose in diabetic animals. (2) Phenylpropionic acid is oxidized in the body only so far as benzoic acid, the aromatic ring remaining intact, while phenyl α-hydroxypropionic acid, $C_6H_5 . CH_2 . CHOH . COOH$, and phenylpyruvic acid, $C_6H_5 . CH_2 . CO . COOH$, substances which would be formed by α-oxidation of phenylpropionic acid, are completely oxidized in the body, the aromatic nucleus being destroyed. (3) α-oxidation of normal higher fatty acids with an uneven number of carbon atoms would presumably yield acids with an even number of carbon atoms, which in turn would yield acetoacetic acid when perfused through a surviving liver, according to Embden's method.

[1] Battelli and Stern have shown that many tissues, especially the liver, contain peroxidases which in the presence of hydrogen peroxide readily effect the oxidation of formic acid to carbon dioxide.

[2] Acetic acid is readily oxidized to oxalic acid by alkaline permanganate.

Acetoacetic acid formation from normal acids with an uneven number of carbon atoms does not occur, however. (4) Levulinic acid, $CH_3 . CO . CH_2 . CH_2 . COOH$, when administered to a diabetic animal, does not materially increase the excretion of acetoacetic acid as would be the case had *a*-oxidation occurred (Weintraud).

From the above and other reasons it is concluded that a-oxidation of normal saturated fatty acids does not take place in the animal body, or is at least unusual.

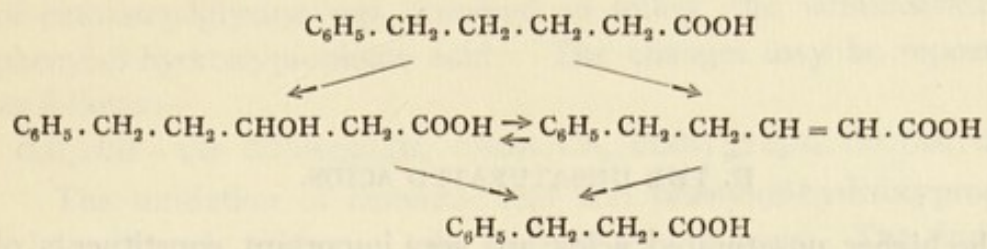
The occurrence of γ-oxidation of normal fatty acids is very unlikely, for in the first place γ-hydroxy acids and their lactones and γ-ketonic acids, such as levulinic acid, phenyl γ-hydroxybutyric acid and phenyl γ-hydroxyvaleric acid, are rather resistant to oxidation in the animal body; secondly, γ-oxidation, if it occurred, would result in butyric acid and hence acetoacetic acid formation from acids with an uneven number of carbon atoms, such as heptylic acid, when given to diabetic animals, or perfused through a surviving liver. This acetoacetic acid formation, however, is not observed, hence it appears safe to conclude that oxidation of saturated normal fatty acids in the γ-position does not take place in the animal body.

That oxidation in the δ-position may occur is more probable than either the *a* or γ-position, although the evidence at present is against the assumption of its occurrence. As previously mentioned, phenylvaleric acid is oxidized in the animal body to benzoic acid, which is excreted as hippuric acid (Knoop). This reaction obviously involves oxidation of the hydrogen attached to the δ-carbon atom, but Dakin showed, through the isolation of intermediate products, that the oxidation was a complex one involving two successive β-oxidations. There was no evidence that δ-oxidation as such had occurred. The following intermediate products of catabolism were identified: β-phenyl-β-hydroxypropionic acid, cinnamoylglycine, benzoylacetic acid and acetophenone. These are the same substances as are formed among the products of catabolism of phenylpropionic acid. Hence it is reasonable to assume that the phenylvaleric acid underwent β-oxidation yielding phenylpropionic acid, which in turn underwent β-oxidation again yielding benzoic acid :—

$$C_6H_5 . CH_2 . CH_2 . CH_2 . CH_2 . COOH \rightarrow C_6H_5 . CH_2 . CH_2 . COOH \rightarrow C_6H_5 . COOH$$

This type of reaction may be termed successive β-oxidation and is probably typical of the normal catabolism of straight chain saturated fatty acids. The actual mode of conversion of phenylvaleric acid into phenylpropionic acid could not be exactly determined, but it was shown

that the corresponding β-hydroxy acid and α-β-unsaturated acid gave the same catabolic products as phenylvaleric acid :—



II. THE UNSATURATED ACIDS.

The higher unsaturated acids are very important constituents of the animal body and their oxidation furnishes a readily available source of energy. Most chemical reagents attack the unsaturated acids infinitely more readily than they do the saturated acids, but in the animal organism this difference is not nearly so marked. Thus, phenylpropiolic acid, $C_6H_5 . C \equiv C . COOH$, is oxidized in the body with much greater difficulty than either cinnamic, $C_6H_5 . CH = CH . COOH$, or phenylpropionic acid, $C_6H_5 . CH_2 . CH_2 . COOH$ (unpublished observations). Of the three acids, the saturated phenylpropionic is oxidized in the body with greater ease than either of the others, but towards most chemical oxidizing agents it is by far the most resistant.

The question of the formation of unsaturated acids from saturated acids has been dealt with on p. 28, and we are now concerned simply with their further decomposition. Oleic acid, $C_{18}H_{34}O_2$, which may occur in structurally isomeric forms, and the still more highly unsaturated acids related to it, are the most important members of this group. Unfortunately we know almost nothing of the biochemical oxidation of these acids and can only speculate as to the mechanism of their catabolism by analogy with other acids.[1]

The simplest unsaturated acid, acrylic acid, $CH_2 = CH . COOH$, readily undergoes complete oxidation in the animal body without yielding any clue to intermediate products (Luzatto). Its phenyl derivative, cinnamic acid, $C_6H_5 . CH : CH . COOH$, when administered to animals has been found to undergo decomposition in an interesting fashion and the results may have a bearing upon the catabolism of other unsaturated acids. It had been observed long ago by Erdmann and Marchand that cinnamic acid was oxidized in the body to benzoic acid which was excreted in the form of hippuric acid. By administering larger amounts of ammonium cinnamate subcutaneously to cats and dogs (0·25 – 0·45 grm. per kilo) Dakin observed the excretion of laevo-β-phenyl-β-hydroxypropionic acid and acetophenone, the latter substance being derived from benzoylacetic acid. This formation of a β-

[1] For information concerning the oxidation *in vitro* of the chief unsaturated acids, consult "The Fats," by J. B. Leathes, this series, 1910.

hydroxy acid from the corresponding unsaturated acid by union with the elements of water, appears to be a reversible reaction, since excretion of cinnamoylglycine was observed to follow the administration of phenyl-β-hydroxypropionic acid.[1] The changes may be represented as follows :—

$$C_6H_5 . CH = CH . COOH \rightleftharpoons C_6H_5 . CHOH . CH_2 . COOH \rightleftharpoons C_6H_5 . CO . CH_2 . COOH$$

The oxidation of cinnamic acid and phenyl-β-hydroxypropionic acid to benzoic acid may take place in several ways. The finding of benzoylacetic acid shows that at least part of the cinnamic acid was converted into benzoic acid through the formation of this substance as an intermediate product. Expressing the reaction in general terms one may therefore represent an α-β-unsaturated acid undergoing decomposition in the following fashion :—

$$R . CH = CH . COOH \rightarrow R . CHOH . CH_2 . COOH \rightarrow R . CO . CH_2 . COOH \rightarrow R . COOH$$

It will be noticed that these changes have much in common with those representing the β-oxidation of a saturated fatty acid, such as butyric acid, or phenylpropionic acid. But, at present at any rate, there is no reason for assuming that an unsaturated acid, such as cinnamic acid, can undergo oxidation only by conversion into a β-hydroxy acid and β-ketonic acid. It seems reasonable to suppose that some of the unsaturated acids may undergo direct oxidation without previously taking up the elements of water.

When an unsaturated acid is carefully oxidized with a reagent such as potassium permanganate, as a general rule a dihydroxy acid is formed as the first product of oxidation, while on further oxidation the original carbon chain is broken at the double linkage with formation of a lower fatty acid. Thus cinnamic acid yields phenylglyceric acid and then benzoic acid :—

$$C_6H_5 . CH = CH . COOH \rightarrow C_6H_5 . CHOH . CHOH . COOH \rightarrow C_6H_5 . COOH$$

The question arises whether these dihydroxy acids are products of the oxidation of unsaturated acids in the body. There seems to be good evidence that they are *not* formed, at any rate to any large extent. Thus (1) it is found that phenylglyceric acid is oxidized in the body to hippuric acid only with great difficulty compared with cinnamic acid (Dakin). (2) Friedmann showed that the behaviour of crotonic

[1] The conversion of a β-hydroxy acid into an unsaturated acid is often observed. Thus phenyl-β-hydroxypropionic acid readily gives cinnamic acid when warmed with hydrochloric acid. The reverse change, i.e. the formation of a β-hydroxy acid from an α-β-unsaturated acid is much less common. The conversion of fumaric acid into malic acid on prolonged heating with caustic soda solution is an example of this change :—

$$COOH . CH = CH . COOH + H_2O = COOH . CHOH . CH_2 . COOH$$

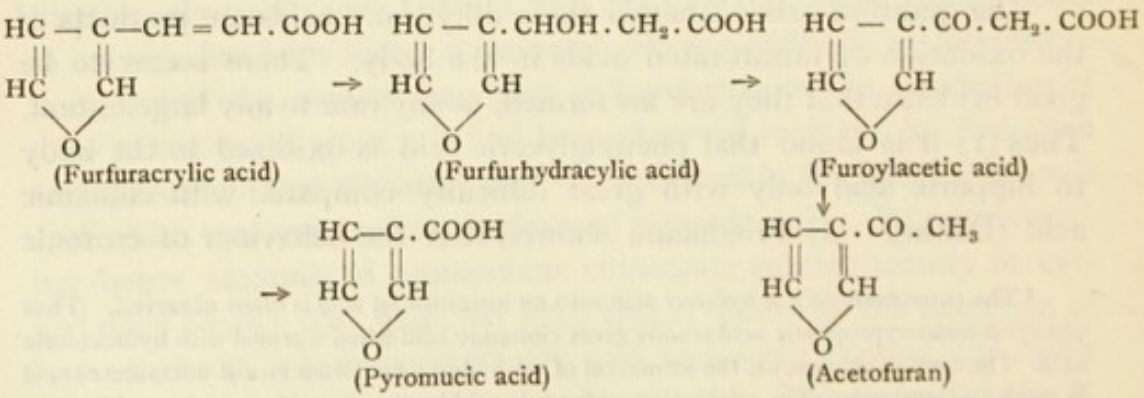
acid, $CH_3 . CH : CH . COOH$, when perfused through a surviving liver, was quite different from that of α-β-dihydroxybutyric acid, $CH_3 . CHOH . CHOH . COOH$ (see later). (3) Phenyl-β-γ-dihydroxybutyric acid $C_6H_5 . CHOH . CHOH . CH_2 . COOH$ is not a product of the oxidation in the animal body of phenylisocrotonic acid, $C_6H_5 . CH : CH . CH_2 . COOH$, since the end-product of the oxidation of the latter substance is phenylacetic acid (Knoop), while the former yields benzoic acid (Dakin).

It appears, therefore, that the dihydroxy acids do not represent intermediate stages in the normal catabolism of singly unsaturated acids. It is quite possible, however, that polyhydroxy acids are formed from more highly unsaturated acids by taking up two, or more, molecules of water, but it is unlikely that the hydroxyl groups would occupy adjacent positions.

There still remains the possibility of an unsaturated acid undergoing direct oxidation with formation of a lower fatty acid without intermediate formation of hydroxy, or ketonic acid :—

$$R . CH = CH . COOH \rightarrow R . COOH$$

The occurrence of this change in the animal body cannot be regarded as definitely proved, although Friedmann's experiments on the fate of furfuracrylic acid tend to support this supposition. On injection of the sodium salt of this acid into dogs, unchanged acid was excreted in combination with glycine (furfuracryluric acid) together with pyromucic acid and traces of acetofuran. No optically active β-hydroxy acid was found, such as was observed in the case of cinnamic acid. The acetofuran was undoubtedly derived from furoylacetic acid by loss of carbon dioxide—a reaction analogous to the formation of acetone from acetoacetic acid. It might be assumed that the oxidation took place as follows :—



Friedmann is, however, unwilling to concede that the detection of acetofuran in the urine indicates the fact that any considerable part of

the furfuracrylic acid was oxidized by way of furoylacetic acid, since on administering the latter substance to dogs about 50 per cent of the substance was excreted unchanged, and while an active β-hydroxy acid was apparently formed, no pyromucic acid was detected. Friedmann regards these results as a direct experimental proof that α-β-unsaturated acids may be converted into acids with two fewer carbon atoms, without going through the stage of β-ketonic acids. To the writer this conclusion appears likely, but unproven. It appears somewhat unsafe to assume that the fate of subcutaneously injected furoylacetic acid is the same as that of the same substance produced at low concentration in the actual sphere of oxidative change. Moreover, none of the substances of this group appear to be very readily oxidized in the body, as almost 30 per cent of the furfuracrylic acid was recovered unoxidized, but in combination with glycine, after giving to a dog 10 grms. of the acid in the course of five days.

An excellent example of the derivation of a β-ketonic acid from an α-β-unsaturated acid is furnished by Friedmann's demonstration of acetoacetic acid formation when salts of crotonic acid are perfused through a surviving liver. It is likely that in this reaction β-hydroxybutyric acid is first formed and then undergoes oxidation to acetoacetic acid :—

$$CH_3 . CH = CH . COOH \rightarrow CH_3 . CHOH . CH_2 . COOH \rightarrow CH_3 . CO . CH_2 . COOH$$

The fate of the phenyl-derivative of crotonic acid offers some peculiarities. This acid, when administered by mouth to dogs, is converted into phenaceturic acid (Knoop), and the same result was obtained when the sodium salt was given subcutaneously to cats (Dakin). At first sight it would appear as if reduction of the unsaturated acid had occurred as well as oxidation :—

$$C_6H_5 . CH = CH . CH_2 . COOH \rightarrow C_6H_5 . CH_2 . COOH$$

It is evident that oxidation did not take place with rupture of the side-chain at the double linkage, as in oxidation *in vitro*, for in this case benzoic (hippuric) acid would have been formed. Neither were phenyl-β-γ-dioxybutyric acid nor phenyl-γ-hydroxybutyric acid (phenylbutyrolactone) formed as intermediate stages in the reaction, for the former yields hippuric acid while the latter is very resistant to oxidation in the body. It appears more likely that the unsaturated phenylisocrotonic acid takes up the elements of water to form phenyl-β-hydroxybutyric acid which then undergoes further oxidation. Phenyl-β-hydroxybutyric acid when administered to animals does

yield phenylacetic (phenaceturic acid) just as phenyl-isocrotonic acid does (Dakin) :—

$$\underset{\text{(Phenylisocrotonic acid)}}{C_6H_5 . CH = CH . CH_2 . COOH} \rightarrow \underset{\text{(Phenyl-}\beta\text{-hydroxybutyric acid)}}{C_6H_5 . CH_2 . CHOH . CH_2 . COOH} \rightarrow \underset{\text{(Phenylacetic acid)}}{C_6H_5 . CH_2 . COOH}$$

It will be noted that the unsaturated acids, crotonic, cinnamic and phenylisocrotonic acid, all yield the same end-products as the corresponding saturated acids, butyric, β-phenylpropionic acid and γ-phenylbutyric acid. This was also found to be the case with three unsaturated acids derived from phenylvaleric acid :—

Phenyl-α-β-pentenic acid	$C_6H_5 . CH_2 . CH_2 . CH : CH . COOH$
Phenyl-β-γ-pentenic acid	$C_6H_5 . CH_2 . CH = CH . CH_2 . COOH$
Cinnamylidineacetic acid	$C_6H_5 . CH = CH . CH = CH . COOH$

In each case it was found that the acids on oxidation in the body gave benzoic (hippuric) acid and in each case evidence was obtained by the detection in the urine of substances such as cinnamoylglycine, phenyl-β-hydroxypropionic acid and acetophenone, that the oxidation was indirect, i.e. that the four terminal carbon atoms of the side-chain were removed in two pairs by successive β-oxidation (Dakin).

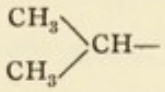
In considering the changes which the unsaturated acids undergo, the possibility of a shifting of the position of the double linkage must not be lost sight of. In a great many cases Fittig and others have shown that this change may be effected *in vitro* by simple treatment with acid, or alkali. The conversion of oleic acid into palmitic and acetic acids on fusion with potash, a reaction involving both oxidation and shifting of the double link, may also be recalled in this connexion.

In general it may be said that unsaturated acids undergoing oxidation in the animal body yield products essentially similar to those derived from the corresponding saturated acids; that they may take up the elements of water to form optically active saturated hydroxy acids which then undergo further oxidation; that they may possibly undergo direct oxidation at the double linkage; but dihydroxy acids such as are formed by unsaturated acids in vitro are not intermediate products of these biochemical oxidations.

III. THE OXIDATION OF ACIDS WITH BRANCHED CHAINS.

The fatty acids with branched chains readily undergo complete oxidation in the animal body. Although these acids are not as important as those of the normal series from a biochemical point of view, the study of their catabolism has shown the occurrence of several interesting types of reaction.

Of the acids containing the isopropyl group,

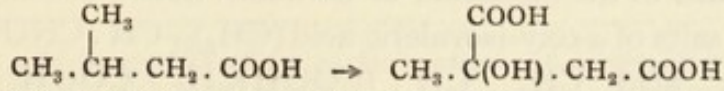


isobutyric, isovaleric and isocaproic have been most carefully studied. The first two of these acids are of special importance on account of their relationship with the protein derivatives, *a*-amino-isovaleric acid (valine) and leucine, from which they are readily formed on oxidation (p. 49). On oxidation *in vitro* with alkaline permanganate, the tertiary hydrogen atom contained in these acids is replaced by a hydroxyl group, while on further oxidation acetone is readily formed[1] (R. Meyer).

On oxidation in the body an entirely different type of reaction occurs, for of these acids referred to only one, namely isovaleric acid, yields acetone on perfusion through a surviving liver.[2]

Baer and Blum similarly found that the administration of salts of isobutyric acid to diabetics was not followed by an increased excretion of acetone bodies, whereas consumption of isovaleric acid was followed not only by increased excretion of acetone and acetoacetic acid, but

[1] On oxidation with nitric acid a similar change takes place, but in addition one of the methyl groups is oxidized to a carboxyl group. Thus, isovaleric acid gives methyl-hydroxy-succinic acid (methyl malic acid) (J. Bredt):—



Isobutyric acid and isovaleric acid are readily oxidized in neutral solution with hydrogen peroxide yielding acetone (Dakin).

[2] Blum, however, has recently stated that isobutyric acid when given to young animals gives rise to acetoacetic acid excretion. The mechanism of this reaction is quite obscure. Further experiments are very desirable in view of the possible contamination of the iso-acid with normal butyric acid. α-oxy-isobutyric acid does not yield acetoacetic acid, but is mostly excreted unchanged together with traces of lactic acid.

also of β-hydroxybutyric acid. Embden later showed that the acetone found after perfusion of the liver with salts of isovaleric acid was derived from acetoacetic acid and hence was produced by a set of reactions entirely different from the *in vitro* oxidations previously referred to :—

$$\begin{array}{l} \quad\ \ CH_3 \\ \quad\ \ \ | \\ CH_3 . CH . CH_2 . COOH \rightarrow CH_3 . CO . CH_2 . COOH \rightleftarrows CH_3 . CHOH . CH_2 . COOH \\ \qquad\qquad\qquad\qquad\qquad\qquad \downarrow \\ \qquad\qquad\qquad\qquad\qquad CH_3 . CO . CH_3 \end{array}$$

The biochemical oxidation of isovaleric acid involves the removal of one methyl group from the isopropyl radical. A clue to the mechanism of this change was furnished by Baer and Blum's observation of the excretion of d-lactic acid following the administration of iso-butyrates to diabetics :—

$$\begin{array}{ccc} \begin{array}{c} \ \ CH_3 \\ \ \ | \\ CH_3 . CH . COOH \\ \text{(Isobutyric acid)} \end{array} & \rightarrow & \begin{array}{c} \ \ OH \\ \ \ | \\ CH_3 . CH . COOH \\ \text{(Lactic acid)} \end{array} \end{array}$$

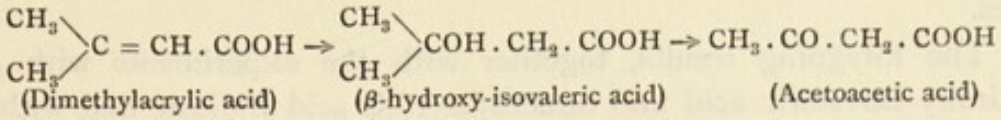
This reaction involves the substitution of a methyl group by hydroxyl. It is doubtful, however, if this reaction is general for other acids of this group, for Baer and Blum found that α-methylbutyric acid, $CH_3 . CH_2 . CH . (CH_3) . COOH$, readily gave rise to β-hydroxybutyric acid when given to diabetics, whereas α-hydroxybutyric acid is incapable of this change. It is perhaps more likely that the methyl group is replaced by a hydrogen atom. That this latter change does *not* take place by intermediate oxidation of the methyl group to carboxyl, followed by loss of carbon dioxide, was shown by the failure of ethyl malonic acid,

$$\begin{array}{l} CH_3 . CH_2 . CH . COOH \\ \qquad\qquad\ \ | \\ \qquad\qquad COOH, \end{array}$$

to yield acetone bodies when administered to a diabetic.

Friedmann showed that β-hydroxy-isovaleric acid gave acetoacetic acid when perfused through a surviving liver, so that this substance may be formed in the oxidation of isovaleric acid. Friedmann found further that salts of α-oxy-isovaleric acid $(CH_3)_2 . CH . CHOH . COOH$, pyrotartaric acid $CH_3 . CH . (COOH)CH_2 . COOH$, citraconic $CH_3 . C . (COOH) = CH \cdot COOH$, mesaconic $CH_3 . C(COOH) = CH . CH_2 . COOH$, and citramalic acids $CH_3 . C(OH) . (COOH) . CH_2 . COOH$ did not yield acetoacetic acid. Dimethylacrylic acid, on the other hand, readily yields acetoacetic acid on perfusion. It is probable that this unsaturated acid takes up the elements of water to

form β-hydroxy-isovaleric acid, which then undergoes further oxidation (cf. crotonic acid, p. 39):—



Baer and Blum have studied the effect of the administration to diabetics of salts of a number of acids with branched chains other than those previously referred to. These results together with Friedmann's are collected in the following table. It will be seen that only

Substance.	Acetoacetic Acid Formation on Liver Perfusion.	Formation of "Acetone Bodies" in Diabetic Organism.
Isobutyric acid $(CH_3)_2CH.COOH$	—	—
Isovaleric acid $(CH_3)_2CH.CH_2.COOH$	+	+
Isocaproic acid $(CH_3)_2CH.CH_2.CH_2.COOH$	—	
Methyl-ethyl-acetic acid (α-methylbutyric acid) $CH_3.CH_2.CH(CH_3).COOH$		+
Di-ethyl-acetic acid (α-ethylbutyric acid) $CH_3.CH_2.CH(C_2H_5).COOH$	+[1]	+[1]
Methyl-ethyl-propionic acid (β-ethylbutyric acid) $CH_3.CH(C_2H_5).CH_2.COOH$		+
Methyl-propyl-acetic acid (α-methylvaleric acid) $CH_3.CH_2.CH_2.CH(CH_3).COOH$		—
Ethyl-malonic acid $CH_3.CH_2.CH(COOH).COOH$		—
α-Hydroxy-isovaleric acid $(CH_3)_2CH.CHOH.COOH$	—	
β-Hydroxy-isovaleric acid $(CH_3)_2C(OH).CH_2.COOH$	+	
Dimethylacrylic acid $(CH_3)_2C=CH.COOH$	+	

those acids which contain four carbon atoms in a straight chain yield acetoacetic acid. Those with chains of three, or five, carbon atoms do *not* yield acetoacetic acid. On the other hand, not *all* acids with a straight chain of four carbon atoms yield acetoacetic acid, for acids

[1] Blum and Koppel have very recently identified the ketone derived from di-ethyl-acetic acid as methyl-propyl-ketone. The ketone is derived apparently from the β-ketonic acid $CH_3.CO.CH(C_2H_5).COOH$ through loss of carbon dioxide (cf. p. 27).

with a carboxyl group in the α-position (ethyl malonic acid), or in the β-position (citramalic acid), evidently undergo oxidation along other lines.

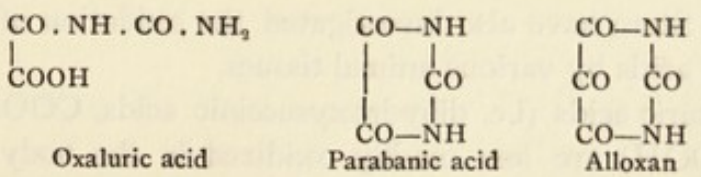
The foregoing results, together with the experiments with β-hydroxy-isovaleric acid and dimethylacrylic acid indicate that within certain natural limitations Knoop's hypothesis of β-oxidation is applicable to the case of fatty acids with branched chains.

In general it may be said that the branched-chain fatty acids, when undergoing oxidation in the body, tend to part with their side-chains and then undergo oxidation along similar lines to those of the corresponding straight-chain normal fatty acids.

IV. THE DIBASIC ACIDS.

The unsubstituted dibasic fatty acids are of relatively little importance in animal metabolism. Only one of them, oxalic acid, is known to be commonly present in the animal organism, but the oxidation in the body of a few of the higher acids has been studied to some extent and the results are of interest in connexion with the general problems of fatty acid oxidation.

Oxalic Acid compared with its homologues and with the monobasic fatty acids is very resistant to oxidation in the animal body. In fact many regard the question whether oxalic acid is oxidized at all as still an open one. A number of observers have declared that oxalic acid is completely unattacked in the animal organism (Gaglio, Wiener, Pohl, Faust and others) while others have found that the oxalic acid excreted in the urine was less than the amount administered (Marfori, Lommel, Autenrieth and Barth, Bakhoven and others). Since, however, the acid was in most cases given by mouth and oxalic acid is susceptible to bacterial decomposition, the results are not convincing and indeed in many cases the analytical methods employed were inadequate. Hildebrandt more recently administered small amounts of oxalic acid subcutaneously to rabbits and found that 60 to 90 per cent was apparently oxidized ; and these results were confirmed by Dakin. It must be admitted, however, that the capacity of the body to oxidize oxalic acid, introduced as such, is restricted within very narrow limits. But a number of derivatives of oxalic acid and substances which may under certain circumstances yield oxalic acid in the body are oxidized comparatively easily. Thus, Luzatto and Koehne found that oxaluric acid and parabanic acid, both simple derivatives of oxalic acid,



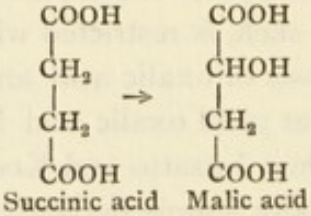
Oxaluric acid — Parabanic acid — Alloxan

are oxidized in the body. Alloxan was also found to be easily oxidized. Glycollic acid (CH_2OH . COOH) and glyoxylic acid (CHO . COOH), which, when administered subcutaneously in large

amounts give rise to a considerable excretion of oxalic acid, may be given in smaller doses, especially by mouth, with the production of little or no oxalic acid excretion. But in the case of the latter acids it is not unlikely that alternative methods of decomposition result in the avoidance of the formation of oxalic acid as an intermediate product.

Malonic Acid.—($COOH . CH_2 . COOH$) in contrast with oxalic acid is readily oxidized in the body. Pohl found that on giving salts of malonic acid to dogs there was a barely perceptible rise in oxalic acid excretion and only minute traces of the unchanged acid appeared in the urine. The mechanism of the oxidation of the acid has not been made clear. Tartronic acid, $COOH . CHOH . COOH$, and mesoxalic acid, $COOH . C(OH)_2 . COOH$ were found by Pohl to undergo oxidation in the animal body readily. No intermediate products were detected.

Succinic Acid.—($COOH . CH_2 . CH_2 . COOH$) and malic acid ($COOH . CHOH . CH_2 . COOH$) were found by Pohl to be easily oxidized in the body, and their administration led to the excretion of no intermediate products of oxidation. Recently, Battelli and Stern have stated that almost all the tissues of the higher animals possess the property of oxidizing succinic acid to inactive malic acid and they have made extensive studies of the conditions affecting the oxidation. This type of reaction is obviously of considerable interest, since it affords another example of the β-oxidation of a carboxylic acid. It must be confessed, however, that the experimental evidence so far available does not seem to prove conclusively that the oxidation is actually of the type represented. Further investigation is necessary to definitely ascertain the course of the reaction :—



Succinic acid Malic acid

Battelli and Stern have also investigated the oxidation of citric, malic and fumaric acids by various animal tissues.

The tartaric acids (i.e. dihydroxysuccinic acids, $COOH . CHOH . CHOH . COOH$) are less readily oxidized in the body than either malic, or succinic, acid. According to the earlier experiments of Brion the laevo acid is oxidized more readily than the dextro acid, while racemic acid is the most stable. Mesotartaric acid is oxidized to

about the same extent as the laevo-tartaric acid. In the light of these results it was somewhat remarkable that Brion found no selective resolution of the racemic acid on administering it to animals ; the acid excreted showed no marked optical activity. More recent experiments by Neuberg and Saneyoshi have shown that as a matter of fact there appears to be no marked difference between the dextro- and laevo-tartaric acids as regards their relative rate of oxidation in the animal body. *d-l*-tartaric acid, therefore, must no longer be classed with the substances which undergo asymmetric decomposition in the animal body.

Glutaric Acid.—($COOH . CH_2 . CH_2 . CH_2 . COOH$) undergoes complete oxidation in the animal organism (Marfori). Special interest attaches to the oxidation of this acid together with its homologues, adipic acid $COOH . (CH_2)_4 . COOH$ and pimelic acid $COOH . (CH_2)_5 . COOH$ and suberic acid $COOH . (CH_2)_6 . COOH$ on account of the remarkable effect they have in inhibiting the glycosuria and acidosis of animals rendered diabetic by phlorhizin (Baer and Blum). The inhibitory action upon the glycosuria and acidosis is accompanied by a marked decrease in the excretion of nitrogenous substances in the urine. The effect of these acids appears to be remarkably specific and is not shown by sebacic, succinic, or malonic acid. On the other hand, Baer and Blum have shown that certain hydroxy acids derived from glutaric and adipic acids, such as β-hydroxyglutaric acid, α-γ-dihydroxyglutaric acid, α-β-γ-trihydroxyglutaric acid and the saccharic acids have a marked effect in inhibiting phlorhizin glycosuria, but that α-hydroxyglutaric acid is inactive. Conclusions have been drawn from these results as to the possible mechanism of the oxidation of the higher dibasic acids, but at present the evidence is only fragmentary.[1]

[1] Very recent experiments of Ringer and of Underhill throw doubt on the accuracy of the interpretation by Baer and Blum of their results.

CHAPTER III.

I. THE α-AMINO, α-HYDROXY AND α-KETONIC ACIDS.

THE reasons for classifying the *α*-amino, hydroxy and ketonic acids are mainly based upon the close structural relationships existing between these substances which frequently make it possible for members of the different groups to undergo mutual interconversion in the animal body. Furthermore, it appears that the processes involved in the oxidation of the three groups of substances have much in common.

The biochemical importance of the substances under consideration hardly requires emphasis including as they do the various amino acids derived from the hydrolysis of proteins,[1] and hydroxy acids such as lactic acid. This significance of the *α*-ketonic acids has only recently been recognized mainly owing to the admirable researches of Neubauer, and later of Knoop, but it now appears that they play a very prominent rôle in intermediary metabolism.

The amino acids derived from the hydrolysis of proteins are readily oxidized in the animal body, the nitrogen appearing in the urine chiefly in the form of urea, while the carbon is eventually oxidized to carbon dioxide. The earlier experiments of Schultzen and Nencki, and of E. Salkowski, have been amplified by many later workers. While it is true that the animal body eliminates practically the whole of the nitrogen of amino acids, derived from the hydrolysis of protein, in the form of urea and to a lesser extent as ammonia, the ease of this transformation varies considerably with the different amino acids and according to the conditions of the experiment. Thus, according to Stolte's experiments in which various amino acids were injected intravenously into rabbits, glycine and leucine yield urea most completely, while alanine, cystine, aspartic and glutamic acids are less readily metabolized. Phenylalanine and tyrosine injections led to no immediate urea excretion. More recently, Abderhalden and his pupils have made careful studies of the utilization of a number of amino acids and polypeptides

[1] Cf. "Chemical Constitution of the Proteins." This Series. R. H. A. Plimmer.

when fed to dogs. In general it may be said that the decomposition of most amino acids is remarkably complete.[1]

There is good evidence for the belief that the first step in the formation of urea from amino acids is the liberation of ammonia and carbon dioxide. The ammonium carbonate, or carbamate, resulting from the union of ammonia and carbon dioxide is then converted into urea in accordance with Schroeder's well-known observation. There is much evidence tending to show that the liver is the main seat of urea synthesis; thus for example, Salaskin by perfusion of a surviving liver with blood containing added glycine and aspartic acid was able to demonstrate the formation of urea. But other organs than the liver such as kidney, pancreas and intestinal mucosa have been shown by Lang, and later by Bostock, to possess the property of liberating ammonia from amino acids. In the case of one amino acid, namely arginine, urea may be formed by simple hydrolysis without prior formation of ammonia (p. 61).

The production of ammonia and carbon dioxide from amino acids, the first step in the formation of urea, is believed to take place by a process of oxidation in the *a*-position with formation of an acid with one fewer carbon atom :—

$$R.CH_2.CHNH_2.COOH + O_2 = R.CH_2.COOH + CO_2 + NH_3$$

Many examples of this type of change are known to occur in the animal body. Thus the conversion of tyrosine into homogentisic acid in cases of alcaptonuria may be cited, also the formation of ortho- and meta-hydroxyphenylacetic acid from ortho- and meta-tyrosine (Blum, p. 68).

Embden upon applying his method of liver perfusion to the study of amino acid catabolism found that in general the behaviour of the aliphatic *a*-amino acids was similar to that of the derived fatty acid containing one less carbon atom. Thus leucine gave acetoacetic acid just as isovaleric acid does ; valine (*a*-amino-isovaleric acid) on the other hand does not, neither does isobutyric acid. Oxidation has evidently been effected in the *a*-position :—

[1] Small amounts of amino acids appear to be constantly present in normal urine—among these glycine has been definitely established (Embden and Marx).

When large amounts of optically inactive amino acids are given to animals the amino acid excreted usually contains an excess of the active component which does *not* occur naturally in the proteins, i.e. the natural component undergoes oxidation more readily, or is more rapidly assimilated (Wohlgemuth).

Occasionally uramido acids, or their anhydrides, the hydantoins, are formed in the urine of animals which have received amino acids, but in some cases these substances are formed after the urine has been passed by the interaction of amino acids and urea (Lippich, Dakin).

$$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH\,.\,CH_2\,.\,CHNH_2\,.\,COOH + O_2 = \begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH\,.\,CH_2\,.\,COOH + NH_3 + CO_2$$

Leucine — Isovaleric acid

A similar reaction may be brought about by bacteria, or yeasts. Thus, Nencki obtained isovaleric acid by the putrefactive decomposition of leucine, and many similar examples are known (p. 76). Moreover, this change can readily be effected *in vitro*. Thus, hydrogen peroxide oxidizes most *a*-amino acids so as to yield lower fatty acids, ammonia and carbon dioxide (Dakin, Neuberg). In this reaction aldehydes are first formed and they may subsequently undergo further oxidation with formation of acids.[1] Alanine, for example, yields acetaldehyde, acetic acid, ammonia and carbon dioxide, while leucine yields isovaleric aldehyde and isovaleric acid :—

$$CH_3\,.\,CHNH_2\,.\,COOH + O = CH_3\,.\,CHO + NH_3 + CO_2$$

The formation of aldehydes as intermediate steps in the oxidation of *a*-amino acids, has apparently no exact counterpart in animal oxidation, although it appears to occur in certain other biochemical decompositions (cf. p. 78). Instead of an aldehyde being formed in the animal oxidation of *a*-amino acids to lower acids, it appears that *a*-ketonic acids are intermediate products. The first evidence for the belief in the intermediate formation of ketonic acids in the oxidation of *a*-amino acids was furnished by Neubauer's investigations upon the fate in the body of phenyl-*a*-aminoacetic acid [2] (p. 52).

That the *a*-ketonic acids formed by the oxidation of *a*-amino acids may be further oxidized in the body with formation of a lower acid and carbon dioxide has been frequently observed. Thus, Neubauer found that the *a*-ketonic acid corresponding to the amino acid, tyrosine, when given to alcaptonurics, is converted into homogentisic acid :—

$$\underset{\text{(Hydroxyphenylpyruvic acid)}}{C_6H_4OH\,.\,CH_2\,.\,CO\,.\,COOH} \rightarrow \underset{\text{(Homogentisic acid)}}{C_6H_3(OH)_2\,.\,CH_2\,.\,COOH}$$

Similarly p-chlorphenylpyruvic acid gives p-chlorphenylacetic acid, which is excreted in combination with glycine (Friedmann).[3]

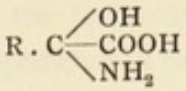
Knoop and Neubauer have suggested that the ketonic acids may

[1] The amino acids also yield aldehydes on oxidation with alloxan (Strecker, Hurtley and Wooton), with lead peroxide and sulphuric acid (Liebig), with sodium hypochlorite (Langheld), and on exposure of their solutions to light in the presence of iron salts (Neuberg).

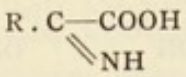
[2] It is of interest to note that K. A. H. Mörner found pyruvic acid, $CH_3\,.\,CO\,.\,COOH$, and possibly propionylformic acid, $CH_3\,.\,CH_2\,.\,CO\,.\,COOH$, among the products of the hydrolysis of various proteins with hydrochloric acid.

[3] The same type of oxidation may be readily brought about *in vitro*. Holleman has shown that the a-ketonic acids are easily oxidized by hydrogen peroxide with formation of carbon dioxide and a lower acid.

be formed from amino acids with intermediate formation of substances of the following type:—



i.e. hydrates of *imino* acids:—



These hydrated imino acids which may be regarded as acid derivatives of aldehyde-ammonia compounds are not known in the free state but undoubtedly would be labile, unstable substances, and on parting with ammonia they would yield ketonic acids.

It appears therefore that a-amino acids frequently undergo oxidation in the animal body in such a way that a-ketonic acids are formed, possibly with intermediate formation of a hydrated imino acid. The a-ketonic acid in turn may be oxidized with liberation of carbon dioxide and formation of a fatty acid with one fewer carbon atom than the original amino acid. The fatty acid may then undergo complete oxidation with formation of carbon dioxide and water (cf. Chap. II.). The changes may be represented as follows:—

$$R.CH_2.CHNH_2.COOH \rightarrow R.CH_2.C(OH)NH_2.COOH$$
$$R.CH_2.C(OH)NH_2.COOH \rightarrow R.CH_2.CO.COOH + NH_3$$
$$R.CH_2.CO.COOH \rightarrow R.CH_2.COOH$$
$$R.CH_2.COOH \rightarrow xCO_2 + yH_2O$$

It will appear later that in many cases there are other alternate methods of decomposition for the *a*-ketonic acids.

Relation of *a*-Amino to *a*-Hydroxy Acids.—It was formerly considered probable that the formation of *a*-hydroxy acids represented the first step in the decomposition of amino acids. This reaction, involving hydrolysis, but not oxidation, is very difficult to imitate directly *in vitro*, except by the use of nitrous acid, and it is doubtful if it occurs in the animal body. But a number of reactions have been observed to take place in the animal body which at first sight appear to be of this type. Thus, Neuberg and Langstein obtained lactic acid from the urine of starving rabbits, when alanine was administered. Embden also obtained lactic acid by perfusing a surviving liver with alanine. Schotten observed the excretion of mandelic acid, following administration of phenyl-aminoacetic acid, while Blendermann believed that p-hydroxyphenyl-*a*-lactic acid was present in the urine of rabbits when they were fed large quantities of tyrosine. The constitution of Blendermann's acid is doubtful however. Paul Mayer found glyceric

acid in the urine of rabbits which had been given *a*-*β*-diaminopropionic acid :—

Amino Acid.	Hydroxy Acid.
Alanine, $CH_3 . CHNH_2 . COOH$	Lactic acid, $CH_3 . CHOH . COOH$
Phenyl-aminoacetic acid, $C_6H_5 . CHNH_2 . COOH$	Mandelic acid, $C_6H_5 . CHOH . COOH$
Tyrosine, $OH . C_6H_4 . CH_2 . CHNH_2 . COOH$	Hydroxyphenyl-*a*-lactic acid, $OH . C_6H_4 . CH_2 . CHOH . COOH$
a-*β*-Diaminopropionic acid, $CH_2NH_2 . CHNH_2 . COOH$	Glyceric acid, $CH_2OH . CHOH . COOH$

But many, if not all of these reactions are undoubtedly indirect, i.e. the hydroxy acids are not formed by immediate hydrolysis of the amino acid, but by the reduction of ketonic acids, or in other ways (see p. 53).

Neubauer's observation that both tyrosine and p-hydroxyphenyl-pyruvic acid yield homogentisic acid when given to an alcaptonuric, while p-hydroxyphenyl-*a*-lactic acid does not, also shows that the *a*-ketonic acids are not readily derived from these hydroxy acids.

Furthermore, Neubauer and Gross found that p-hydroxyphenyl-lactic acid does not readily yield acetoacetic acid, while the corresponding *a*-amino and *a*-ketonic acids (tyrosine and p-hydroxyphenyl-pyruvic acid) yield acetoacetic acid freely. Similarly, Friedmann found that p-chlorphenylalanine and p-chlorphenylpyruvic acid were converted into p-chlorphenaceturic acid,

$$C_6H_4Cl . CH_2 . CO . NHCH_2 . COOH,$$

on oxidation in the body, while p-chlorphenyl-*a*-lactic acid did not yield this oxidation product.

It is concluded therefore that the direct conversion of *a*-amino acids into *a*-hydroxy acids is not a common biochemical reaction.

The Relationships between *a*-Amino and *a*-Ketonic Acids.—O. Neubauer's investigations upon the biochemical oxidation of phenyl-aminoacetic acid, which led to the recognition of the importance of the *a*-ketonic acids in intermediary metabolism require first consideration.

Schotten found, on administering large amounts of phenyl-aminoacetic acid to dogs, that part of the acid was excreted unchanged, while another portion was converted into mandelic acid. Mandelic acid itself is very resistant to further oxidation in the animal body and only yields traces of benzoic acid (hippuric acid) when given to dogs.[1] It appeared at first sight that a simple interchange of (NH_2) for (OH) groups had taken place. On repeating these experiments Neubauer

[1] Schulzen and Graebe erroneously concluded from their earlier experiments that mandelic acid was readily converted into hippuric acid.

found that on administering inactive phenyl-aminoacetic acid the following products were excreted in the urine: phenyl-aminoacetic acid containing an excess of the *lævo*-component, *lævo*-mandelic acid, phenylglyoxylic acid and hippuric acid derived from benzoic acid :—

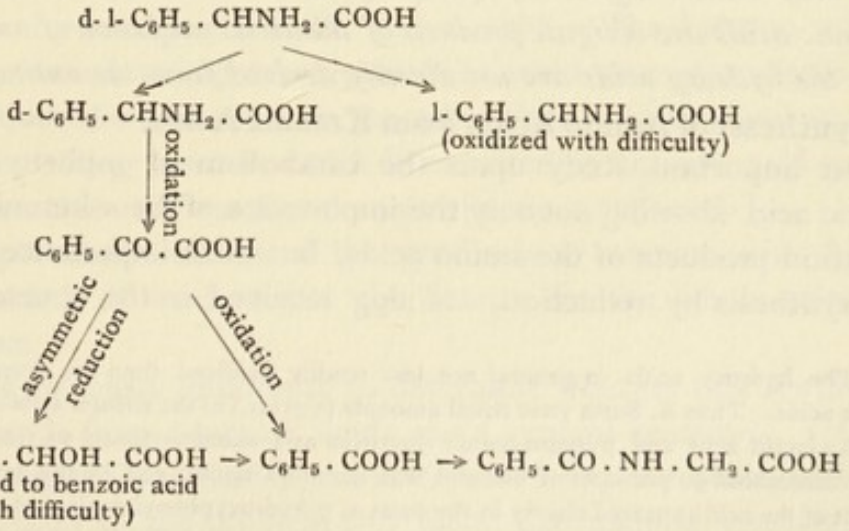
$C_6H_5 . CHNH_2 . COOH$ (d-l-phenyl-aminoacetic acid) $\rightarrow$ { $l\text{-}C_6H_5 . CHNH_2 . COOH$ (l-phenyl-aminoacetic acid); $l\text{-}C_6H_5 . CHOH . COOH$ (l-mandelic acid); $C_6H_5 . CO . COOH$ (Phenylglyoxylic acid); $C_6H_5 . COOH$ (Benzoic acid) }

The excretion of lævo-rotatory phenyl-aminoacetic acid following the administration of the inactive acid made it appear probable that the lævo-component was less readily attacked in the organism, and this was definitely proved by administering the lævo-acid which was mainly excreted unchanged together with traces of hippuric acid. There was no appreciable amount of lævo-mandelic acid compared with the previous experiment, although l-phenyl-aminoacetic acid and l-mandelic acid possess a similar stereochemical configuration.

On feeding the *dextro*-phenyl-aminoacetic acid there was found in the urine in addition to phenylglyoxylic acid, *lævo*-mandelic acid, i.e. an apparent optical inversion had occurred. The explanation of this remarkable result was found in the fact that l-mandelic acid was a secondary product formed by the asymmetric *reduction* of phenylglyoxylic acid.[1]

$$C_6H_5 . CHNH_2 . COOH \xrightarrow{\text{oxidation}} C_6H_5 . CO . COOH \xrightarrow{\text{reduction}} C_6H_5 . CHOH . COOH$$

Special experiments made with phenylglyoxylic acid confirmed the accuracy of the above conclusion.[2] The oxidation of *a*-aminophenyl-acetic acid may be represented as follows :—

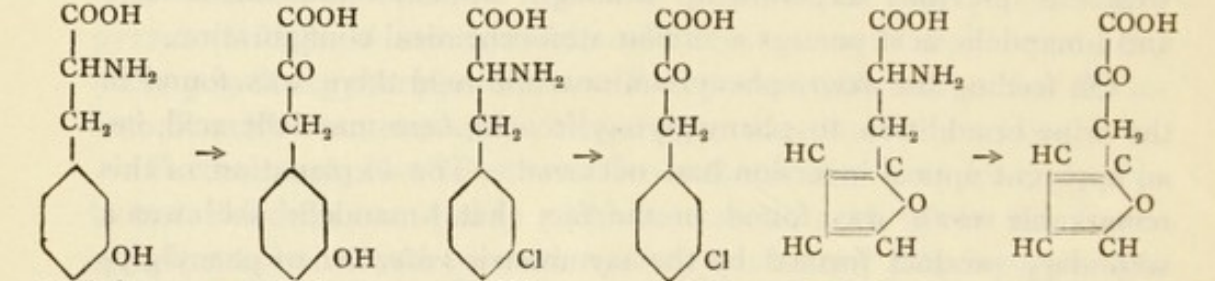


[1] Phenylglyoxylic acid, however derived, is necessarily optically inactive since it contains no asymmetric carbon atom.

[2] Experiments with p-hydroxyphenyl-aminoacetic acid have shown that this acid undergoes oxidation with formation of the corresponding α-ketonic acid, but no reduction of the ketonic acid to para-hydroxymandelic acid could be demonstrated (K. Fromherz).

Neubauer and Fischer by perfusion experiments subsequently showed that the series of changes represented above took place in the liver and might even be demonstrated in minced liver tissue.

Additional examples of the formation of *a*-ketonic acids from *a*-amino acids by oxidation in the body have been furnished by Knoop and by Flatow. Knoop observed the excretion of a ketonic acid on administering γ-phenyl-*a*-aminobutyric acid to dogs and showed that the change was a reversible one. These important results are considered in the following pages. Flatow administered meta-hydroxyphenylalanine (meta-tyrosine), meta-chlorphenylalanine and furylalanine to rabbits; in each case he was able to demonstrate the excretion of ketonic acids. In the case of the first two of the substances the corresponding *a*-ketonic acid was isolated and definitely identified:—



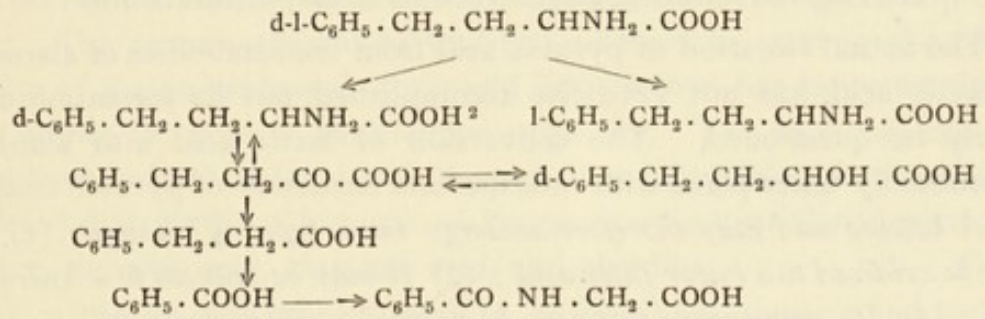
meta-Hydroxy-phenylalanine — meta-Hydroxy-phenylpyruvic acid — meta-Chlor-phenylalanine — meta-Chlor-phenylpyruvic acid — Furylalanine — Furylpyruvic acid

Many further experiments with other ketonic acids show that *in general the fate in the body of a-amino and a-ketonic acids is identical, whereas the a-hydroxy acids being presumably secondary reduction products may behave differently* (cf. p. 51).[1] *It is therefore assumed that a-ketonic acids are obligate products of the direct oxidation of amino acids, while the hydroxy acids are not directly derived from the amino acids.*

Synthesis of Amino Acids from Ketonic Acids.—Knoop has made a most important study upon the catabolism of γ-phenyl-*a*-aminobutyric acid showing not only the importance of the *a*-ketonic acids as oxidation products of the amino acids, but their importance for amino acid synthesis by reduction. A dog received in the course of three

[1] The hydroxy acids in general are less readily oxidized than the corresponding a-ketonic acids. Thus A. Suwa gave equal amounts (2 grms.) of the sodium salts of p-hydroxyphenyl-a-lactic acid and p-hydroxyphenylpyruvic acid subcutaneously to rabbits. In the former case about 90 per cent of the acid was excreted unchanged, in the latter about 14 per cent of the acid appeared chiefly in the form of p-hydroxyphenylacetic acid. Unchanged ketonic acid was not excreted. When administered to man essentially similar results were obtained, but part of the ketonic acid was asymmetrically reduced to the dextro-hydroxy acid. The differences in ease of oxidation are not nearly so marked in the case of phenyllactic and phenylpyruvic acids.

days 18 grms. of the inactive amino acid; in the urine the following substances were recovered: *lævo*-phenyl-α-aminobutyric acid, the acetyl derivative of the *dextro*-phenyl-α-aminobutyric acid, *dextro*-phenyl-α-hydroxybutyric acid, hippuric acid and a residue giving the reaction of an α-ketonic acid. The catabolism of the amino acid evidently was similar to that of phenyl-α-aminoacetic acid, and it appeared likely that the hydroxy acid was formed by the asymmetric reduction of the ketonic acid. This supposition was subsequently confirmed by actually administering the sodium salt of the ketonic acid (12 grms.) to a dog and obtaining the dextro-hydroxy acid (2·5 grms.) from the urine. But in addition 0·44 grm. of the acetyl derivative[1] of *dextro*-phenyl-α-aminobutyric acid was obtained, i.e. a synthesis of the amino acid from the ketonic acid involving reduction had taken place. The various changes may be represented as follows:—



Since administration of γ-phenyl-α-hydroxybutyric acid also gave rise to the excretion of a small amount of the dextro-acetyl derivative of γ-phenyl-α-aminobutyric acid it appears likely that the hydroxy acid may be oxidized in the body to the ketonic acid (a reversible reaction) and so in turn may yield the amino acid. The ketonic acid on further oxidation would be expected to yield phenylpropionic acid. This acid was not isolated from the urine since it readily undergoes β-oxidation yielding benzoic which is excreted as hippuric acid (p. 18). This latter substance was found as previously mentioned.

Knoop's demonstration of the synthesis of α-amino acids from α-ketonic acids is of fundamental significance for the whole science of metabolism.

It was obviously important to determine whether this synthesis of α-amino acids from α-ketonic acids was a general reaction. Utilizing Knoop's suggestion, Embden and Schmitz applied their method of liver perfusion to the problem and found that alanine, phenylalanine, and tyrosine, all important protein constituents, might be synthesized

[1] The mechanism of the acetylation of amino acids is referred to on p. 58.

[2] Excreted in part in form of its acetyl derivative.

in the liver from the ammonium salts of the corresponding ketonic acids :—

$$R.CH_2.CO.COONH_4 \longrightarrow R.CH_2.C\begin{matrix}OH\\COOH\\NH_2\end{matrix} \xrightarrow{\text{Reduction}} R.CH_2.CHNH_2.COOH$$

(Ammonium salt of α-ketonic acid) (Imino-acid hydrate) (Amino acid)

The alanine and tyrosine were optically identical with the amino acids obtained by the hydrolysis of proteins.

Embden, in addition to showing that *d*-alanine might be formed from the ammonium salt of pyruvic acid, when perfused through a surviving liver, showed that ammonium lactate might yield alanine and also that alanine might yield lactic acid. It is evident from these results that the closest possible relation exists between the amino, hydroxy and ketonic acids.

$$CH_3.CHNH_2.COOH \rightleftarrows CH_3.CO.COOH \rightleftarrows CH_3.CHOH.COOH$$

The actual isolation of pyruvic acid from the catabolism of alanine, or lactic acid, has not yet been accomplished, but its formation can hardly be questioned. The conversion of lactic acid into alanine undoubtedly takes place with intermediate formation of pyruvic acid.

A ketonic acid may therefore undergo three types of change : (1) *It may be oxidized to a lower fatty acid ;* (2) *It may be reduced to a hydroxy acid ;* (3) *Its ammonium salt may be reduced to an amino acid.*

(1) $R.CH_2.CO.COOH + O = R.CH_2.COOH + CO_2$
(2) $R.CH_2.CO.COOH + H_2 = R.CH_2.CHOH.COOH$
(3) $R.CH_2.CO.COONH_4 + H_2 = R.CH_2.CHNH_2.COOH + H_2O$

The conditions determining whether oxidation, or reduction, of an *a*-ketonic acid shall occur are but slightly understood. The problem is similar to that of acetoacetic acid which also may undergo either reduction or further oxidation (cf. p. 24). In addition, the synthesis of an amino acid by reduction is clearly dependent upon the presence of an adequate supply of ammonia.

Lactic acid is frequently formed in conditions involving active tissue breakdown combined with insufficient oxidation, such as excessive exercise (Ryffel, Feldman and Hill), or in cases of restricted oxygen supply (Araki). Its origin is readily understood in the light of the foregoing facts.

It is of interest to note that all of the three types of reaction which the *a*-ketonic acids are believed to undergo in the body may be readily imitated *in vitro*. The oxidation of *a*-ketonic acids to lower acids is readily effected with hydrogen peroxide (Hollemann); their reduction to hydroxy acids may be brought about by sodium amalgam and other

reducing agents. The possibility of reducing the ammonium salts of ketonic acids to *a*-amino acids was shown by Knoop, who obtained phenylalanine by the reduction of ammonium phenylpyruvate with sodium amalgam (cf. also Erlenmeyer and Kunlin, and de Jong).

The Relations between the Carbohydrates and the *a*-Amino, Hydroxy, and Ketonic Acids.—The close relation existing between lactic acid with the carbohydrates on the one hand and with alanine on the other led Knoop to surmise that amino acids might be derived indirectly from the carbohydrates and Embden showed this to be the case. On perfusing a liver rich in glycogen it was found that alanine was formed, while, when a glycogen-free liver was used, no appreciable amount of alanine was found. The glycogen was undoubtedly converted into lactic acid, which in turn gave alanine with intermediate formation of ammonium pyruvate. The demonstration of this relationship between the amino acids and the carbohydrate is of great importance. The reverse change to that just referred to, namely the conversion of amino acids and lactic acid into glucose has for some time been known to occur in the diabetic organism. The following amino acids have been shown to yield glucose when administered to animals rendered diabetic by phlorhizin or by pancreas extirpation:[1] glycine, alanine, aspartic acid, glutamic acid, and histidine.

It is not an unlikely supposition to ascribe to pyruvic and lactic acids an important role in the intermediary metabolism involved in the conversion of these amino acids into sugar, but how far the reactions occurring in the diabetic organism resemble the normal changes is an open question.

A number of amino acids, including leucine and tyrosine, apparently do not yield glucose when given to a diabetic animal (Halsey, Ringer and Lusk), but apparently they yield *β*-hydroxybutyric acid and acetoacetic acid, i.e. products of higher fatty acid catabolism (Baer and Blum). The same amino acids yield acetoacetic acid on perfusion through a surviving liver (Embden and others), so that it is possible to make a relatively sharp separation between the amino acids which may yield glucose on the one hand or acetoacetic acid on the other.

The results are recorded in the following table:—

[1] Lusk and his pupils have made the most exact studies upon the conversion of amino acids into glucose, making use of starving dogs rendered fully diabetic by phlorhizin. An increased sugar excretion following administration of glycine, alanine, and asparagine was observed by Embden and Salomon in the case of depancreatized dogs (cf. also Glaesner and Pick, and Höckendorf).

Substance.	Increased Glucose excretion when given to Diabetic Animal.	Acetoacetic Acid formation when perfused through surviving liver.
Glycine [1]	+	−
Alanine	+	−
Valine	?	−
Leucine	−	+
Aspartic acid	+	−
Glutamic acid	+	−
Phenylalanine	?	+
Tyrosine	−	+
Histidine	+	+(?)
Lactic acid	+	−

Acetylation of Amino Acids.—It will be recalled that Knoop found that the amino acid synthesized in the body from γ-phenyl-*a*-ketobutyric acid was excreted in the form of its acetyl derivative :—[2]

$$C_6H_5 . CH_2 . CH_2 . C\begin{cases} H \\ COOH \\ NH . CO . CH_3 \end{cases}$$

(γ-phenyl-α-acetylaminobutyric acid)

A little later Neubauer and Warburg found on perfusing surviving livers with blood containing phenyl-aminoacetic acid that the acetyl derivative of the lævo-amino acid was formed, while subsequently the optical antipode d-phenyl-acetylaminoacetic acid was found by Neubauer and Fromherz among the products of the action of yeast upon the same amino acid. A similar excretion of an optically active acetyl derivative of an amino acid was observed on feeding inactive p-methyl-phenylalanine to an alcaptonuric subject (Dakin). The formation of these acetyl derivatives appears to be of some significance. Knoop from analogy with a reaction observed by Erlenmeyer and Kunlin and by de Jong suggests that acetylation is effected by means of pyruvic acid. When pyruvic acid is mixed with ammonium carbonate, a rise in temperature followed by loss of carbon dioxide occurs and acetylalanine is formed :—

[1] The conversion of glycine into glucose with or without intermediate formation of pyruvic or lactic acid must necessarily be an intricate change involving complex syntheses. A possible clue to the first step in the reaction is found in the fact that glycine on oxidation *in vitro* with hydrogen peroxide yields glyoxylic acid and formaldehyde, both reactive substances capable of undergoing condensation (Dakin). Traces of glyoxylic acid may be formed by simply evaporating an aqueous solution of glycine upon the water-bath, and Neuberg has shown that this type of change is accelerated by the action of sunlight in the presence of uranium salts.

The accuracy of Grube's statement of the formation of glycogen from formaldehyde by direct synthesis in the liver of the tortoise appears doubtful.

[2] The possibility of the occurrence of acetylation in the body had already been shown by the fact that certain foreign aromatic substances are excreted as N-acetyl derivatives, e.g. p-nitrobenzaldehyde gives p-acetylaminobenzoic acid. Moreover, Baumann and Preusse's mercapturic acids are acetylated cysteine derivatives :—

$$R . S . CH_2 . CH . NH . COCH_3 . COOH$$

$$\begin{array}{l} CH_3 . CO . COOH \\ \\ CH_3 . CO . COOH \\ \text{Pyruvic acid} \end{array} + NH_3 \rightarrow \begin{array}{l} CH_3 . CH . COOH \\ | \\ NH \\ | \\ CH_3 . CO \\ \text{Acetylalanine} \end{array} + CO_2 + H_2O$$

This reaction involves a simultaneous oxidation and reduction—one molecule of pyruvic acid is reduced to an amino acid (alanine) while the second yields acetic acid (acetyl group) and carbon dioxide. It is probable that reactions of this type will be found to occur in the animal body for it is becoming increasingly clear that metabolic processes may include many reductions which at first sight might appear unlikely for thermodynamic reasons; the necessary energy being derived from the oxidation of a second molecule of reacting material (cf. "Cannizzaro Reaction," p. 105).

Other Amino Acids.—Brief mention must be made of the oxidation of some other amino acids than those previously referred to:—

Cystine, $\begin{array}{l} S—CH_2 . CHNH_2 . COOH \\ | \\ S—CH_2 . CHNH_2 . COOH \end{array}$

or *cysteine*, $CH_2 . SH . CH . NH_2 . COOH$, the sulphur containing amino acid derived from proteins, when fed to a normal animal undergoes extensive oxidation. J. Wohlgemuth fed cystine to rabbits and showed that not only the inorganic sulphates of the urine was much increased but thiosulphates were also excreted and the "neutral" or "unoxidized" sulphur of the urine was higher than normal. The sulphur content of the bile was also higher than normal. Blum obtained essentially similar results.

Taurine, $CH_2 . NH_2 . CH_2 . SO_3H$, which is found in the bile combined with cholic acid as taurocholic acid, appears to be derived from cysteine. G. von Bergmann found that on giving cystine to dogs provided with a biliary fistula the taurine of the bile was not increased but by previously administering excess of sodium cholate to the animal, taurine amounting to twice the normal quantity was excreted. Taurine must therefore be regarded as a product of cystine metabolism. E. Friedmann had previously shown the possibility of converting cystine into taurine by purely chemical methods. Cystine (cysteine) on oxidation with bromine yields cysteic acid, which on heating with water to 240° loses carbon dioxide with formation of taurine:—

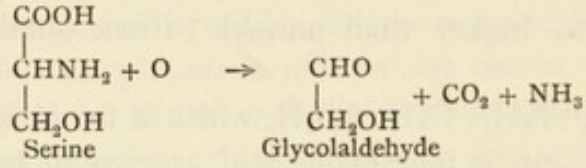
$$\begin{array}{l} COOH \\ | \\ CHNH_2 \\ | \\ CH_2(SH) \\ \text{(Cysteine)} \end{array} \rightarrow \begin{array}{l} COOH \\ | \\ CHNH_2 \\ | \\ CH_2(SO_3H) \\ \text{(Cysteic acid)} \end{array} \rightarrow \begin{array}{l} CH_2 . NH_2 \\ | \\ CH_2(SO_3H) \\ \text{(Taurine)} \end{array}$$

There is no evidence to show that the whole of the cysteine supplied to the body is eventually converted into taurine, and indeed such a suggestion appears improbable.[1]

The sulphur of cystine is readily removed by oxidation *in vitro*. Breinl and Baudisch oxidized cystine with hydrogen peroxide and obtained the whole of the sulphur in the form of sulphuric acid. On complete oxidation with alkaline permanganate cysteine gives oxalic, sulphuric, acetic and carbonic acids, ammonia and free sulphur. Pyruvic acid appears to be an intermediate product (Denis). Cystine may be converted into pyruvic acid by a variety of chemical methods (Baumann, Dewar and Gamgee). It is possible that this relationship may have biochemical significance, but we have no direct evidence upon the subject.

An interesting metabolic abnormality is found in the condition known as cystinuria in which the patient excretes considerable quantities of cystine. Apparently cystinurics are capable of oxidizing cystine when administered as such, but for some not yet understood reason, they fail to fully catabolize the cystine arising in the course of ordinary endogenous metabolism.[2]

Nothing definite is known of the fate in the animal body of serine, the oxygen analogue of cystine. On exposure of its aqueous solution to sunlight or on oxidation with hydrogen peroxide, serine yields glycollic aldehyde—the simplest sugar (Neuberg).



Paul Meyer is inclined to the belief that glycollic aldehyde undergoes condensation in the body with formation of glucose, since the latter sugar is excreted when large quantities of glycollic aldehyde are given to rabbits. It is probable but not yet proved that serine may take part in sugar synthesis in the body.

It was previously mentioned that aspartic and glutamic acids when given to diabetic animals lead to the excretion of considerable amounts of glucose. The changes leading to sugar synthesis are not understood.

[1] E. Salkowski found that on administering taurine to rabbits the excretion of sulphates and especially "neutral" sulphur was increased; thiosulphates were also present. These results were not observed when taurine was fed to man or to dogs.

[2] This subject cannot be further discussed here. The reader is referred to Garrod's "Inborn Errors of Metabolism". Oxford Medical Publications, 1909.

It has been suggested that lactic acid may be an intermediate step but this is purely hypothetical. The intermediate steps in the catabolism of aspartic and glutamic acids in the normal animal are unknown.[1]

The Diamino Acids and Histidine.—The mode of catabolism of the diamino acids is very obscure. Arginine undoubtedly first of all undergoes hydrolysis by the enzyme arginase yielding urea and ornithine (Kossel and Dakin).

$$\underset{\text{Arginine}}{H_2N.C(=NH).NH.CH_2.CH_2.CH_2.CHNH_2.COOH} + H_2O$$
$$= NH_2.CO.NH_2 + \underset{\text{Ornithine}}{H_2N.CH_2.CH_2.CH_2.CHNH_2.COOH}$$

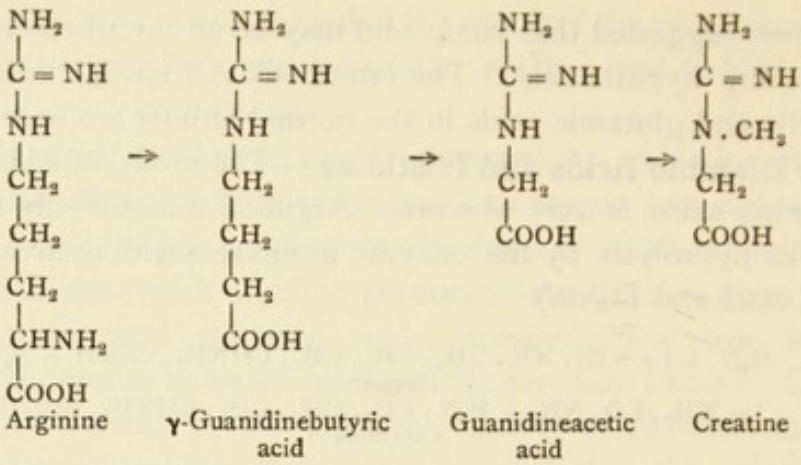
W. H. Thompson has shown that when arginine is administered to a dog an amount of urea corresponding to that liberated by the action of arginase is rapidly excreted in the urine, while an additional quantity presumably derived from the ornithine is more slowly excreted. The intermediate steps in the catabolism of both ornithine and its homologue, lysine, are unknown. Cystinuric patients are apt to excrete tetramethylenediamine and pentamethylenediamine in the urine, and these substances are undoubtedly derived from lysine and ornithine, but it is improbable that these bases represent obligate steps in the normal catabolism of the diamino acids:—

$$\underset{\text{Lysine}}{H_2N.CH_2.CH_2.CH_2.CH_2.CHNH_2.COOH}$$
$$= \underset{\text{Pentamethylenediamine}}{H_2N.CH_2.CH_2.CH_2.CH_2.CH_2.NH_2} + CO_2$$

Attention may be drawn to a suggestion as to the possible origin of creatine from arginine. It is possible to oxidize arginine to γ-guanidinebutyric acid *in vitro* with barium permanganate (Kutscher),[2] and such a change might well occur in the living organism. Guanidinebutyric acid on further oxidation in the body would probably yield guanidineacetic acid through β-oxidation. The latter substance is closely related to creatine, its methyl derivative. According to Jaffé and also Dorner methylation of guanidineacetic acid with formation of creatine may actually take place in the body.

[1] Jastrowitz has shown that a small increase in the oxalic acid in the urine is found after administering large amounts of aspartic and glutamic acids to dogs, but the result is of doubtful significance.

[2] On futher oxidation guanidine and succinic acid are obtained (Bénech & Kutscher).



It must be admitted, however, that no satisfactory evidence has yet been adduced of the actual formation in the animal body of creatine from arginine.

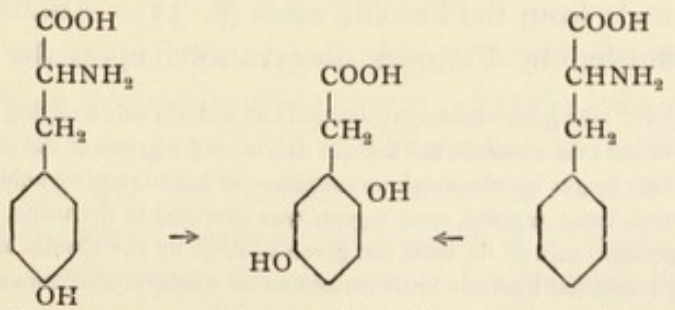
Abderhalden and Einbeck and also K. Kowalevsky administered large quantities of histidine to dogs without detecting in the urine any definite product of catabolism other than urea. The excretion of allantoine, purine bases, and uric acid was not materially influenced. Judging by analogy with the other cyclic amino acids, it is probable that the ring undergoes disruption with formation of aliphatic substances capable of undergoing further oxidation. Dakin and Wakeman found that, on perfusing dogs' livers with blood containing histidine carbonate, a slightly increased formation of acetoacetic acid occurred. It is possible, but not proved, that histidine may undergo decomposition in the liver by a reaction resembling the formation of acetoacetic acid from tyrosine (p. 66).

When given to dogs rendered diabetic with phlorizin a large increase in sugar excretion followed, while the increased excretion of acetoacetic acid was much less marked (Dakin and Wakeman).

II. THE OXIDATION OF PHENYLALANINE, TYROSINE, TRYPTOPHANE AND OTHER RELATED AROMATIC SUBSTANCES.

Most aromatic substances do not readily undergo complete oxidation in the animal body, the benzene nucleus usually remaining intact. The naturally occurring aromatic amino acids, derived from proteins, phenylalanine, tyrosine and tryptophane, however, readily undergo oxidation which involves the disintegration of the benzene ring. The reason for this difference is simply a structural one. It will be seen later that only those aromatic substances possessing a side-chain of certain structure undergo oxidation in the animal body with ease.

In considering the nature of the reactions which phenylalanine and tyrosine are likely to undergo in the animal body much attention has been given to the phenomena presented by the curious metabolic anomaly known as alcaptonuria. In this condition the human organism loses its customary ability to oxidize phenylalanine and tyrosine completely, but instead converts it into homogentisic acid (2·5, dihydroxyphenylacetic acid). The constitution of this acid was determined by Wolkow and Baumann; they also proved its origin from tyrosine and suspected that phenylalanine might also yield it. Later, Falta and Langstein definitely proved the conversion of phenylalanine into homogentisic acid. Tryptophane, on the other hand, when fed to an alcaptonuric does not yield homogentisic acid (Garrod, Neubauer).



Tyrosine → Homogentisic acid ← Phenylalanine
2·5, Dihydroxyphenylacetic acid

Homogentisic acid is oxidized moderately readily by the normal

organism[1] but not by the alcaptonuric. It therefore seemed not improbable that homogentisic acid was a normal product of tyrosine and phenylalanine catabolism and that the peculiarity of alcaptonuria consisted in failure to carry the oxidation of homogentisic acid to completion. The acceptance of the view that homogentisic acid was a normal intermediary product of phenylalanine and tyrosine catabolism, was more general when Embden, Solomon and Schmidt showed that phenylalanine, tyrosine, and homogentisic acid all gave large amounts of acetoacetic acid when perfused through a surviving liver.[2] It was assumed that tyrosine and phenylalanine were converted into homogentisic acid which in turn gave acetoacetic acid, the latter finally undergoing complete oxidation. There are, however, a number of objections to this assumption, and the writer is of opinion that the path of phenylalanine and tyrosine catabolism does not lie by way of homogentisic acid. The opposite view is, however, very commonly held.

Decomposition of Phenylalanine and Tyrosine in the Normal Organism.—Judging by analogy from the experiments of Neubauer and Knoop upon the catabolism of the phenyl derivatives of *a*-aminoacetic and *a*-aminobutyric acids, it is surmised that the first products of tyrosine and phenylalanine catabolism are the corresponding *a*-ketonic acids: phenylpyruvic acid, $C_6H_5.CH_2.CO.COOH$ and p-hydroxyphenylpyruvic acid, $C_6H_4OH.CH_2.CO.COOH$. The formation of these acids has not been actually proved by direct isolation of the substances but there is excellent indirect evidence available. Thus, both of these acids undergo ready oxidation in the animal body with breaking up of the benzene nucleus, both of them yield homogentisic acid when fed to an alcaptonuric patient, and both of them readily yield acetoacetic acid when their salts are perfused through a surviving liver (Schmitz, Neubauer and Gros). In all of these respects they resemble phenylalanine and tyrosine from which they may be derived. Furthermore, Embden has demonstrated the reverse change, namely the formation of the amino acids from the ketonic acids (p. 55). Additional indirect evidence is afforded by Flatow's observations upon the excretion of

[1] H. Embden gave 5·65 grms. homogentisic acid as sodium salt to a dog by subcutaneous injection and recovered 1·82 grms. in the urine. On taking 4 grms. of the acid slowly in the course of twenty-four hours he observed no excretion of homogentisic acid in his own case but on taking 8 grms. more rapidly, over 1 grm. was excreted in the urine.

When homogentisic acid or its salts are given to dogs by the mouth, much of the acid is decomposed by intestinal bacteria with formation of methylhydroquinone (Wolkow and Baumann).

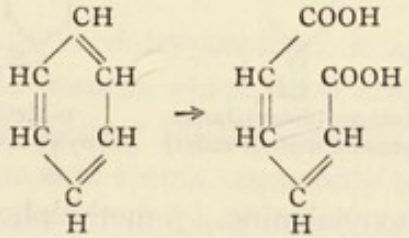
[2] Baer and Blum have shown that when phenylalanine and tyrosine are fed to diabetic patients the excretion of *β*-hydroxybutyric acid, acetoacetic acid, and acetone may be increased.

the corresponding *a*-ketonic acids on feeding meta-hydroxyphenylalanine, meta-chlorphenylalanine and furylalanine to rabbits (p. 54).

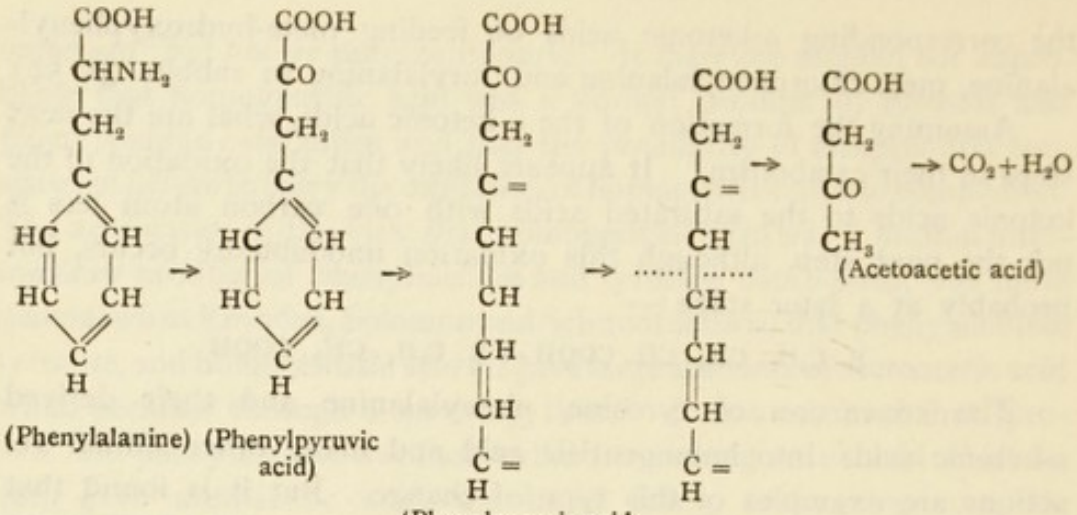
Assuming the formation of the *a*-ketonic acids, what are the next steps in their catabolism? It appears likely that the oxidation of the ketonic acids to the saturated acids with one carbon atom less is not the next step, although this oxidation undoubtedly occurs, but probably at a later stage:—

$$R.C_6H_4.CH_2.CO.COOH \rightarrow R.C_6H_4.CH_2.COOH$$

The conversion of tyrosine, phenylalanine and their derived *a*-ketonic acids into homogentisic acid and many other similar reactions are examples of this type of change. But it is found that both phenylacetic acid and p-hydroxyphenylacetic acid, which would be formed by the oxidation of the ketonic acids as suggested, are entirely resistant to further oxidation in the animal body. They do not yield homogentisic acid when administered to an alcaptonuric nor acetoacetic acid when perfused through a surviving liver, but when fed to animals are excreted unoxidized chiefly in combination with glycine (E. & H. Salkowski). They cannot therefore be products of phenylalanine or tyrosine catabolism. We are now confronted with the problem of picturing the conversion of phenylpyruvic acid and p-hydroxyphenylpyruvic acid into acetoacetic acid without prior oxidation of the side-chain. It appears probable that the next change involves an opening of the benzene ring. An analogy for this somewhat surprising reaction is found in the observation of Jaffé that small quantities of muconic acid could be isolated from the urine of dogs which had been given benzene (p. 102):—

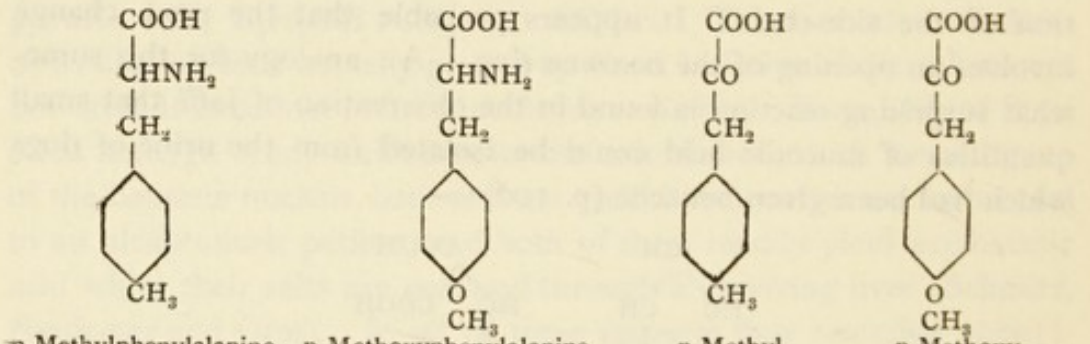


A clear picture of the steps involved in the formation of acetoacetic acid from phenylpyruvic acid or p-hydroxyphenylpyruvic acid is at present lacking, but Dakin and Wakeman have adduced evidence tending to show that the acetoacetic acid molecule is formed at the expense of two carbon atoms of the nucleus and two of the side-chain. These four carbon atoms are printed in heavy type in the following formulæ:—



The evidence upon which this conclusion is based is largely indirect and can only be reproduced in part. The following points may be mentioned :—

(*a*) Substitution of phenylalanine or tyrosine in the para-position does not necessarily interfere with its complete oxidation in the animal body. Thus p-methylphenylalanine and p-methoxyphenylalanine (tyrosine methyl ether) are readily oxidized when fed to animals.



(*b*) Para-methylphenylalanine, p-methylphenylpyruvic acid, p-methoxyphenylalanine and p-methoxyphenylpyruvic acid all yield acetoacetic acid when perfused through a surviving liver.

It may be inferred from these results firstly that phenylalanine does not necessarily undergo nuclear hydroxylation with formation of tyrosine or a derivative of it; secondly, substances of quinonoid structure which are believed to be necessary precursors of homogentisic acid (p. 69) are not necessarily formed in the catabolism of the aromatic amino and ketonic acids which undergo complete oxidation in the body,

since none of the four previously mentioned substances are capable of forming para-quinonoid derivatives.[1]

(*c*) *It appears that only those aromatic amino and ketonic acids are capable of yielding acetoacetic acid when perfused through a surviving liver which possess the side-chain—*

$$-CH_2 . \overset{|}{\underset{|}{C}} . COOH$$

It is essential that the hydrogen of the CH_2 group adjacent to the nucleus be unsubstituted. Thus phenylserine, $C_6H_5 . CHOH . CHNH_2 . COOH$, although structurally so similar to phenylalanine, is converted into hippuric acid in the animal body (Dakin). A consideration of its formula will show that it cannot yield acetoacetic acid in the same way as phenylalanine and tyrosine do, if the scheme suggested for the catabolism of the latter substance be correct.[2]

(*d*) If the acetoacetic acid be formed from phenylalanine and tyrosine in the manner indicated, an adequate explanation is afforded of the striking differences in the behaviour of other aromatic substances. The reason of the failure of phenylaminoacetic acid, γ-phenyl-α-aminobutyric acid and their derivatives to undergo complete oxidation in the body may reasonably be referred to the structural impossibility of their yielding acetoacetic acid along the lines believed to represent phenylalanine catabolism. Similarly, the phenyl-fatty acids, which when possible undergo β-oxidation, can neither yield acetoacetic acid nor undergo complete oxidation. Furthermore, the failure of tryptophane to yield acetoacetic acid, although it does apparently undergo complete oxidation in the animal body, may be readily explained.

(*e*) With the exception of tryptophane it appears that practically only those aromatic substances which form acetoacetic acid when perfused through a surviving liver readily undergo complete oxidation in the body. Acetoacetic acid seems commonly to be an obligate stage in the complete oxidation of simple aromatic substances.[3]

[1] Friedmann and Maase fed p-chlorphenylalanine and p-chlorphenylpyruvic acid to dogs, and finding p-chlorphenacetic acid in the urine inferred that the formation of quinonoid substances was necessary for phenylalanine and tyrosine catabolism. This conclusion is open to question since the yield of p-chlorphenacetic acid did not account for more than 21-36 per cent. of the substance fed. Moreover, part of the p-chlorphenylacetic acid may be formed by putrefactive decomposition in the intestine.

[2] Baumann has stated that α-aminocinnamic acid undergoes complete oxidation in the animal body. This statement is, however, incorrect and apt to lead to confusion. The substance fed by Baumann, prepared by Plöchl's method and believed by him to be α-aminocinnamic acid was found afterwards by Erlenmeyer and by Kunlin to be phenylalanine.

[3] It is of interest to recall the many syntheses *in vitro* of aromatic substances from acetoacetic acid and its derivatives.

Assuming that acetoacetic acid is formed from phenylalanine and tyrosine in the manner suggested, the fate of only five out of the nine carbon atoms in phenylalanine and tyrosine has been accounted for—one present in the carboxyl group appearing as carbon dioxide and four as acetoacetic acid. The disposition of the other four is quite unknown. It is conceivable that another molecule of acetoacetic acid may be formed. The acetoacetic acid formed from the catabolism of phenylalanine and tyrosine would normally undergo further oxidation in the usual fashion, eventually giving carbon dioxide and water (cf. p. 31).

Further investigation of the detailed mechanism of the catabolism of aromatic amino acids is very desirable.

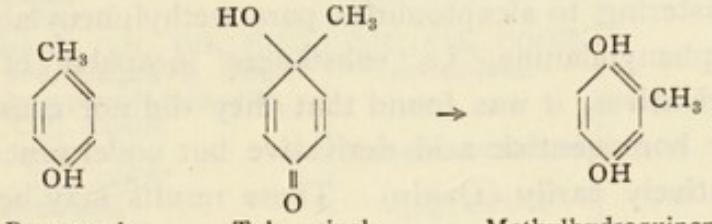
Homogentisic Acid Formation.—The chemical reactions necessary for the conversion of phenylalanine, or tyrosine, into homogentisic acid are rather complex (formulæ, p. 69). The oxidation of the side-chain with loss of one carbon atom is readily intelligible, and, as previously mentioned, there is little doubt that an *a*-ketonic acid is formed as an intermediate product:—

$$-CH_2.CHNH_2.COOH \rightarrow -CH_2.CO.COOH \rightarrow -CH_2.COOH$$

Homogentisic acid has two hydroxyl groups in positions (2) and (5), while the hydroxyl group in tyrosine is in position (4), and the nucleus of phenylalanine is unsubstituted. Baumann and Wolkow were inclined to assume that the hydroxyl group of tyrosine was reduced by a special micro-organism in the intestine of the alcaptonuric and that subsequently two hydroxyl groups were introduced in positions (2) and (5). This unlikely hypothesis was disapproved by Abderhalden, Bloch and Rona's observation that tyrosine injected subcutaneously in the form of glycyl-tyrosine was converted into homogentisic acid. Blum has shown that of the three *o*, *m*, and *p* hydroxyphenylalanines only the para-compound (tyrosine) yields homogentisic acid, and Neubauer found the same to be true of the three hydroxyphenylpyruvic acids. *The conversion of phenylalanine into homogentisic acid cannot, therefore, take place by direct hydroxylation of the nucleus in positions* (2) *and* (5). But Neubauer found that 2·5 dihydroxyphenylpyruvic acid readily gave homogentisic acid when given to an alcaptonuric and hence might be regarded as a probable precursor of the latter substance. It was necessary, therefore, to assume that the hydroxylation of the nucleus was effected by some molecular rearrangement involving a shifting of the relative positions of the (OH) group and the side-chain in the tyrosine molecule.

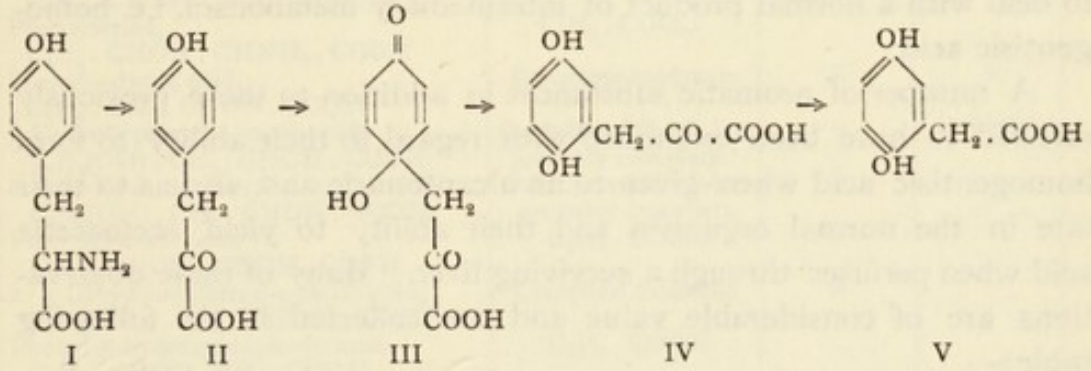
Analogies for this type of change were to be found in the investiga-

tions of Bamberger, Kumagai and Wolffenstein, Zincke, Auwers and others upon substances of quinonoid structure, obtained by the oxidation of phenols, which readily undergo intramolecular rearrangement. For example, p-cresol on oxidation with potassium persulphate yields "tolu-quinol" which readily passes over into methylhydroquinone:—



Paracresol Tolu-quinol Methylhydroquinone

It was suggested by E. Meyer, and later by Friedmann and by Neubauer, that a reaction of this type was probably concerned with homogentisic acid formation. Neubauer has put forward the view that tyrosine (I) is converted first of all into hydroxyphenylpyruvic acid, (II) which on oxidation gives a substance of quinonoid structure, (III); the latter body then undergoes intramolecular rearrangement with formation of 2·5 dihydroxyphenylpyruvic acid, (IV). The oxidation of the latter substance to homogentisic acid, (V) is readily understood.



Phenylalanine is pictured by Neubauer as undergoing a similar set of changes, either by being converted into tyrosine by direct hydroxylation of the nucleus[1] or into phenylpyruvic acid, which on further oxidation gives p-hydroxyphenylpyruvic acid. The latter substance is then converted into homogentisic acid as in the case of tyrosine oxidation.

Neubauer's theory of homogentisic acid formation accords with all the known facts. Each of the substances assumed to be intermediate products (with the exception of the substance of quinonoid structure

[1] This change has not yet been shown to occur in the normal organism. Intravenous injection of phenylalanine to cats failed to evoke an excretion of any phenolic substance, although much unchanged phenylalanine and phenyl-α-uramido-propionic acid were present in the urine (Dakin).

which has not been isolated and which undoubtedly would be very unstable) has been shown to yield homogentisic acid when given to an alcaptonuric patient. It must be admitted, however, that chemical analogy for the "wandering" of the CH_2. CO. COOH group is lacking.[1]

On administering to alcaptonurics para-methylphenylalanine and para-methoxyphenylalanine, i.e. substances incapable of forming quinonoid derivatives, it was found that they did not cause the excretion of any homogentisic acid derivative but underwent complete oxidation relatively easily (Dakin). These results may be taken as evidence in favour of the view that a quinonoid substance is a necessary precursor of homogentisic acid, and furthermore that alcaptonurics have not lost the power to effect complete oxidation of phenylalanine and tyrosine derivatives provided their structure is such that homogentisic acid formation is prevented. Alcaptonuria, according to this view, represents a condition in which there is not only an abnormal formation of homogentisic acid but also an abnormal failure to catabolize it when formed.[2] Neubauer and many others, including Garrod, on the other hand, incline to believe that alcaptonuria represents a failure to deal with a normal product of intermediary metabolism, i.e. homogentisic acid.

A number of aromatic substances in addition to those previously referred to have been examined with regard to their ability to form homogentisic acid when given to an alcaptonuric and also as to their fate in the normal organism and their ability to yield acetoacetic acid when perfused through a surviving liver. Many of these observations are of considerable value and are collected in the following table :—

[1] Although p-cresol on oxidation with potassium persulphate gives methylhydroquinone, the methyl group changing its position, the writer was unable to observe the analogous formation of homogentisic acid on oxidizing p-hydroxyphenylacetic acid (unpublished experiments).

[2] Neubauer and Falta have made some comparative observations upon the fate of gentisic acid (2·5 dihydroxybenzoic acid), 2·4 dihydroxybenzoic acid, 3·4 dihydroxybenzoic acid and caffeic acid, 3·4 dihydroxycinnamic acid, in the normal and alcaptonuric organism. The results appeared to show a somewhat diminished capacity of the alcaptonuric for the oxidation of the two first of these substances.

Substance.	Fate in the Normal Organism.[1]	Homogentisic Acid Formation when given to Alcaptonuric.	Acetoacetic Acid Formation when Perfused through Surviving Liver.
Phenylethylalcohol, $C_6H_5 . CH_2 . CH_2OH$	$C_6H_5 . CH_2 . COOH$	–	
Phenylacetaldehyde, $C_6H_5 . CH_2 . CHO$	$C_6H_5 . CH_2 . COOH$		
Phenylacetic acid, $C_6H_5 . CH_2 . COOH$	not oxidized	–	
o- *m-* & *p*-Hydroxyphenylacetic acids, $C_6H_4(OH) . CH_2 . COOH$	,, ,,		
Homogentisic acid, $C_6H_3(OH)_2 . CH_2 . COOH$	Complete oxidation	+	+
Phenylaminoacetic acid, $C_6H_5 . CHNH_2 . COOH$	$C_6H_5 . COOH$	–	–
Phenylpropionic acid, $C_6H_5 . CH_2 . CH_2 . COOH$	$C_6H_5 . COOH$	–	–
Cinnamic acid, $C_6H_5 . CH = CH . COOH$	$C_6H_5 . COOH$	–	
Phenylalanine, $C_6H_5 . CH_2 . CHNH_2 . COOH$	Complete oxidation	+	+
Tyrosine, $C_6H_4OH . CH_2 . CHNH_2 . COOH$	,, ,,	+	+
3 · 5 Diiodotyrosine, $C_6H_2I_2(OH) . CH_2 . CHNH_2 . COOH$	,, ,,		–[2]
p-Methylphenylalanine, $C_6H_4(CH_3)CH_2 . CHNH_2 . COOH$	,, ,,	Complete oxidation	+
p-Methoxyphenylalanine, $C_6H_4 . (OCH_3) . CH_2 . CHNH_2 . COOH$	,, ,,	Complete oxidation	+
Phenyl-*β*-alanine, $C_6H_5 . CHNH_2 . CH_2 . COOH$	Not oxidized readily		
Phenylserine, $C_6H_5 . CHOH . CHNH_2 . COOH$	$C_6H_5 . COOH$		
Phenyl-*α*-lactic acid, $C_6H_5 . CH_2 . CHOH . COOH$	Complete oxidation	+	+
p-Hydroxyphenyl-*α*-lactic acid, $C_6H_4(OH) . CH_2 . CHOH . COOH$	Oxidation difficult but probably complete[3]	–	Doubtful
o- & *m*-Hydroxyphenyl-*α*-lactic acid, $C_6H_4(OH) . CH_2 . CHOH . COOH$	Oxidation difficult but probably complete	–	
Phenylglyceric acid, $C_6H_5 . CHOH . CHOH . COOH$	$C_6H_5 . COOH$	–	
2 · 5 Dihydroxyphenyl-*α*-lactic acid, $C_6H_3(OH)_2 . CH_2 . CHOH . COOH$	Complete oxidation	+	
Phenyl-*β*-hydroxypropionic acid, $C_6H_5 . CHOH . CH_2 . COOH$	$C_6H_5 . COOH$		
o- *m-* & *p*-Hydroxyphenylpropionic acids, $C_6H_4OH . CH_2 . CH_2 . COOH$	$C_6H_4(OH) . COOH$		
Phenylpyruvic acid, $C_6H_5 . CH_2 . CO . COOH$	Complete oxidation	+	+
p-Hydroxyphenylpyruvic acid, $C_6H_4(OH) . CH_2 . CO . COOH$	,, ,,	+	+
o- & *m*-Hydroxyphenylpyruvic acid, $C_6H_4(OH) . CH_2 . CO . COOH$	,, ,,	–	
p-Methylphenylpyruvic acid, $C_6H_4(CH_3) . CH_2 . CO . COOH$	,, ,,		+
p-Methoxyphenylpyruvic acid, $C_6H_4 . (OCH_3) . CH_2 . CO . COOH$	,, ,,		+
2 · 5 Dihydroxyphenylpyruvic acid, $C_6H_3(OH)_2 . CH_2 . CO . COOH$	,, ,,	+	

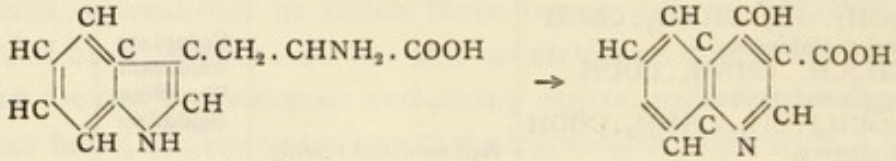
[1] End-products of oxidation are alone considered. Substances excreted in combination with glycine, etc., are recorded in the Table in the uncombined form.

[2] Unpublished observation.

[3] i.e. The aromatic nucleus of that part of the acid which undergoes oxidation is destroyed. No benzoic acid is formed.

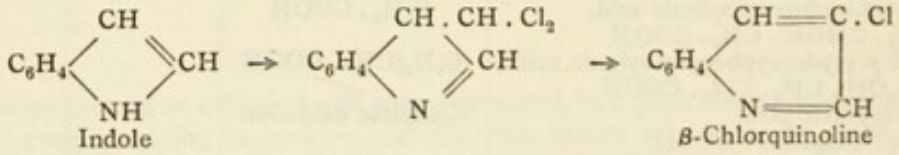
Oxidation of Tryptophane.—Tryptophane, i.e. indole-*a*-aminopropionic acid, undergoes metabolism in the animal body along quite different lines from those followed by tyrosine and phenylalanine. In the human organism it apparently undergoes complete oxidation. No indole derivatives are found in the urine except such as are due to putrefactive decomposition of the amino acid in the intestine, prior to absorption. Neither does tryptophane yield homogentisic acid when given to an alcaptonuric (Garrod, Neubauer) nor give acetoacetic acid when perfused through a surviving liver. The mechanism of the changes involved in the complete oxidation of tryptophane is completely unknown.

When tryptophane is fed to dogs Ellinger noted an increase in the normal excretion of kynurenic acid and was also able to induce the formation of the same substance in rabbits. Kynurenic acid has been shown by Camps to be γ-hydroxyquinoline-β-carboxylic acid :—



Tryptophane → Kynurenic acid

The conversion of tryptophane into kynurenic acid requires the entrance of an additional carbon atom into the indole ring with formation of a quinoline nucleus. This remarkable type of reaction has often been observed *in vitro*. Thus Ellinger obtained β-chlorquinoline together with indole aldehyde by the action of chloroform and potash upon indole (Tiemann-Reimer reaction) and similar results were obtained by Ciamician and co-workers with alkyl indole derivatives [1] (Magnanini).

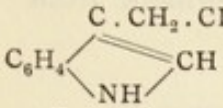


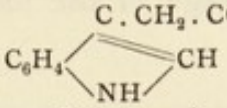
Indole → → β-Chlorquinoline

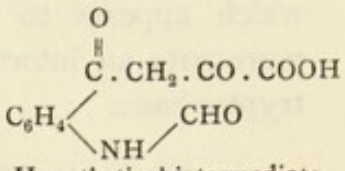
It is possible to picture the conversion of tryptophane into kynurenic acid in many different ways. The following scheme is put forward in a purely tentative way pending further investigations. Judging by analogy with other amino acids one may suppose indylpyruvic acid to be the first product of tryptophane catabolism. On further oxidation the pyrrole ring may be opened at the double linkage. The quinoline nucleus is then formed by incorporating the β-carbon atom

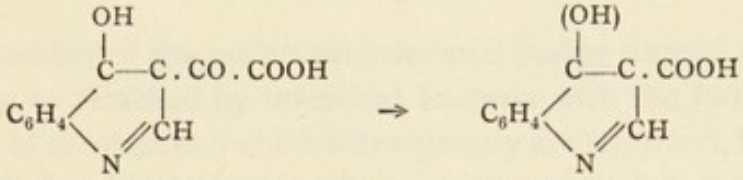
[1] Abderhalden and Kempe have described a by-product obtained in the preparation of tryptophane which they consider to be a hydroxytryptophane of unknown structure. On evaporation with hydrochloric acid followed by heating the odour of quinoline was observed. The reaction requires further study.

of the side-chain, while, either before or after this stage has been reached, the CO . COOH group is oxidized to COOH :[1]


Tryptophane


Indylpyruvic acid


Hypothetical intermediate product

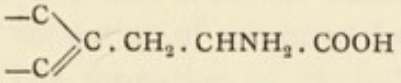

Quinoline-γ-hydroxy-β-glyoxylic acid → Kynurenic acid

It is doubtful if kynurenic acid is produced in the intermediary metabolism of those animals which do not normally excrete it. When given by mouth to man little or none is excreted in the urine, but if the acid be given subcutaneously it may be excreted in considerable amount. When given to rabbits and dogs by the mouth only part reappears in the urine. Generally speaking kynurenic acid appears to undergo oxidation in the body with some difficulty (Solomin, Hauser, A. Schmidt).

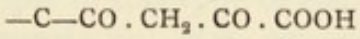
Nothing definite is known of any intermediate products of normal tryptophane catabolism other than kynurenic acid.

Eppinger has recently made an interesting observation in a case

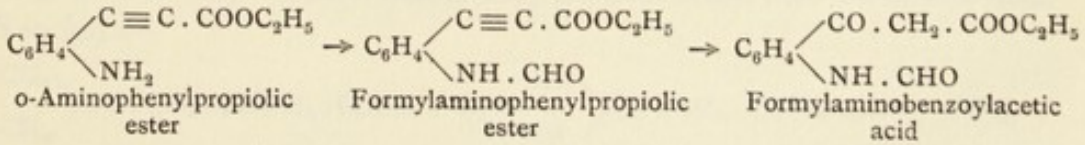
[1] Ellinger considers indole glyoxylic acid a possible intermediary product. The above representation is preferred partly because of its close analogy with the decomposition of phenylalanine and tyrosine with formation of acetoacetic acid (p. 66). In each case

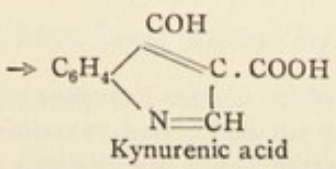


is converted into



with opening of the ring at the double linkage. Moreover, the suggested mode of formation of the quinoline ring very closely resembles Camp's synthesis of kynurenic acid in which o-aminophenylpropiolic ester was condensed with formic acid and the resulting formyl derivative then boiled with water :—


o-Aminophenylpropiolic ester → Formylaminophenylpropiolic ester → Formylaminobenzoylacetic acid


→ Kynurenic acid

of melanuria upon the dependence of pigment formation upon tryptophane. He also succeeded in isolating a substance from the urine which appears to be a pyrrole derivative and which apparently represents an intermediate stage in the formation of pigment from tryptophane.

III. THE OXIDATION AND REDUCTION OF AMINO ACIDS BY MICRO-ORGANISMS.

Since a number of the amino acids formed during normal intestinal digestion may be attacked by intestinal bacteria with the formation of various types of substances [1] which subsequently are absorbed, it seemed proper to include a brief account of the chief types of decomposition of the amino acids by micro-organisms.

Most of the products of the bacterial decomposition of the amino acids were isolated in the first instance from mixtures obtained by allowing proteins to undergo putrefaction. As a more exact knowledge of the structure of the amino acids was acquired, the probable origin of most of these decomposition products became clear and experiments were then made upon the decomposition of the amino acids themselves.

The amino acids derived from proteins furnish bacteria, moulds, yeasts and other vegetable forms with a readily available source of nitrogen. The chemical changes involved depend largely upon the character of the organism, the condition of growth especially with regard to the presence or absence of oxygen and the available sources of nutriment other than the amino acids.

In general it may be said that anærobic bacteria are prone to *reduce* *a*-amino acids with formation of saturated fatty acids and liberation of ammonia (I). The formation of phenylpropionic acid from phenylalanine is an example of this type of change :—

$$C_6H_5 . CH_2 . CHNH_2 . COOH + H_2 = C_6H_5 . CH_2 . CH_2 . COOH + NH_3$$

Aerobic bacteria more frequently *oxidize* the *a*-amino acid to a fatty acid containing one fewer carbon atom, carbon dioxide and ammonia being set free (II). Thus leucine may be converted into isovaleric acid (Nencki) :—

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!\rangle CH . CH_2 . CHNH_2 . COOH + O_2 = \begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!\rangle CH . CH_2 . COOH + NH_3 + CO_2$$

Yeasts, on the other hand, have been shown by F. Ehrlich to convert

[1] Ackermann and Kutscher have suggested the use of the word " Aporrhegma " as a class designation for the various substances derived from the decomposition of amino acids by physiological processes by both vegetable and animal organisms.

the amino acids into alcohols, carbon dioxide and ammonia (III). Alanine, for example, yields ethyl alcohol :—

$$CH_3 . CHNH_2 . COOH + H_2O = CH_3 . CH_2OH + NH_3 + CO_2$$

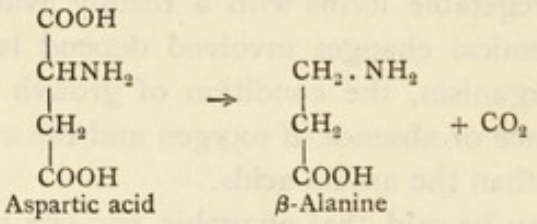
The net result of this last reaction indicates neither reduction nor oxidation but simply hydrolysis and liberation of carbon dioxide. It will be shown, however, that probably both oxidation and reduction are concerned in this change :—

I. $R . CH_2 . CHNH_2 . COOH + H_2 \rightarrow R . CH_2 . CH_2 . COOH + NH_3$ (Reduction)
II. $R . CH_2 . CHNH_2 . COOH + O_2 \rightarrow R . CH_2 . COOH + NH_3 + CO_2$ (Oxidation)
III. $R . CH_2 . CHNH_2 . COOH + H_2O \rightarrow R . CH_2 . CH_2OH + NH_3 + CO_2$ (Hydrolysis)
IV. $R . CH_2 . CHNH_2 . COOH \rightarrow R . CH_2 . CH_2 . NH_2 + CO_2$ (Amine formation)
V. $R . CH_2 . COOH \rightarrow R . CH_3 + CO_2$ (Carboxylic acid decomposition)

Another type of reaction (IV) very commonly effected by bacteria involves the liberation from the amino acid of carbon dioxide but not ammonia. Amines are formed in this way. Tyrosine, for example, yields p-hydroxyphenylethylamine :—

$$OH . C_6H_4 . CH_2 . CHNH_2 . COOH = OH . C_6H_4 . CH_2 . CH_2NH_2 + CO_2$$

Carbon dioxide is also frequently liberated from the dicarboxylic acids such as aspartic and glutamic acids and from the acids produced by the oxidation of amino acids according to the second reaction (V). The formation of β-alanine and cresol from aspartic acid and tyrosine respectively are examples of this type of change.



Aspartic acid — β-Alanine

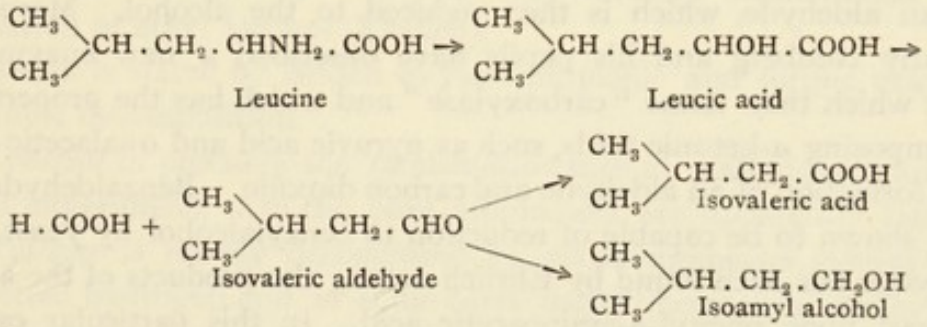
A combination of a number of these reactions may be effected by a single organism and different results may often be obtained using the same organism growing under varying conditions. When optically inactive amino acids undergo decomposition by micro-organisms it is usual for both forms to be attacked but at unequal rates. The difference in rate may vary widely so that in some cases a resolution into an active form may be effected. In other cases, e.g. glutamic acid, the two active forms may be decomposed at almost equal rates and no resolution is possible (Neuberg).

Before considering the various decomposition products of the amino acids, reference may be made to the types of change involved in the typical reactions recorded above.

Reduction of the amino acids to saturated acids (I) although easily brought about by anærobic bacteria, is effected *in vitro* with some dif-

ficulty. For example, hydriodic acid (sp. gr. 1·96) reduces amino acids when heated in sealed tubes at 220°. Thus glycine gives acetic acid and ammonia. The biochemical oxidation of the amino acids (II) with formation of a fatty acid, ammonia and carbon dioxide is readily imitated *in vitro*, either by the action of hydrogen peroxide, lead peroxide or by exposure of aqueous solutions to sunlight. It is not unlikely that in the biochemical oxidation of the amino acids by bacteria, *a*-ketonic acids are intermediate products as is the case when they undergo oxidation in the animal body.

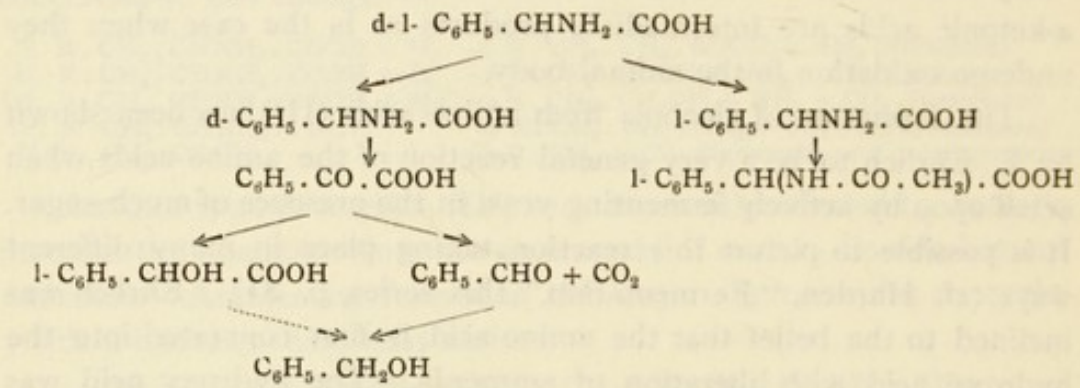
The formation of alcohols from amino acids (III) has been shown by F. Ehrlich to be a very general reaction of the amino acids when acted upon by actively fermenting yeast in the presence of much sugar. It is possible to picture this reaction taking place in many different ways (cf. Harden, "Fermentation," this series, p. 81). Ehrlich was inclined to the belief that the amino acid is first converted into the hydroxy acid with liberation of ammonia. The hydroxy acid was supposed then to undergo decomposition into formic acid and an aldehyde, and the latter substance then reduced to the corresponding alcohol or oxidized to the acid, according to the conditions of the experiment. Leucine, for example, might yield amyl alcohol and isovaleric acid as follows :—



Recent experiments by O. Neubauer and Fromherz have shown, however, that amino acids may on the one hand undergo *oxidation* through the action of yeast yielding *a*-ketonic acids and that the latter substances may undergo *reduction* with formation of an alcohol and carbon dioxide. For example, they isolated l-phenylaminoacetic acid, l-phenylacetylaminoacetic acid,[1] phenylglyoxylic acid, l-mandelic acid and benzyl alcohol from the products of the action of yeast upon d-l-phenylaminoacetic acid. Benzyl alcohol is the final product of the action of yeast upon this amino acid. Neubauer and Fromherz further showed that the phenylglyoxylic acid underwent partial reduction to l-mandelic acid and that the reverse change, namely the

[1] Cf. Acetylation of amino acids in the animal body, p. 58.

oxidation of mandelic acid to phenylglyoxylic acid could also be demonstrated, although this reaction was less vigorous than the former. That the ketonic acids are the probable precursors of the alcohols was also shown by subjecting para-hydroxyphenylpyruvic acid to the action of yeast and obtaining an excellent yield (70 per cent.) of p-hydroxyl-phenylethyl alcohol (tyrosol). The decomposition of phenylamino-acetic acid may be represented as follows :—



The hydroxy acids do not appear to be obligate steps in the conversion of the ketonic acids into alcohols since the yield of tyrosol obtained from p-hydroxyphenyl-*a*-lactic was only about 5 per cent. of that obtained from the ketonic acid. It appears far more likely that the ketonic acid undergoes decomposition so as to yield carbon dioxide and an aldehyde, which is then reduced to the alcohol. Moreover, recently Neuberg and his pupils have described a new enzyme in yeast which they name "carboxylase" and which has the property of decomposing *a*-ketonic acids, such as pyruvic acid and oxalacetic acid, with formation of an aldehyde and carbon dioxide. Benzaldehyde has been shown to be capable of reduction to benzylalcohol by yeast, and moreover has been found by Ehrlich among the products of the action of yeast upon phenyl-*a*-aminoacetic acid. In this particular case it may, however, have been artificially formed from the phenylglyoxylic acid during analysis.

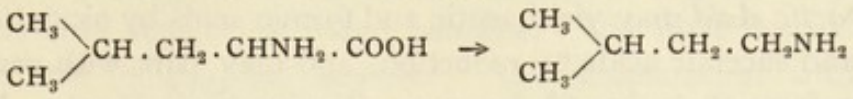
The typical reactions involved in the formation of an alcohol from an a-amino acid appear to be as follows :—

$$\underset{\text{(a-Amino acid)}}{R . CH_2 . CHNH_2 . COOH} \xrightarrow{\text{oxidation}} \underset{\text{(a-Ketonic acid)}}{R . CH_2 . CO . COOH} \rightarrow CO_2 + \underset{\text{(Aldehyde)}}{R . CH_2 . CHO} \xrightarrow{\text{reduction}} \underset{\text{(Alcohol)}}{R . CH_2 . CH_2HO}$$

The alcohols may be obtained from the *a*-amino acids *in vitro* by first oxidizing with hydrogen peroxide or some similar reagent and then reducing the aldehyde thus formed with sodium amalgam or zinc and acetic acid.

The formation of amines from amino acids (Reaction IV) may be

readily imitated *in vitro* by simply heating the amino acid to a high temperature. Leucine, for example, gives isoamylamine :—



The decomposition of carboxylic acids (Reaction V) can usually only be effected *in vitro* by indirect methods. But aspartic and glutamic acids may be directly reduced to propionic and butyric acids respectively by heating to high temperatures with hydriodic acid (Kwisda). The same reaction is effected by bacteria.

O. Emmerling and Abderhalden and Y. Teruuchi showed that ammonium oxalate was formed by *Aspergillus niger* when grown in solutions of various *a*-amino acids, including glycine, alanine, aspartic acid, glutamic acid, proline and certain polypeptides. Leucine, phenylalanine, the diamino acids and the carbohydrates do not give oxalic acid under similar conditions. The mechanism of oxalate formation is unknown.

The principal decompositions of the common amino acids by micro-organisms are as follows:—

Glycine and Alanine are relatively resistant to bacterial decomposition (Nencki), but Brasch determined the formation of acetic and propionic acids respectively. Alanine may undergo fermentation with formation of ethyl alcohol (Ehrlich).

Serine is stated by Brasch to yield propionic and formic acids on anærobic decomposition.

Creatine is probably the mother-substance of methylguanidine but direct experiments with the pure amino acid are apparently lacking.

Cysteine is readily decomposed by micro-organisms yielding sulphuretted hydrogen and in some cases methylmercaptan, diethyl sulphide and thiosulphates. The reactions in the case of this amino acid are unusually complex (Wohlgemuth, Nencki).

Valine when acted upon by putrefactive organisms may yield isovaleric acid by reduction, and also an amine which is probably isobutylamine (Neuberg and Karczag). It may also undergo alcoholic fermentation with formation of isobutyl alcohol (Ehrlich).

Leucine was found by Nencki to give isovaleric acid. Barger found isoamylamine among the putrefactive products of meat, the base no doubt being derived from leucine. Leucine yields isoamylalcohol on fermentation with yeast (Ehrlich).

Isoleucine probably yields methylethylpropionic acid by reduction since Neuberg and Rosenberg identified this acid among the products

of the putrefaction of caseinogen. On alcoholic fermentation it yields dextroamyl alcohol (Ehrlich).

Aspartic Acid may yield acetic and formic acids by oxidation, propionic and succinic acids by reduction, and may part with carbon dioxide to form β-alanine (Ackermann, Borchardt, A. Harden, Neuberg and Cappezzuoli, E. & H. Salkowski).

Glutamic Acid undergoes bacterial decomposition in similar fashion to aspartic acid. The following products have been identified: Butyric, acetic, formic acids; succinic acid and γ-aminobutyric acid (Ackermann, Borchardt, Brasch, Neuberg). On alcoholic fermentation glutamic acid yields succinic acid (Ehrlich).

Proline has been shown by Ackermann to yield δ-aminovaleric acid through opening of the pyrrole ring by reduction. Neuberg has confirmed this observation and also observed the formation of normal valeric acid.

Ornithine may part with carbon dioxide yielding tetramethylenediamine (Ellinger) and may also give δ-aminovaleric acid by reduction and loss of ammonia (Ackermann).

Lysine similarly yields pentamethylenediamine (Ellinger).

Arginine on decomposition by bacteria may yield inactive ornithine and hence tetramethylenediamine and δ-aminovaleric acid (Ackermann). Agmatine,

$$H_2N—\overset{\overset{NH}{\|}}{C}—NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$$

is also probably derived from arginine by bacterial action.

Histidine has been shown by Ackermann to yield β-iminazolepropionic acid by bacterial reduction and also the physiologically active base, β-iminazolethylamine.

Phenylalanine yields a variety of products of bacterial decomposition. Phenylpropionic acid is obtained by reduction, phenylethylamine is obtained through loss of carbon dioxide, while phenylacetic acid and benzoic acid are products of oxidation (E. Baumann, Nencki, Salkowski, Barger and Walpole). On alcoholic fermentation phenylalanine yields benzyl alcohol (Ehrlich).

Tyrosine. The following products have been identified: p-hydroxyphenylpropionic acid, p-hydroxyphenylacetic acid, p-hydroxyphenylethylamine, p-cresol and phenol (Weyl, Baumann, Nencki). E. and H. Salkowski consider that phenylpropionic acid may be formed from tyrosine by reduction both in the nucleus and the side-chain. This observation has not been confirmed, although Traetta-Mosca has described an

organism which he states is capable of effecting the complete decomposition of tyrosine with intermediate formation of p-hydroxyphenylpropionic acid, benzoic acid and benzene. The identification of the benzene was inadequate, however. On fermentation with yeast tyrosine yields p-hydroxybenzyl alcohol (tyrosol) (Ehrlich).

Tryptophane undergoes decomposition by bacteria in much the same way as tyrosine. The following products which had long been obtained from the products of the putrefaction of proteins were obtained from tryptophane by Hopkins and Cole: indolepropionic acid, indoleacetic acid, scatole and indole.

CHAPTER IV.

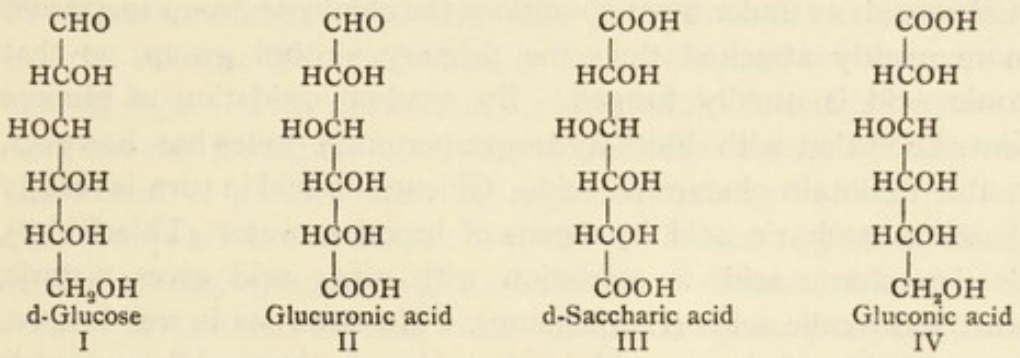
THE CARBOHYDRATES.

A MARKED characteristic of the sugars is their ready utilization not only by the normal organism but even by excised organs. It has long been known that tissues such as muscle in which active metabolism is in progress may obtain a large part of their energy by the combustion of sugar with liberation of carbon dioxide, but our knowledge of the mechanism of the chemical changes is very meagre. Within the last few years, however, a better understanding of the types of chemical reaction which are likely to occur in carbohydrate metabolism has been obtained. At the present time the search for intermediate products of carbohydrate metabolism in both plant and animal organism is being carried on actively with encouraging results.

With the possible exception of the pentoses, the various carbohydrates contained in the food-stuffs are believed to yield glucose in the processes of digestion, absorption, and assimilation. The glucose is in large measure stored as glycogen in the muscles and liver of the body and on utilization yields glucose once more. It follows, therefore, that the problems of carbohydrate oxidation are principally centred around the chemical changes which glucose may undergo.

The agents which effect the oxidation of the sugars in the body are practically unknown. A great deal of work has been done on the subject of "glycolysis," the term implying the disintegration of the sugar molecule. Many tissues, especially certain combinations of tissue plasma and extracts, such as muscle and pancreas, have been believed capable of effecting the decomposition of glucose. But recently Levene and Meyer have shown that the apparent disappearance of glucose in Cohnheim's experiments is due to the formation of polysaccharides, and it is probable that this source of error applies to many other cases in which a disappearance of glucose has been inferred simply from a change in reducing power and optical rotation. The general question of animal glycolysis is outside the scope of this chapter in which the structural relations of the sugars and their decomposition products are alone considered.

More than thirty years ago Wiedemann and Schmiedeberg and Meyer found that when camphor was given to animals it was excreted in combination with a substance related to the carbohydrates. The same substance, glucuronic acid, has since then been found to be present in small amounts in the blood, in the liver, and in normal urine. It is excreted in large amounts in combination with various aromatic alcohols, phenols, ketones, and acids when these substances are given to animals. Apparently the acid is never excreted in the free state, but always in combination. The close structural relationship between glucuronic acid (II) and glucose (I) made it appear probable that glucuronic acid was derived from the oxidation of the sugar :—



Evidence of the formation of glucuronic acid from sugar was furnished by the following experiments of Paul Mayer, who found that rabbits after prolonged starvation, and hence containing extremely little stored glycogen, furnished very little glucuronic acid on administration of camphor, but on subcutaneous administration of camphor and glucose the normal excretion of the glucuronic acid derivative of camphor was at once obtained.

The observation of Hildebrandt that the toxicity of thymotin-piperidide was diminished by simultaneous administration of large quantities of glucose, presumably because the sugar furnished an additional supply of glucuronic acid which on combination with the foreign substance diminished its toxicity, may be regarded as additional evidence for the occurrence of this change. Moreover, in certain cases of artificially induced dyspnœa in animals an excretion of glucose and glucuronic acid was observed by Mayer. The presence of the latter substance was ascribed to impaired power of oxidation on the part of the animal. The results of these latter experiments have not been adequately confirmed.[1]

Assuming that glucuronic acid represents the first step in the

[1] Mandel and Jackson and also Loewi have suggested the possible formation of glucuronic acid from proteins.

oxidation of glucose, further oxidation might be expected to give saccharic acid (III). Now Mayer showed that when large amounts of glucose, glucuronic acid, or saccharic acid, were given to rabbits a small but varying amount of oxalic acid was excreted in the urine. The oxidation of glucuronic acid to oxalic acid was also observed to take place in an excised liver. It appeared conceivable that sugar, at least in part, might undergo oxidation in the animal body so as to yield successively glucuronic acid and oxalic acid,[1] with possible intermediate formation of saccharic acid.

The oxidation of glucose *in vitro* affords comparable results. Until recently the direct oxidation of glucose to glucuronic acid had not been observed, as under most conditions the aldehyde group in glucose is more readily attacked than the primary alcohol group, so that gluconic acid is usually formed. By gradual oxidation of glucose in neutral solution with dilute hydrogen peroxide, Jolles has, however, been able to obtain glucuronic acid. Glucuronic acid in turn is readily oxidized to saccharic acid by means of bromine water (Thierfelder), while d-saccharic acid on oxidation with nitric acid gives tartaric, racemic and oxalic acids (Hornemann). Moreover, as is well known, direct oxidation of glucose with nitric acid and other oxidizing agents readily yields saccharic and oxalic acids.

While there can be little doubt that in the animal body glucuronic acid may be formed in large quantities from the oxidation of glucose, it may be questioned whether this is true of either saccharic acid or oxalic acid. Saccharic acid is relatively easily oxidized in the body, but oxalic acid is much more resistant. Many writers have declared that oxalic acid is entirely unattacked in the animal organism, while others have found that the oxalic acid excreted in the urine was less than the quantity administered. Many of these experiments are not very convincing, and in most cases the analytical methods employed were inadequate. More recently, Hildebrandt and later Dakin administered small amounts of oxalic acid subcutaneously to rabbits and found that from 60 to 90 per cent. was apparently oxidized, but it must be admitted, however, that the capacity of the body to oxidize oxalic acid, introduced as such, is restricted within very narrow limits (see p. 45).

It has been recently shown by J. Wirth that saccharic, gluconic and mucic acids when added in sufficient quantity to the blood sup-

[1] P. Mayer believed that gluconic acid (IV), an alternative primary oxidation product of glucose, when given in large amounts to rabbits led to an excretion of saccharic acid (III). This result has not been confirmed by the later experiments of E. Schott.

plying perfused dogs' livers may lead to a marked acetoacetic acid formation. If, at any time, it should be definitely shown that saccharic acid is a product of the normal biochemical oxidation of glucose, this formation of acetoacetic acid would furnish an important link between the carbohydrates on the one hand and the higher normal fatty acids present in fats and certain amino acids such as leucine, phenylalanine, and tyrosine, on the other, as all of these latter substances may yield acetoacetic acid on decomposition. But the fact that under most circumstances the influence of glucose is to hinder acetoacetic acid formation rather than to augment it, appears to be opposed to the idea of saccharic acid formation from sugar in the body, and it must be remembered that it has never been possible to detect saccharic acid nor the isomeric mucic acid among the products of the animal oxidation of sugar. That glucose may yield glucuronic acid is without question, but the formation of saccharic acid as a product of the normal catabolism of glucose must be regarded as very doubtful. In any case it appears almost certain that the catabolism of glucose along the following lines: glucose → glucuronic acid → d-saccharic acid → oxalic acid → carbon dioxide and water—is not the principal path of carbohydrate metabolism. Other modes of decomposition appear to be of greater quantitative importance.

It has long been recognized that the hexoses containing six carbon atoms could by a variety of processes be converted into substances containing three carbon atoms, while conversely in some cases the synthesis of hexoses could be effected by condensation of two molecules of certain substances containing three carbon atoms.[1] The formation of lactic acid by the action of dilute alkali upon glucose and the synthesis of a hexose by the condensation of two molecules of glycerose may be cited as well-known examples of these changes. It appears that reactions of this type are of great importance in carbohydrate metabolism.

Thus Embden has shown that lactic acid is formed at the expense of glycogen, and hence from glucose, when a surviving liver rich in glycogen is submitted to perfusion. Conversely, it is known that lactic acid may give rise to glycogen in the normal animal, and when given to a diabetic animal it is largely converted into glucose (Mandel and Lusk, and others). *Lactic acid must therefore be regarded as one of the*

[1] According to conditions, a hexose molecule may be resolved by the action of alkali into a pentose and formaldehyde, a tetrose and glycollic aldehyde, or two molecules of a triose.

most important substances concerned with the intermediate metabolism of the carbohydrates.

Glycogen $\rightleftharpoons$ glucose $\rightleftharpoons$ lactic acid.

The mechanism of the formation of lactic acid from glucose and the subsequent fate of lactic acid in the body are obviously matters of great importance. Unfortunately we know nothing definite about the intermediate steps of the conversion in the animal body of glucose into lactic acid. It is likely, however, that the change is complete, that is to say each molecule of glucose ($C_6H_{12}O_6$) yields two molecules of lactic acid ($C_3H_6O_3$). This is inferred not only from the simple relation existing between the empirical formulæ of lactic acid and glucose, but also because, by means of the lactic fermentation of glucose, or by the action of alkalies on glucose, it is possible to obtain more than a 50 per cent. yield of lactic acid. An inspection of the structural formulæ of glucose and lactic acid shows that a number of complex rearrangements are necessary for the production of the latter substance. A large number of investigations have been made upon the action of alkalies upon sugars, but the results of these experiments can only be briefly referred to in so far as they indicate a possible analogy with the process of lactic acid formation in the body. The work of Pinkus, Nef, Wohl and others has led to the belief that glyceric aldehyde, or possibly dihydroxyacetone, is first formed (I). These substances readily yield pyruvic aldehyde (methyl glyoxal) by loss of a molecule of water (II). The pyruvic aldehyde may then take up a molecule of water with formation of lactic acid (III) :—

(1) $CH_2OH(CHOH)_4 . CHO \rightarrow 2CH_2OH . CHOH . CHO$
(Glucose) (Glyceric aldehyde)

(2) $CH_2OH . CHOH . CHO \rightarrow CH_3 . CO . CHO$
(Glyceric aldehyde) (Pyruvic aldehyde)

(3) $CH_3 . CO . CHO \rightarrow CH_3 . CHOH . COOH$
(Pyruvic aldehyde) (Lactic acid)

The main evidence for this theory is as follows :—

(*a*) Glucose when acted upon by alkali in the presence of phenylhydrazine yields the osazone of pyruvic aldehyde.

(*b*) Pyruvic aldehyde on treatment with alkali readily yields lactic acid.

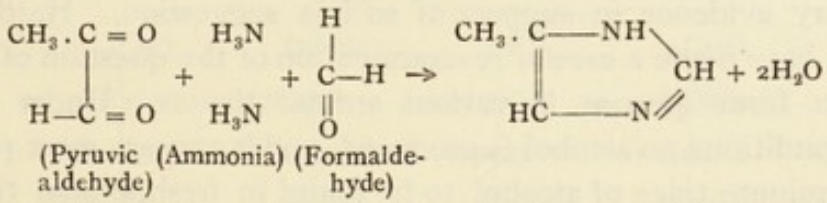
(*c*) Dihydroxyacetone on distillation with dilute sulphuric acid yields pyruvic aldehyde.

(*d*) Glyceric aldehyde and dihydroxyacetone like glucose yield lactic acid when treated with alkalies.

(*e*) According to Jensen dihydroxyacetone is invariably present in

glucose solutions and is also produced during alcoholic fermentation of glucose. (These observations are of doubtful accuracy.)

(*f*) The synthesis of methyliminazole from glucose and ammonia in the presence of zinc oxide is readily explained on the assumption that pyruvic aldehyde and formaldehyde are formed from sugar (Knoop and Windaus) :—



(*g*) The formation of saccharines from sugar is probably due to synthesis from glyceric aldehyde and lactic acid (Kiliani and Windaus).

If the mechanism of lactic acid production from sugar in the body be similar to that believed to represent its formation by means of alkali, it would be anticipated that glyceric aldehyde and pyruvic aldehyde would also yield lactic acid when perfused through a surviving liver. Experiments in this direction are at present lacking, but Embden and Schmidt state that S. Oppenheimer (unpublished experiments) has shown that glycerol when perfused through a surviving liver yields lactic acid. Since glycerol on oxidation must be assumed to yield either glyceric aldehyde, or dihydroxyacetone, this may be regarded as evidence in favour of the formation of these latter substances from sugar in the animal body. The fact that glyceric aldehyde in most biochemical respects behaves like a typical sugar is in harmony with this supposition.

The subsequent fate of lactic acid in the body has been already discussed (p. 57). Its oxidation to pyruvic acid and the possibility of the latter substance leading to amino acid formation (alanine) have already been referred to. Pyruvic acid readily undergoes complete oxidation in the body yielding carbon dioxide and water.

It is of interest to note that O. Neubauer has recently suggested the possibility of pyruvic acid being an intermediate product in the alcoholic fermentation of sugar by yeast. The possibility of the formation of pyruvic acid from sugar is readily inferred from the preceding statements. The reaction necessitates depolymerization followed by oxidation, but it is not necessary to assume that the pyruvic acid is formed by the oxidation of preformed lactic acid. It may be formed by the oxidation of pyruvic aldehyde, or in other ways.

The conversion of *a*-ketonic acids into alcohols by yeast appears to be a general reaction. Hydroxy acids are formed as by-products.

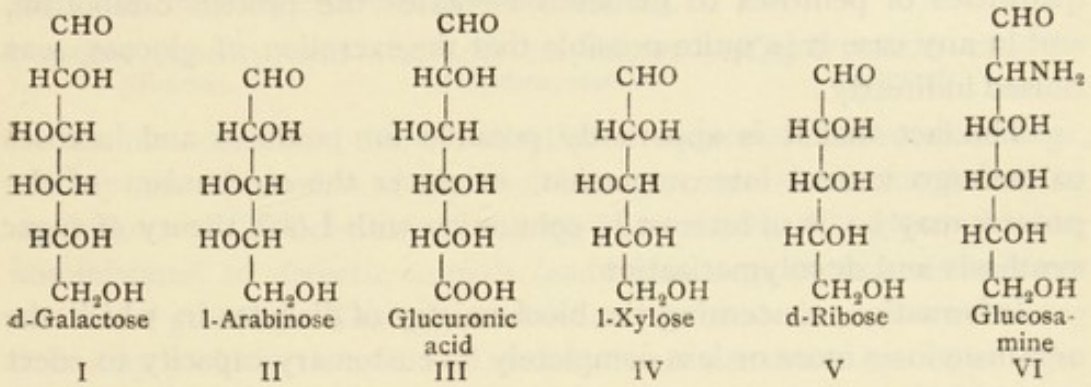
They are converted into alcohols only with difficulty and to a limited extent (O. Neubauer and K. Fromherz).

The fact that it is apparently possible to isolate small quantities of ethyl alcohol by the distillation of aqueous extracts of animal tissues has led at times to the supposition that the sugars might undergo alcoholic fermentation in the animal body. There is, however, no satisfactory evidence in support of such a suggestion. Harden and McClean have made a careful re-examination of the question of alcohol formation from glucose in various animal tissues. Under strictly aseptic conditions no alcohol is produced, and it appears most probable that the minute trace of alcohol to be found in fresh animal tissues is a product of intestinal fermentation.

Reference must be made to the possibility of a fourth type of carbohydrate decomposition which may possibly be of importance in connexion with sugar catabolism. Walther Löb has shown in a long series of papers that the depolymerization of glucose does not necessarily result in the formation of two molecules of substances containing three carbon atoms, but that under certain circumstances, e.g. by very dilute alkali, or by electrolysis, or certain other methods of oxidation, a more gradual decomposition may occur leading first of all to the formation of a pentose and formaldehyde. By successive depolymerization the sugar molecule may be further resolved with formation of additional molecules of formaldehyde and Löb refers the production of many substances, derived from glucose by biochemical reactions, to their synthesis from formaldehyde and other products of partial depolymerization. It is not clear that reactions such as these play any part in oxidations in the animal body although they do present striking analogies with reactions taking place in the vegetable organism. The possible formation from hexoses of the pentoses which are found combined in the nucleic acids of animal cells may be correlated with reactions of this type. The derivation from any of the common hexoses of d-ribose (V), the pentose which Levene and Jacobs have obtained from a number of nucleic acids, would necessitate stereochemical rearrangements, but on the other hand it may possibly be formed synthetically from formaldehyde, or other simple products of the depolymerization of the hexoses. Neuberg has suggested that the inactive arabinose (II) which he states is excreted in the peculiar metabolic abnormality known as pentosuria, is derived from galactose (I).[1] As an actual

[1] Recent experiments by Raper throw doubt on the excretion of arabinose in pentosuria. The sugar in pentosuric urine appears to resemble ribose more closely than any other of the known pentoses.

example of the biochemical conversion of hexose to pentose it is of interest to note that glucuronic acid (III) derived from d-glucose may be converted into l-xylose (IV) by means of putrefactive micro-organisms. The transformation of hexoses to pentoses in the animal body must be regarded as probable, although unproven.



At one time it was thought that glucosamine (VI) was an important link connecting the proteins on the one hand and carbohydrates on the other, but E. Fabian's experiments showed that glucosamine is not very readily oxidized in the body; when given subcutaneously much was excreted unchanged in the urine. The manner of its oxidation is unknown.

Brief mention must be made of the fate of the pentoses in the animal body. The pentosans, i.e. the complex polysaccharides which on hydrolysis yield the pentoses, are most important constituents of the food of herbivorous animals. The questions concerning the mechanism of the hydrolysis of these substances are complex, but it is certain that in one way, or another, large quantities of pentoses undergo absorption. In general the pentoses are not so readily utilized in the body as the hexoses (cf. V. Jaksch and Salkowski), and when moderate amounts are administered to animals a considerable proportion may be excreted in the urine. Thus on feeding 10-15 grms. of l-arabinose to starving rabbits about 18 per cent. was excreted unchanged in the urine. Cremer, by respiration experiments, showed that rhamnose, a methyl pentose, underwent oxidation in the body with formation of carbon dioxide, but the mechanism of the oxidation is entirely obscure. It appears, however, that under certain circumstances the pentoses may yield hexoses, i.e. the reverse process to that referred to above. Thus it has been found that l-arabinose, l-xylose and rhamnose, when fed to starving rabbits, apparently may lead to the formation of ordinary glycogen, which on utilization would yield glucose (E. Salkowski, Cremer, and others). Moreover, several cases

have been observed in which the administration of pentoses to diabetics has led to the excretion of glucose, although at the time immediately preceding the experiment the excretion of glucose had been abolished (R. v. Jaksch, Lindemann and May, P. Bergell). It must not be forgotten, however, that according to von Jaksch, administration of large quantities of pentoses to diabetics increases the protein catabolism, and in any case it is quite possible that the excretion of glucose was caused indirectly.

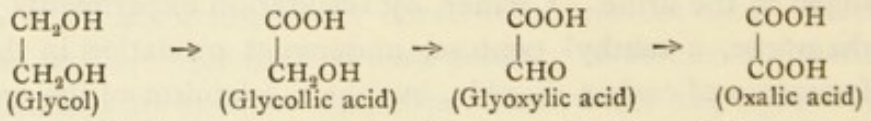
The fact that it is apparently possible for pentoses and hexoses to undergo mutual interconversion, whatever the mechanism of the process may be, is of interest in connexion with Löb's theory of sugar synthesis and depolymerization.

Information concerning the biochemistry of diabetes in which the organism loses more or less completely its customary capacity to effect the oxidation of glucose must be sought in the special works on chemical pathology (e.g. Van Noorden's "Metabolism and Practical Medicine"). It is of importance to note that this failure to effect the oxidation of glucose does not extend to glucuronic acid, nor to other derivatives of glucose, except in so far as some of them, such as lactic acid, may be converted into glucose.

The polyhydric alcohols are so closely related to the carbohydrates that they may be conveniently considered in connexion with the sugars. Glycerol is the only one of these substances of much importance in animal metabolism. The simplest polyhydric alcohol, glycol,

$$CH_2.OH.CH_2.OH,$$

so far as is known, is not formed in the animal body. When given to dogs, or rabbits, it is partly oxidized to oxalic acid (Pohl). Mayer found that when large doses were given to rabbits it was possible to isolate glycollic acid from the urine in the form of its phenylhydrazide. It is probable that glycollic and glyoxylic acids are intermediate products of the oxidation of glycol (Dakin). It is doubtful if more than a part of any glyoxylic acid formed is oxidized to oxalic acid.



Complete oxidation with intermediate production of formic acid is probably an alternative path for the oxidation of glyoxylic acid.

Glycerol is readily utilized in the animal body. When given to animals in large amounts, a part may be excreted unchanged, but under most circumstances it is completely oxidized (Luchsinger, Nicloux, and others). Ordinarily the concentration of free glycerol in the

body must be very small, but it is constantly being formed through the hydrolysis of fats. When necessary it is readily synthesized and is probably formed from the sugars. Its formation by the reduction of glyceric aldehyde, or dihydroxyacetone, the trioses believed to be derivable from glucose, is an attractive hypothesis, but satisfactory experiments on the subject are at present lacking.

$$\underset{\text{(Glucose)}}{CH_2OH \cdot (CHOH)_4 \cdot CHO} \rightarrow \underset{\text{(Dihydroxyacetone)}}{2CH_2OH \cdot CO \cdot CH_2OH} \rightarrow \underset{\text{(Glycerol)}}{2CH_2OH \cdot CHOH \cdot CH_2OH}$$

On the other hand there is excellent evidence of the reverse change, namely the conversion of glycerol into glucose—a reaction involving oxidation. Both Cremer and Luthje have shown that glycerol when administered to diabetic animals leads to a large increase in the excretion of glucose. That glycerol when perfused through a surviving liver may yield lactic acid has already been mentioned (p. 87). The lactic acid is probably derived from glyceric aldehyde, or dihydroxyacetone, by intramolecular rearrangement. The lactic acid may then undergo complete oxidation to carbon dioxide and water in the normal fashion.

$$\underset{\text{(Glycerol)}}{CH_2OH \cdot CHOH \cdot CH_2OH} \rightarrow \underset{\text{(Glyceric aldehyde)}}{CH_2OH \cdot CHOH \cdot CHO} \rightarrow \underset{\text{(Lactic acid)}}{CH_3 \cdot CHOH \cdot COOH} \rightarrow CO_2 + H_2O$$

Whether the chief path of the normal catabolism of glycerol is by way of lactic acid is an open question.

The higher polyhydric alcohols of vegetable origin, erythritol $C_4H_6(OH)_4$, quercitol $C_6H_7(OH)_5$, and mannitol $C_6H_8(OH)_6$, when given in large amounts to animals are at least in part excreted unchanged in the urine (Von Mering, Pohl, Luchsinger). It is probable that when consumed in smaller quantities they are oxidized more, or less, completely, but the mechanism of the reactions has not been elucidated.

CHAPTER V.

THE PURINE DERIVATIVES.

THE purine bases occur in two forms in the animal body—free and combined. The free purine bases, especially hypoxanthine, occur to a limited extent in certain tissues, especially muscle. The combined purine bases have a wider distribution. They are found in combination with phosphoric acid, a carbohydrate, and in some cases with bases of the pyrimidine group in the form of complex nucleic acids [1] (Kossel). The nucleins and nucleoproteides, compounds formed by the union of proteins and nucleic acids, are important constituents of all cell nuclei.

The view that the complex nucleic acids (polynucleotides) are made up of simpler fragments (mononucleotides) each of which possesses the composition of a simple nucleic acid, has been definitely proved by Levene and Jacobs. They have further shown that the mononucleotides are commonly composed of phosphoric acid, pentose (d-ribose) and a purine base united in the following fashion :—

$$O = P\begin{matrix}\diagup OH \\ \\ \diagdown OH\end{matrix}O \cdot C_5H_8O_3\text{—Purine, or pyrimidine, group}$$

The principal methods made use of in unravelling the mechanism of purine metabolism are the following :—

(1) The investigation of the action of various isolated tissues, or tissue extracts, upon nucleic acids and on free purine derivatives.

(2) The administration of nucleic acids, nucleins, or nucleoproteides, to animals.

(3) The administration of free purine derivatives (adenine, hypoxanthine, guanine, xanthine and uric acid) to animals.

By utilizing the information gathered from each of these lines of inquiry, it has been possible to formulate a fairly satisfactory, although doubtless incomplete, picture of purine catabolism.

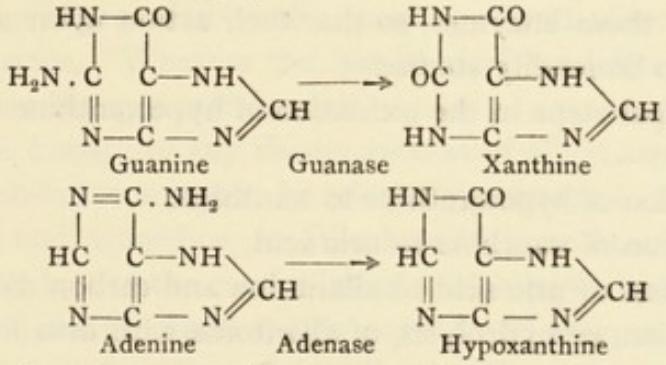
The nucleic acids, derived from the hydrolysis of nucleins and nucleoproteides, readily undergo further hydrolysis by means of enzymes

[1] For the most recent investigations upon the structure of nucleic acids, see papers by Levene and Jacobs, " Berichte," 1908-1911.

present in most animal tissues. These enzymes are collectively known as "nucleases" and act in a variety of ways. Thus, the nucleic acid may undergo complete hydrolysis yielding purine and pyrimidine bases, phosphoric acid and a carbohydrate. On the other hand, distinct nucleases may effect the liberation of phosphoric acid leaving the bases in combination with the sugar molecule (cf. Levene and Medegreceanu). Adenosine and guanosine are examples of substances formed in this reaction. They are collectively known as nucleosides and may be hydrolysed by acids, or enzymes found in various animal glands, yielding a purine base and a pentose (d-ribose).

Another type of enzyme action involves the conversion of guanine, or adenine groups, while still attached to the sugar complex, into xanthine and hypoxanthine groups with liberation of ammonia.

Two other enzymes, not belonging to the class of nucleases, bring about the conversion of free guanine and adenine into xanthine and hypoxanthine. These are known as guanase and adenase respectively, and together with the last-mentioned enzymes belong to the class of so-called "desamidases" (Jones and Partridge, Jones and Winternitz). The relationships of these enzyme actions may be readily seen by reference to the diagram on page 101.



None of the above reactions are oxidations, but all of them are hydrolytic decompositions which are essential for the subsequent oxidation of the purine derivatives. *Eventually by the action of these several enzymes the purine groups guanine, xanthine, adenine and hypoxanthine present in the nucleic acids, or formed from them by enzyme action, may be converted into free xanthine and hypoxanthine.*[1] (Cf. Diagram, p. 101.)

Horbaczewski showed in 1889 that uric acid was formed during the digestion of spleen pulp with blood in presence of oxygen, but that in the absence of blood xanthine and hypoxanthine, but no uric acid,

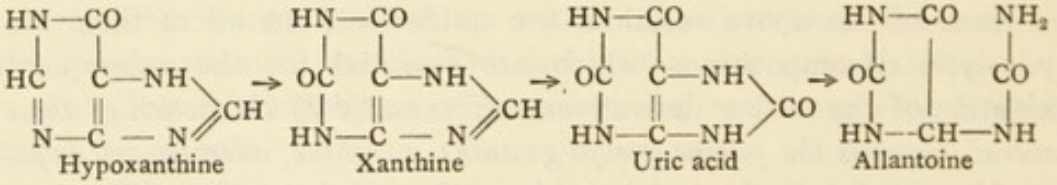
[1] For detailed information of the various steps involved in the biochemical hydrolysis of nucleic acids reference must be made to papers by Burian, Walter Jones, Levene, Salkowski, Schittenhelm and many others.

were formed. Horbaczewski was under the impression that a certain amount of putrefaction was a necessary condition for uric acid production, but Spitzer and all subsequent observers showed this to be unnecessary. Kossel's fundamental investigations on the constitution of the nucleins led to the belief that the uric acid might be formed from purine bases derived from the nucleins of the spleen, and the probability of such a theory was greatly increased by the observation of Horbaczewski and a host of subsequent experimenters that nucleins, when fed to men, led to a large increase in the excretion of uric acid. Spitzer then demonstrated in 1899 the direct conversion of added xanthine into uric acid when digested with organ extracts in the presence of oxygen, and Wiener observed the similar formation of uric acid from hypoxanthine when digested with liver tissue.

In recent years the whole subject of the behaviour of various organs towards purine derivatives has been carefully re-investigated, especially by Burian, Jones, Schittenhelm and Wiechowski. It has been found that the purine bases undergo oxidation by means of enzymes which exhibit a very irregular distribution in various animal species. They are principally found in the glandular organs, especially liver, spleen and pancreas. It has been found possible to separate and to some extent purify these enzymes, so that their action upon added purine derivatives can be readily studied.

The principal steps in the oxidation of hypoxanthine and xanthine are as follows :—

1. Oxidation of hypoxanthine to xanthine.
2. Oxidation of xanthine to uric acid.
3. Oxidation of uric acid to allantoine and carbon dioxide.
4. Oxidation, or hydrolysis, of allantoine with urea formation.



The oxidations represented by reactions (1) and (2) are commonly ascribed to the action of a single enzyme, xanthine-oxidase. There is some evidence which suggests the existence of separate enzymes for the oxidation of hypoxanthine and xanthine and by analogy with other enzymes the assumption appears probable (Wells). It is perhaps better to designate the enzymes concerned with the oxidation of hypoxanthine as hypoxanthine-oxidase reserving the name xanthine-oxidase for the enzyme taking part in the oxidation of xanthine to uric acid.

These enzymes are found in the livers of most animals, including man. They are also found in many other glandular tissues of animals excepting man.

In effecting the oxidation of the purine bases to uric acid by means of tissue extracts the supply of oxygen profoundly affects the results. In the presence of but little oxygen an extract of dog's spleen oxidizes hypoxanthine to xanthine with practically no formation of uric acid, but when an abundance of oxygen is present uric acid is formed instead of xanthine.

The oxidation of uric acid to allantoine (Reaction 3) is affected by an oxidizing enzyme known as "uricase," or as a "uricolytic enzyme". The action of this enzyme has been demonstrated in either the liver, or kidney, of all mammals thus far examined, *with the exception of man.* It is probably absent from all tissues of birds and reptiles, i.e. animals in which uric acid is the chief end-product of nitrogenous metabolism.

Wiechowski showed that on perfusing dog's liver or the kidney of the ox, with blood containing uric acid the latter was oxidized almost quantitatively to allantoine.

The mechanism of the oxidation of uric acid to allantoine by means of potassium permanganate is complex, and apparently allantoine formation is preceded by the opening of both closed rings in the uric acid molecule. Whether the biochemical oxidation of uric acid involves similar changes is unknown.

Nothing is known of any decomposition of allantoine by enzymes. In fact, the substance seems to be relatively resistant to changes in the animal body, and according to Wiechowski, is to be considered the normal end-product of the catabolism of purine bases by the carnivora.

Reference must be made at this point to an obscure phenomenon observed by Ascoli, Izar, Bezzola and Preti. These observers found that uric acid when added to oxygenated blood and perfused through a surviving liver practically completely disappears, but that on subsequently saturating the blood with carbon dioxide the uric acid reappears. Similar results were obtained with finely minced liver tissue under successively ærobic and anærobic conditions. This formation of uric acid is not observed to take place from added allantoine, parabanic acid, or glycine and urea. Dialuric acid and urea, on the other hand, do lead to uric acid formation, but there is no convincing evidence that dialuric acid is formed from uric acid when perfused through a surviving liver with oxygenated blood. The phenomenon requires further investigation.

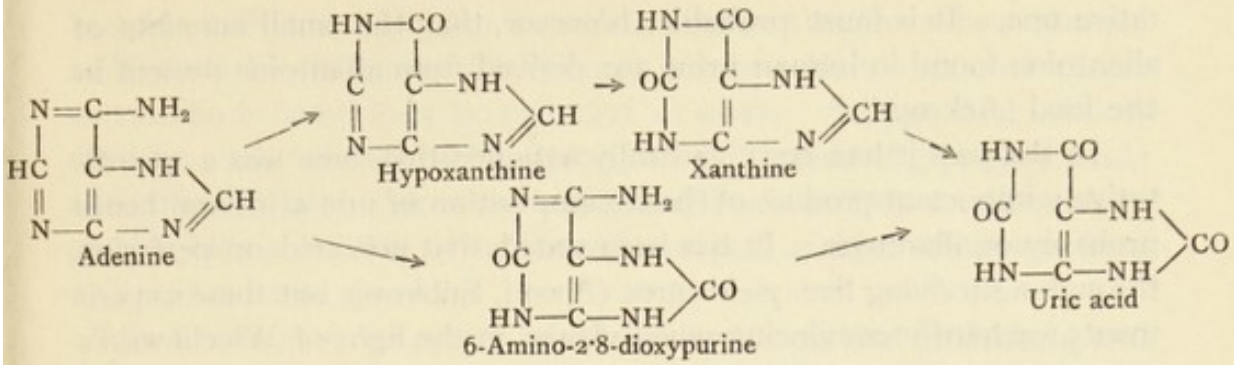
The fate in the animal body of the various purine derivatives and

their products of metabolism must now be considered. The results obtained by this line of investigation harmonize excellently with those derived from the study of the action of individual organs, although for a long while a satisfactory demonstration of the formation of uric acid from the xanthine bases was not obtained owing to the fact that the experiments were made on dogs, or other animals, promising a high capacity for uric acid destruction. Thus xanthine, guanine, and hypoxanthine may be fed to dogs without causing any marked increase in the excretion of uric acid, but when these same bases are administered to man an increased excretion of uric acid is readily observed.

Hypoxanthine, or xanthine, when administered to man leads to an increased output of uric acid corresponding to from 45 to 65 per cent. or even more of the base. The yield of uric acid from adenine and guanine has generally been found to be less than that from xanthine and hypoxanthine; the most reliable experiments give results varying from 10 to 40 per cent. according to conditions (Minkowski, Burian and Schur, Krüger and Schmid, Mendel and Lyman, and others).

Although administration of purine bases to dogs commonly fails to produce an increased uric acid excretion, in several cases it has been possible to demonstrate the excretion of allantoine, which, as was previously mentioned, is produced in the liver of the dog by oxidation of uric acid. Minkowski, Cohn and Salkowski independently found a marked allantoine excretion follow feeding with thymus, or pancreas, both of these organs containing large quantities of purine bases in combination. Mendel and White observed similar results on injecting salts of nucleic acid, and recently Levene and Medegreceanu obtained much allantoine from inosine when given to dogs. Allantoine has also been obtained from the urine of dogs which had received hypoxanthine, guanine and adenine (Minkowski, Mendel and Lyman, Levene and Medegreceanu). There can be little doubt of the intermediate formation of the uric acid in the oxidation of xanthine bases to allantoine.

When adenine is administered to animals a peculiar form of nephritis is usually produced characterized by deposition in the kidneys of a sparingly soluble substance which has been identified by Nikolaier as 6-amino-2·8-dioxypurine. This substance would be expected to yield uric acid when acted upon by enzymes of the type of adenase. It would seem likely, therefore, that uric acid may be formed from adenine not only by conversion into hypoxanthine and subsequent oxidation to xanthine and uric acid, but also by first undergoing oxidation and subsequently parting with a NH_2 group to yield uric acid:—



The intravenous administration of guanine to rabbits was shown by Schittenhelm and Bendix to be followed by a large excretion of uric acid together with a smaller amount of xanthine, and Mendel and Lyman have obtained similar results. This observation harmonizes with the view that xanthine is a normal precursor of uric acid derived from the purine bases.

From what has been mentioned of the variations in the fate of purine bases when administered to different animal species, it would be anticipated that similar variations would be encountered with uric acid. This is found to be the case. Uric acid is oxidized in the human organism with much greater difficulty than is the case with other animals, including monkeys, dogs, cats, rabbits and pigs. When uric acid is administered to man more than half can usually be recovered unchanged from the urine, and in some cases as high a recovery as 99 per cent. has been recorded (Soetbeer and Ibrahim). The recent experiments of Wiechowski show an excretion of 60 to 90 per cent. of the uric acid administered subcutaneously. Burian and Schur's experiments led them to conclude that when uric acid was administered to man about 50 per cent. was excreted unchanged, with rabbits the proportion was about 15 per cent., while carnivorous animals excreted only about 4 per cent. Corresponding with these results are the observations that uric acid when administered to man fails to yield a marked increase in allantoine excretion, while in dogs uric acid readily yields allantoine (Salkowski, Mendel and White, Wiechowski, and others). Man's relative inability to effect the catabolism of uric acid may be referred to a more or less complete lack of the uricolytic enzyme present in the livers of most other animals, and actual experiments on the action of the human liver upon uric acid confirm this supposition. Wiechowski has, however, been able to isolate traces of allantoine from normal human urine which he considers to be derived from purine metabolism. It is possible therefore that the differences between various mammals with regard to uric acid decomposition is a quantitative rather than a quali-

tative one. It is most probable, however, that the small amounts of allantoine found in human urine are derived from allantoine present in the food (Ackroyd).

In the past it has been generally assumed that urea was a quantitatively important product of the decomposition of uric acid, and hence probably of allantoine. It has been stated that uric acid on perfusion through a surviving liver yields urea (Ascoli, Subkow), but these experiments are hardly convincing when viewed in the light of Wiechowski's subsequent work on allantoine formation. According to Wiechowski the amount of allantoine excreted by dogs and rabbits after administration of uric acid is nearly sufficient to account for the whole of the uric acid undergoing oxidation. The amount of urea formed from uric acid under these conditions must therefore be small.

Levene and Medegreceanu, on the other hand, found comparatively little allantoine (15 per cent.) when sodium urate was given to dogs but much urea.

According to Wiechowski allantoine is the normal end-product of purine metabolism in rabbits and dogs. In agreement with this view it is found that allantoine is not very readily decomposed when given to animals. Wiechowski showed that when administered subcutaneously to man about 90 per cent. was excreted unchanged. Poduschka, on the other hand, found only 30 to 50 per cent. of allantoine excreted unchanged in the urine in cases of man, while with dogs he found almost quantitative excretion. It must be noted, however, that Poduschka failed to detect allantoine after feeding uric acid to dogs, and in addition all the methods for allantoine estimation prior to Wiechowski's work were very inaccurate. Recent experiments of Levene and Medegreceanu showed that when allantoine was given to dogs about one-third was excreted unchanged and two-thirds converted into urea. Luzatto's experiments also seemed to show a more ready decomposition. On administering three grams of allantoine to rabbits none could be recovered in the urine, but an increased excretion of oxalic acid was noted. This last result is of interest since it has frequently been suggested that oxalic acid might be an intermediate step in the oxidation in the body of uric acid. However, direct administration of uric acid does not lead to oxalic acid excretion (Luzatto and others). Outside the body allantoine is a relatively unstable substance undergoing slow decomposition in aqueous solution even at ordinary temperatures. This decomposition is much accelerated in an alkaline medium, and even Wiechowski is inclined to concede the possibility of oxalic acid formation from allantoine in the body under

certain conditions. Glycine is another substance which has been claimed to be derivable from uric acid (Wiener), but the evidence upon which this conclusion is based must be regarded as weak. At the same time it must be admitted that as allantoine is the di-ureide of glyoxylic acid,[1] and since the latter substance might readily yield either oxalic acid, or glycine,[2] the possibility of the formation of both of these substances from uric acid must be conceded.

In the diagram on p. 101, which is based upon that in Amberg and Jones's paper, are represented the principal paths of purine metabolism, showing how a typical nucleic acid, represented as a di-nucleotide containing adenine and guanine groupings is converted successively by hydrolysis and oxidation into xanthine, uric acid, allantoine and finally urea.

The oxidation of hypoxanthine to xanthine and subsequently to uric acid such as is believed to take place in the animal body has not yet been imitated *in vitro*. The oxidation of uric acid to allantoine, on the other hand, is readily effected by a variety of oxidizing agents such as potassium permanganate, lead peroxide, etc.

A word must be said of the possibility of the formation of purine bases by the reduction of uric acid. That purine bases may be synthesized in the young animal is well known. An inspection of careful urinary analyses following the administration of salts of uric acid shows in many cases a distinct rise in the purine nitrogen "other than uric acid". Although the evidence is admittedly slight, the idea of the reduction of uric acid to purine bases is by no means as incredible as might appear at first sight, for the reactions involved are not vastly different in character from those concerned in the observed reduction in the body of ketonic acids to hydroxy, or amino, acids.

Before concluding the consideration of the oxidation of the purine bases found in the animal body, reference may be made to the fate of the three methylxanthines of vegetable origin: theophylline, theobromine, and caffeine. All of these substances undergo a progressive demethylation, the order in which the methyl groups are removed varying in different animal species (Krüger and Schmidt). Whether demethylation is effected by oxidation, or hydrolysis, is unknown. Caffeine = 1. 3. 7, trimethylxanthine, when given to dogs chiefly yields 1. 3, dimethylxanthine (theophylline) 3, methylxanthine, 1. 7,

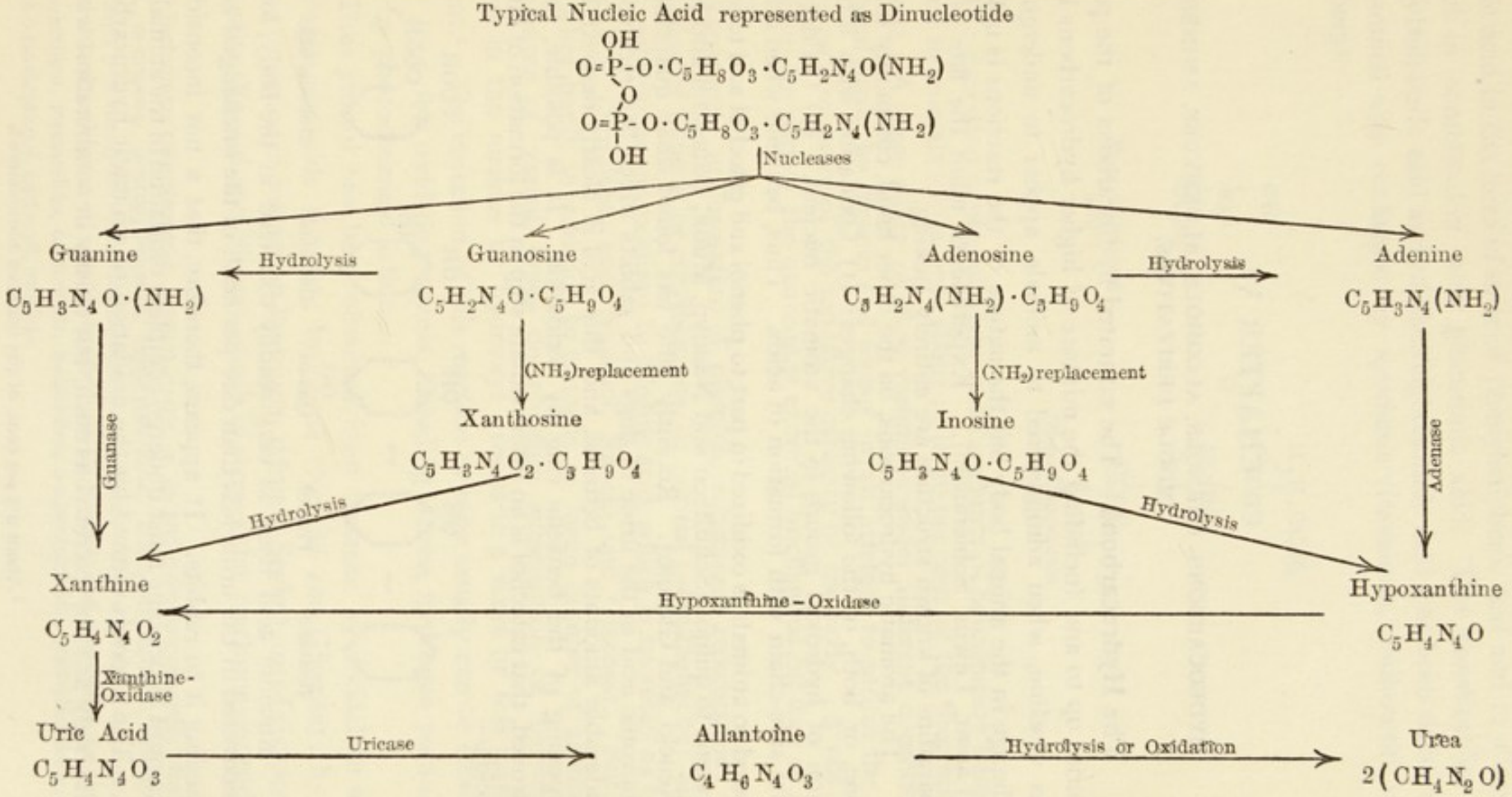
[1] Salts of glyoxylic acid when administered to rabbits and dogs lead to a varying excretion of oxalic acid. Eppinger has stated that allantoine was formed by synthesis in the body from glyoxylic acid, but this result could not be confirmed by Dakin.

[2] Ammonia acts upon glyoxylic acid to give formylglycine (Erlenmeyer and Kunlin).

dimethylxanthine and 3. 7 dimethylxanthine being formed in smaller amounts. Thus in dogs methyl groups (1) and (7) are most readily removed, while with rabbits it is found that methyl group (3) is most easily replaced, so that 1, methylxanthine, 7, methylxanthine and 1. 7, dimethylxanthine are excreted. Theobromine = 3. 7, dimethylxanthine yields chiefly 3, methylxanthine when given to dogs and 7, methylxanthine when given to rabbits. Theophylline (1.3, dimethylxanthine) yields 1, and 3, methylxanthine. Neither uric acid, methyl-uric acids nor methyl-allantoines have as yet been observed among the products of catabolism of methylated xanthines.

Little is known of the mode of decomposition of the pyrimidine bases present in various nucleic acids. Steudel has carried out feeding experiments with a number of these substances; he has not observed the synthesis of purine derivatives through combination with a urea-grouping, nor identified any characteristic product of their catabolism.

Diagram Representing the Hydrolysis & Oxidation of Nucleic Acids.

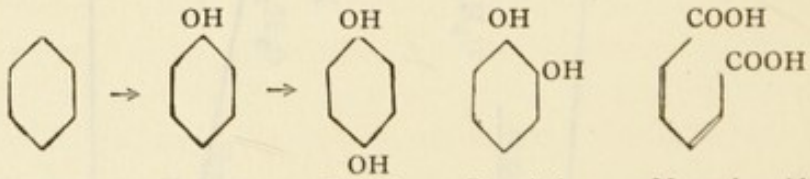


CHAPTER VI.

HYDROCARBONS, PHENOLS, ALCOHOLS, ALDEHYDES, AMINES, INDOLE DERIVATIVES.

The Hydrocarbons.—The saturated hydrocarbons of the paraffin series up to and including the mixture of higher hydrocarbons known as vaseline, when administered to animals, appear to undergo some change in the animal body but the nature of the reactions is unknown (Lassar, Lewin, Sobieranski). Experiments upon the fate of pure paraffins of known structure are entirely lacking.

The aromatic hydrocarbons, on the other hand, commonly undergo one, or both, of the following changes: (1) Oxidation with introduction of hydroxyl groups in the aromatic nucleus. (2) Oxidation of the side-chain with formation of acids. Thus, benzene when administered to animals is oxidized in part to phenol and catechol and to a lesser extent to quinol (Schultzen and Naunyn, Munk, Baumann and Herter, Nencki and Giacosa). Recently Jaffé has found small quantities[1] of muconic acid in the urine of dogs and rabbits which had received considerable amounts of benzene, and this acid is clearly derived by the opening of the benzene ring by oxidation. It is possible, but not proved, that catechol is an intermediate step in the formation of muconic acid:—



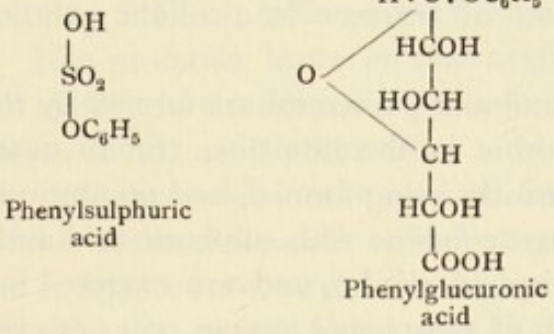
Benzene — Phenol — Quinol — Catechol — Muconic acid

Muconic acid itself is very readily oxidized in the body, for Jaffé recovered in the urine less than one per cent. of the unchanged acid on feeding it to rabbits. It appears, therefore, that a not inconsiderable amount of benzene must undergo complete oxidation in the animal body.

The phenols formed by the oxidation of aromatic hydrocarbons in the body are not excreted as such, but chiefly in combination with sul-

[1] About 0·3 per cent. of the benzene administered.

phuric acid in the form of salts of phenylsulphuric acid and to a lesser extent in combination with glucuronic acid. The constitution of phenylsulphuric acid and of phenylglucuronic acid has been definitely determined and confirmed by synthesis (Baumann, Salkowski and Neuberg).



The excretion of phenol when small amounts of benzene are administered to animals varies from about 15 to 30 per cent. of the theoretical amount. A close study of these variations under different conditions has been made by Nencki and Sieber in an attempt to utilize the reaction as a measure of the oxidative capacity of the body. A not inconsiderable part of the benzene is excreted unchanged by way of the lungs.

Apparently no formation of phenolic substances accompanies the oxidation of toluene, or ethylbenzene, or normal propylbenzene; all of these substances are oxidized to benzoic acid, which is excreted as hippuric acid (Schultzen and Naunyn, Nencki and Giacosa). But on the other hand, in addition to benzene, a large number of aromatic substances of varied types do undergo substitution of hydrogen atoms in the nucleus by hydroxyl groups to a more or less marked extent.[1] The imitation of this reaction *in vitro* usually can be effected only by a few oxidizing agents, including ozone, hydrogen peroxide, and by photochemical action.

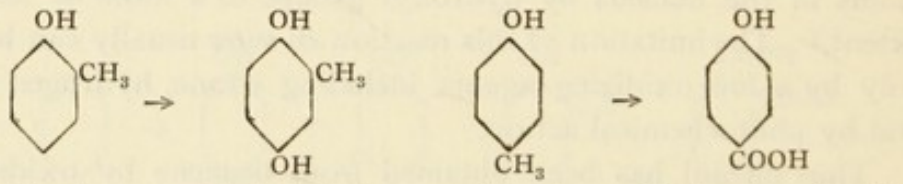
Thus phenol has been obtained from benzene by oxidation with hydrogen peroxide (Leeds); by ozone (Nencki and Giacosa); by air in the presence of palladium-hydrogen (Hoppe-Seyler), or of copper, or iron salts (Nencki and Sieber)—both of these latter reactions depending

[1] Including: chlorobenzene, bromobenzene, o- m- and p-dichlorobenzene, nitrobenzene, m-cymene, isopropylbenzene, isobutylbenzene, methylethyltoluene, mesitylene, diphenyl, p-bromodiphenyl, diphenylmethane, naphthalene, bromonaphthalene, phenol, o- and p-chlorphenol, p-cresol, anisol, phenetol, guaicol, thymol, and p-naphthol, aniline, dimethylaniline, formanilide, acetanilide, o-toluidine, p-acetotoluidide, phenylurethane, carbonyl-o-amidophenol, carbazol, acridine.

probably upon the formation of hydrogen peroxide—by the action of sunlight in the presence of caustic soda (Radziszewski) and also by the combined action of oxygen and aluminium chloride (Friedel and Crafts). Catechol and quinol may also be obtained by the oxidation of benzene and phenol with hydrogen peroxide in the presence of iron salts (Cross, Bevan, and Heiberg), and quinol is formed during the electrolytic oxidation of benzene in alcoholic solution (Gattermann and Friedrichs).

Phenols.—Phenol and paracresol are formed by the bacterial decomposition of tyrosine in the intestine. Small quantities of these substances are constantly being formed, and on absorption by the animal body they in part combine with sulphuric acid and to a lesser extent with glucuronic acid (Kulz), and are excreted in the urine. A considerable portion of the phenol, 50 per cent., or even more, undergoes oxidation with formation of quinol and catechol, which in turn combine with sulphuric acid, and to some extent with glucuronic acid, and are also excreted in the urine (Baumann and Preusse, Tauber, Schaffer). Catechol appears to be relatively resistant to futher oxidation, but quinol appears to undergo oxidation more readily. Sufficiently accurate experiments to determine definitely the extent of the further oxidation of quinol and catechol in the body are at present lacking.

All of the isomeric cresols are in large measure excreted unchanged in combination with sulphuric acid. Ortho-cresol appears to give in addition a small amount of methylquinol, while p-cresol yields a small amount of p-hydroxybenzoic acid (Baumann and Herter). The latter substance is resistant to further oxidation in the animal body and is commonly found in small amounts in normal urine:—



o-Cresol — Methylquinol — p-Cresol — p-Hydroxybenzoic acid

Alcohols.—In general the alcohols appear to undergo oxidation in the animal body with formation of the corresponding acids, but in many cases the latter substances undergo further oxidation and so escape detection. Administration of methyl alcohol to animals leads to a very definite excretion of formic acid (Pohl), and benzyl alcohol gives benzoic acid (hippuric acid). O. Neubauer showed that phenylethyl alcohol gave phenylacetic acid (phenaceturic acid), while Nencki found

that saligenin $C_6H_4OH \cdot CH_2OH$ was converted into salicylic acid. Many other cases of the oxidation of aromatic alcohols to the corresponding acids have been observed.

No intermediate product of the oxidation in the animal body of ethyl alcohol has been satisfactorily detected, although small amounts of unchanged alcohol, both free and combined with glucuronic acid, may be found in the urine. The volatile acids of the urine are not materially increased. It is probable, however, that acetic acid is formed by the oxidation of alcohol in the animal body and undergoes practically complete oxidation as fast as it is formed.

Iso-amyl alcohol when added to blood used for perfusing a freshly excised liver, yields some acetoacetic acid, but possibly owing to its toxic action, the amount of acetoacetic acid is less than that obtained from iso-valeric aldehyde, or iso-valeric acid (F. Sachs). The latter substances are probably intermediate products in the reaction (cf. p. 42).

$$\begin{matrix} (CH_3)_2CH \cdot CH_2 \cdot CH_2OH \rightarrow (CH_3)_2CH \cdot CH_2 \cdot CHO \rightarrow \\ (CH_3)_2CH \cdot CH_2 \cdot COOH \rightarrow CH_3 \cdot CO \cdot CH_2 \cdot COOH \end{matrix}$$

Battelli and Stern have recently described an enzyme which they name "alcoholoxydase" occurring in animal tissues, especially the liver, which accelerates the oxidation of ethyl alcohol in the presence of free oxygen. Acetaldehyde appears to be an intermediate product of the reaction and is subsequently oxidized to acetic acid. Buchner and Gaunt had previously obtained a similar enzyme from the micro-organisms concerned with acetic fermentation.

Aldehydes.—It has long been known that aldehydes might be converted into acids in the animal body and the same reaction was observed to occur in various isolated animal tissues. Until recently the action was generally believed to be a simple oxidation due to oxidizing enzymes collectively known as "aldehydases" (Schmiedeberg, Jaquet, Abelous and Biarnès, Jacoby, Spitzer, and others). It had, however, been noticed that free oxygen was not only unnecessary for the reaction, but under certain conditions was distinctly disadvantageous (Medwedew, Abelous and Aloy, Dony-Henault and Van Duuren). The explanation of this fact is found in the observations of Parnas and of Battelli and Stern, who have determined that the chief change which aldehydes undergo when brought in contact with animal tissues is not a direct oxidation at all but is the so-called Cannizzaro reaction. Two molecules of the aldehyde undergo rearrangement in such a fashion

that one molecule is *reduced* to the corresponding alcohol, while the second is *oxidized* to the corresponding acid. The acid and alcohol thus produced are either combined in the form of an ester, or free, according to the special conditions of the experiment. The Cannizzaro reaction is very commonly observed to occur *in vitro* when aldehydes are treated with alkalies:—

$$2R.CHO + H_2O = R.CH_2OH + R.COOH$$

Parnas observed the Cannizzaro reaction to occur with considerable rapidity when the following aldehydes were digested with liver tissue in dilute sodium bicarbonate solution in the presence of oxygen: propionic, butyric, isobutyric, isovaleric, *n*-valeric, heptylic, benzoic, salicylic aldehydes and aldol. Batelli and Stern had already observed a similar decomposition with acetaldehyde, with formation of ethyl alcohol and acetic acid. Parnas suggests the name "aldehydemutase" for the enzyme concerned with the reaction.

In general it may be said that aldehydes may undergo rearrangement in the animal body with formation of the corresponding alcohols and acids, and that the alcohols may then undergo further oxidation with renewed formation of aldehyde and acid, so that the net result is a complete oxidation of the aldehyde to the corresponding acid. A large number of examples of this change have been observed in the case of aromatic as well as fatty aldehydes.

Amines.—Small amounts of a number of amines are constantly being formed by bacterial decomposition of amino acids in the intestine and subsequently are absorbed. Amines are also present in animal food; thus Krimberg found methylguanidine in fresh ox-muscle, while choline and other bases are found either free, or combined, in a variety of tissues.

The simple amines, methylamine, ethylamine and isoamylamine appear to undergo fairly complete decomposition in the animal body. Only small amounts appear unchanged in the urine (Salkowski, Schiffer, Erdmann). It has been stated that the corresponding alkyl ureas may also be excreted (Salkowski, Schmiedeberg), but these results lack adequate confirmation.

Formic acid appears to be an intermediate product of the oxidation of methylamine in the body, for Pohl observed a marked increase in formic acid excretion after administering 2 grms. of methylamine hydrochloride to a dog:

$$CH_3.NH_2 \rightarrow NH_3 + H.COOH \rightarrow CO_2$$

No similar increase in volatile acids has been observed to follow

the administration of the other aliphatic amines, possibly because the higher fatty acids readily undergo complete oxidation. But among the aromatic amines, benzylamine has been observed to give benzoic acid which is excreted as hippuric acid (Schmiedeberg, Mosso), and p-hydroxyphenylethylamine, a physiologically active base obtained by the decomposition of tyrosine, has been shown by Ewins and Laidlaw to give p-hydroxyphenylacetic acid. It is remarkable that the latter base appears capable of undergoing complete decomposition when perfused through a surviving heart.

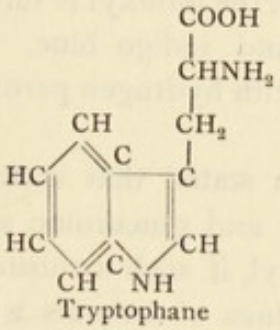
The formation of acetoacetic acid from isoamylamine when perfused through a surviving liver is probably preceded by formation of isovaleric acid which in turn readily yields acetoacetic acid itself (Sachs).

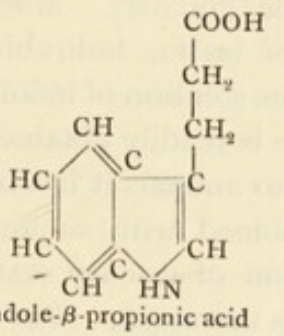
In general it will be noted that the behaviour of the simple primary amines in the body resembles that of the corresponding alcohols and acids.

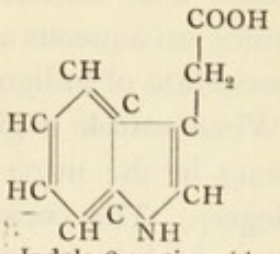
Both guanidine and methylguanidine, $NH_2.C=NH.NHCH_3$, appear to be very resistant against oxidation in the animal body, and when small doses are administered to animals the bases are excreted unchanged in the urine (Gergens and Baumann, Pommerenig).

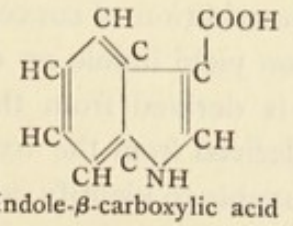
Concerning the fate of the other bases which may be formed in the animal organism there is an almost entire lack of definite information.

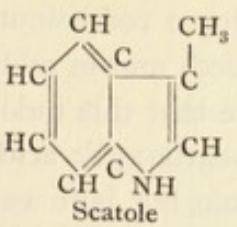
Indole Derivatives.—When tryptophane undergoes bacterial decomposition in the alimentary tract, or elsewhere, the following compounds may be formed: indole-β-propionic acid,[1] indole-β-acetic acid scatole and indole (Hopkins and Cole, Salkowski, Nencki).

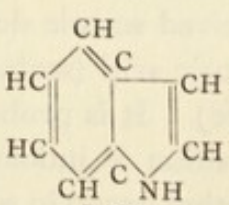

Tryptophane


Indole-β-propionic acid


Indole-β-acetic acid

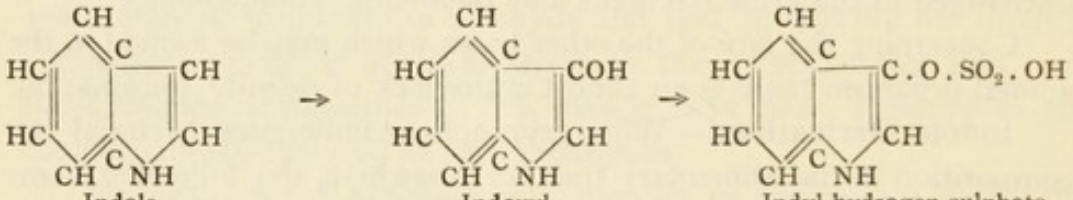

Indole-β-carboxylic acid


Scatole


Indole

[1] Reduction of tryptophane to indole-β-propionic acid apparently is effected only by anærobic organisms.

The fate of indolepropionic acid in the body is unknown, but judging by analogy with other aromatic acids, e.g. phenylpropionic acid, one would expect indole-β-carboxylic acid to be formed by β-oxidation, and it may be noted that this latter substance appears to be actually present in urine under certain conditions. Indoleacetic acid, like phenylacetic acid, appears to be very resistant to change in the animal body and is a common constituent of most urines although the amount is not large (Salkowski, C. Herter). Indole when given to animals is oxidized to indoxyl, which is excreted in the urine in union with both sulphuric acid and glucuronic acid. Jaffé was the first to show that indole, which Nencki had found among the products of the putrefactive decomposition of proteins, was the mother substance of urinary indican, while Baumann and Brieger succeeded in isolating the potassium salt of the latter substance in a pure state and showed that, on hydrolysis with acids, it gave indoxyl and sulphuric acid. The urinary indican is usually known as indoxylsulphuric acid, owing to the fact that it yields indoxyl on hydrolysis, but indylsulphuric acid or indyl-hydrogen-sulphate is the more exact designation :—



Indole → Indoxyl → Indyl-hydrogen-sulphate

The oxidation of indole to indoxyl may be effected *in vitro* by means of hydrogen peroxide (Porcher). Much of the indoxyl is further oxidized with formation of isatin, indirubin and indigo blue. On warming an aqueous acetone solution of indole with hydrogen peroxide a precipitate of indigo blue is readily obtained.

When scatole is given to animals it has been stated that scatoxyl appears in the urine combined with sulphuric and glucuronic acids (Brieger). The constitution of urinary scatoxyl, if such a substance exists, which is doubtful, is unknown. The urines of animals which have received scatole develop a red colour on addition of concentrated hydrochloric acid (scatole red) and in addition yield indole on distillation (Jaffé). It is probable that this indole is derived from the slow decomposition of indole-β-carboxylic acid, derived from the oxidation of the methyl group in scatole, but Jaffé was unable to identify definitely the substance (cf. Blumenthal and E. Jacoby). It appears not improbable that indole-β-acetic acid and indole-β-carboxylic acid represent

intermediate stages in the conversion of tryptophane into scatole and indole respectively, since micro-organisms are frequently capable of effecting the removal of carbon dioxide from carboxylic acids (cf. p. 76). This change, however, apparently does not take place so easily when the ready formed indole acids are subjected to the action of bacteria.

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