[FROM THE PHYSIOLOGICAL LABORATORY OF THE CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK.]

THE FATE OF THE AMINO ACIDS IN THE ORGANISM.[1]

BY GRAHAM LUSK.

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Only a few years ago the chemical composition of protein was absolutely unknown. In many quarters the accepted idea was that voiced by Bidder and Schmidt in 1852, namely that all the nitrogen of protein together with enough carbon, hydrogen and oxygen to form urea were split from combination in protein, while the remainder was burned to carbon dioxide and water, thereby yielding heat to the organism. Voit believed that this non-nitrogenous remainder yielded carbohydrate, as was particularly evident in diabetes and also fat. Indeed the conception of the structure of the protein molecule was quite like that which Levene has so splendidly shown to be characteristic of nucleic acid, *i. e.*, a molecule made up of carbohydrate united with phosphoric acid on the one side, and with nitrogen-containing purine and pyramidine bases on the other. The modern conception of the protein molecule as a huge complex of amino acids riveted together dates from the work of Emil Fischer. Fischer has strung together eighteen of these amino acids in an artificial compound, a peptide called *l*-leucyl-triglycyl-*l*-leucyl-triglycyl-*l*-leucyl-octoglycyl-glycine, a body similar to peptone. Already Fischer has prepared over a hundred such artificial polypeptides.

Proteins differ, as they contain different amino acids. Fischer, in an address given in 1907, expressed the opinion that the proteins then known were merely mixtures of substances, and were not pure individuals. He would not attribute all the great multitude of amino acids found on hydrolysis to a single chemical unit. Osborne, however, takes a different view and believes that his beautifully prepared crystals of plant protein "show a constancy of properties and ultimate composition between successive fractional precipitations which give no reason for believing the substance to be a mixture of two or more individuals." He adds: "Of twenty-three seed proteins which have been hydrolyzed all have yielded leucine, proline, phenylalanine, aspartic acid, glutamic acid, tyrosine histidine, arginine and ammonia. Glycocoll, lysine and tryptophane are the only amino acids which have been proved lacking in any of these proteins." Other amino acids not mentioned above, but frequently revealed, are alanine, valine, serine, cystine, proline, and oxyproline. The chains of amino acids forming this complex must be of immense size to comprise the many and varied elements set forth above. Osborne calculates the molecular weight of protein at 15000 or some higher multiple of this.

[1] A paper read before the New York Section of the Chemical Society, February 11, 1910.

In general, the same constituent amino acids are present in both plant and animal protein. There is no fundamental dividing line. Hence the animal may eat the plant protein, break it by digestion into its constituent amino acids, and then reconstruct these into proteins of another order, those characteristic of animal tissues. Osborne[1] calls attention to the fact that the food proteins which are found in flour are those which are prepared within the endosperm of wheat for the nutrition of the wheat embryo. In the commercial process of milling these embryos are separated and discarded. Bread therefore contains the same nutrient proteins as those provided for the embryo of the wheat.

Osborne finds that seeds which are botanically closely related contain similar food proteins, although those of the leguminous seeds differ greatly from those of the cereals. Hence the developing plant embryo is supplied with a definite food which for each individual of the same species is the same, but for those of different species is different.

It is the same thing in the animal. The genitalia of the fasting salmon grow at the expense of the muscle tissue, as was shown by Miescher, and Jägerroos has adduced evidence which shows that the young of a pregnant dog may be largely nourished at the expense of the protein of the maternal organism. And at all times the unborn animal is furnished with nutrient material of the mother's blood.

Rubner has classified food protein into three divisions representing three varieties of physiological use. The first is the "wear and tear" quota or the amount required to balance the normal waste of tissue protein. The second in the "growth quota," or that necessary for the growth of the body. The third is the "dynamic quota" or that which is used merely to maintain the energy of the cells and whose function may be replaced by carbohydrate or by fat.

In adult life the fundamental use for food protein is to prevent the loss of body protein. If the organism be given fat or carbohydrate alone there will be a constant loss of body protein, the "wear and tear" quota of Rubner. This will continue unless new protein or amino acids are given to replace that lost to the body. Here only the proper array of amino acids will furnish new materials for replacement of the old. For example, if tryptophane be absent from the food, no replacement is possible.

In the growing and even in the adult animal there are conditions in which protein is added to the body. That portion of the food protein retained for growth is the "growth quota." This growth is accomplished at the expense of amino acids formed from ingested protein. Very often protein is taken in excess of that required for the replacement of the "wear and tear" quota and in such excess that the optimum protein

[1] Osborne "The Vegetable Proteins," 1909.

content of the cells is reached and no new deposit from the "growth quota" is possible. What then becomes of the excess? Instead of such protein being retained in the organism, it is broken up, its nitrogen is found in the urine as urea, and its deaminized remainder furnishes fuel for the organism. This is the "dynamic quota."

Modern knowledge indicates that the amino-acid nitrogen not used for growth is split off from its combination in the form of ammonia and this ammonia, uniting with carbon dioxide, is carried as ammonium carbonate to the liver and there synthesized to urea. Whenever single amino acids are ingested their nitrogen content appears in the form of urea in the urine. Convincing and recent evidence of this theory is that afforded by Otto Cohnheim, who filled the intestines of fish with albumoses and on suspending the intestines in saline (Ringer's) solution witnessed a large evolution of ammonia. Amino acids similarly treated gave the same results. Very striking also are the experiments of Weinland on the larvae of the blow-fly, which derive their nutriment from meat. These larvae may devour an amount of meat equal to sixty per cent. of their own weight within a space of two days. A large part of this meat protein they convert into fat, the nitrogen content being eliminated as ammonia. When this well-established biological principle of the origin of fat from protein is considered in connection with the fact that in diabetes sugar may arise from protein to the extent of nearly sixty per cent., it becomes evident that there may be a condition of nutrition in which protein is used neither for repair nor for growth, but simply to be deaminized and subsequently to act like fat or carbohydrate as nutritive materials for the organism. This represents the "dynamic quota" of Rubner, the portion of food protein used for energy alone and which may be equally well furnished in the form of fat or carbohydrate.

How, then, does such a conversion of amino acids into carbohydrates and fat occur? To have a complete knowledge concerning this, one must know the fate of each individual amino acid ingested into the organism.

Dr. Ringer and I have recently taken up the subject of sugar production from amino acids. We have given various such acids to diabetic dogs. We have noticed that the nitrogen elimination in the urine increased in amounts which corresponded to that ingested and that this increase was all due to urea nitrogen. We have given glycocoll, alanine, aspartic acid, glutamic acid, containing respectively two, three, four and five carbon atoms. It was found that all the glycocoll and all the alanine were converted into glucose, whereas three of the carbon atoms contained in aspartic and glutamic acids were so converted.

The process of the deamination of these substances is believed to be one of hydrolysis. After this fashion glycolic acid would be produced

from glycocoll. If this were converted into glycolic aldehyde, then three molecules of the latter would form one of sugar, and indeed the subcutaneous injection of glycolic aldehyde into a rabbit leads to the elimination of sugar in the urine.[1] These reactions would thus be written:

$$\begin{array}{llllll} CH_2NH_2 + H_2O & CH_2OH & \longrightarrow & CH_2OH & & \\ 3\,| & 3\,| & -3\,O & 3\,| & \longrightarrow & C_6H_{12}O_6 \\ COOH & COOH & & CHO & & \\ \longrightarrow & & & & & \end{array}$$

Glycocoll. Glycolic acid. Glycolaldehyde. Glucose.

Next, in the case of the amino-acid alanine, we found that it also may be completely converted into dextrose. The reaction may involve the production of lactic acid from alanine by hydrolysis and the synthesis of two molecules of lactic acid into dextrose. This reaction would read:

$$\begin{array}{llll} CH_3 & & CH_3 & \\ | & & | & \\ 2CHNH_2 & H_2O & 2CHOH & \longrightarrow \; C_6H_{12}O_6 \\ | & & | & \\ COOH & \longrightarrow & COOH & \end{array}$$

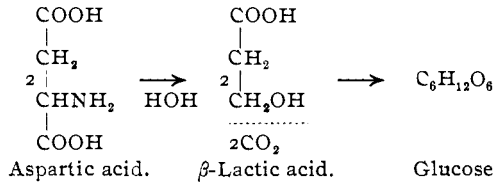
Alanine. Lactic acid. Glucose.

Several years ago Arthur Mandel and I gave a diabetic dog subcutaneous injections of *d*-lactic acid and saw that it was completely converted into glucose. Lactic acid therefore could circulate throughout the body's tissues without being oxidized and when it reached the liver be synthesized to glucose and be eliminated through the kidney of the diabetic. This introduces the question whether lactic acid can ever be directly oxidized by the organism or whether it must not first be converted into glucose. This fate of alanine is also suggested by the work of Neuberg, who gave alanine to a normal rabbit and found glycogen stored in the liver and lactic acid eliminated in the urine. The idea that lactic acid of itself cannot be oxidized accords with the work of Neubauer,[2] which indicates that the deamination of the side chain of tyrosine is not accomplished by simple hydrolysis but by a simultaneous oxidation process, with the production of a ketone instead of an alcohol. The ketone is combustible in the organism, the alcohol not. This reaction will be taken up in connection with the fate of tyrosine. According to this view, pyroracemic acid, $CH_3COCOOH$, would have to be the product of the oxidative deamination of alanine, if the latter were to be directly oxidized. Since the deaminized acid is protected from oxidation, it appears to be probable that lactic acid is the primary product involved in this case. Hence the normal fate of alanine occurs by way of hydrolysis to lactic acid which the organism cannot oxidize except indirectly by synthesis to glucose.

[1] Mayer, *Z. physiologische Chemie, Bd.*, 38, 151 (1903).

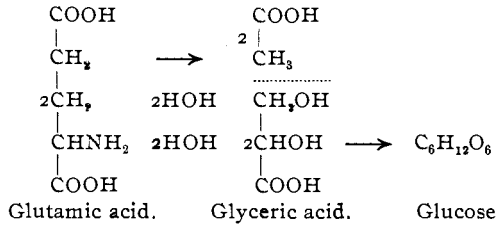
[2] Neubauer, "Ueber die Abbau der Aminosäuren," Habilitationsschrift, 1908.

When Dr. Ringer and I gave aspartic acid to a dog with phlorhidzin glucosuria we observed an elimination of extra sugar in the urine equal to the conversion of three out of its four carbon atoms into glucose. The probable course of metabolism in this instance is as follows:



It is interesting in this connection to note that Höckendorf[1] has observed an increase in the sugar elimination following administration of propyl alcohol to a phlorhidzinized dog, and although some of his results must be accepted with a certain reserve, Dr. Ringer and I have been able to confirm the above observation. Höckendorf's method of single injections of phlorhidzin each day instead of at eight hour intervals calls for severe criticism.[2]

Concerning the fate of glutamic acid where again three atoms of the molecule go over into glucose, the following argument is proposed. When fatty acids are oxidized in the body Knopp has shown that there is a primary oxidation at the β-carbon atom with a cleavage of acetic acid from the chain, which latter is then readily destroyed. If such a method were employed in the metabolism of glutamic acid, acetic acid and glyceric acid would result, the latter of which might be converted into glucose. The reaction would be as follows:



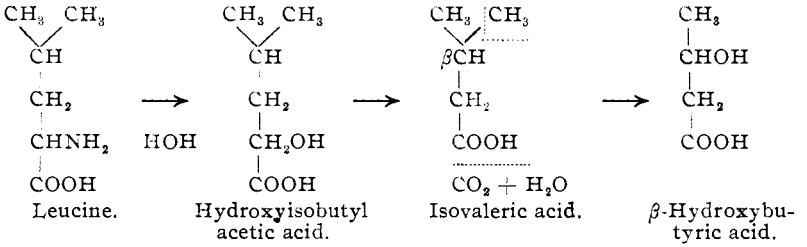
Ringer and I, in confirmation of this theory, have given a diabetic dog sodium acetate without increasing the quantity of glucose in the urine, whereas the administration of glyceric acid caused a large increase in the output of glucose.

Since serine, on deamination, would be converted into glyceric acid, it is apparent that this amino acid also is probably convertible into glucose.

[1] Höckendorf, *Biochemische Zeitschrift* (1909), *Bd.*, **23**, 295.

[2] See Stiles and Lusk, *Am. J. Physiol.*, **10**, 67 (1903).

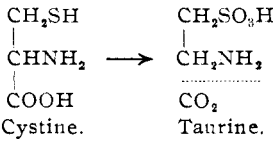
Halsey[1] obtained conflicting results after giving leucine to phlorhidzinized dogs, and doubts the conversion of leucine into glucose. Baer and Blum[2] have found an increased output of β-hydroxybutyric acid in the urine after giving leucine to a diabetic man and the following transformation has been attributed to it:



The fate of *valine* is unknown. Whether it is converted into glucose or not is yet to be determined. Baer and Blum report that it is not convertible into β-hydroxybutyric acid.

Of lysine, a diaminocaproic acid, one can only speculate that it may yield glucose, perhaps to the extent of half of its molecule. Less probable appears the prospect of obtaining glucose from arginine. The metabolism of pyrrole derivatives, such as proline, of indole, of derivatives like tryptophane, or of histidine with its imidazole ring, is too obscurely known to make any prediction.

Cystine, with its sulphur content, is the mother substance of the taurine of the bile and apparently is deaminized less readily than other amino acids. Its conversion into taurine is given by Friedmann as follows:

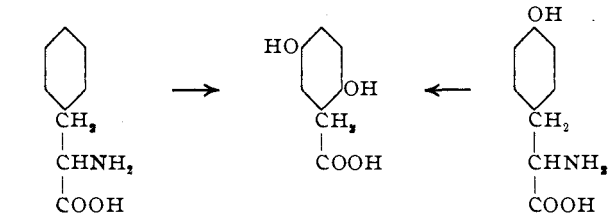


Any further intermediary products of cystine metabolism are unknown.

Finally there remain tyrosine and phenylalanine to be considered. The fate of these acids has been in part revealed by the work of Neubauer and Falta[3] and of Neubauer[4] alone. Under ordinary circumstances these acids are oxidized in the body with the complete destruction of the benzene ring. The breaking up of the ring in these substances is dependent upon the presence of the amino radicle in the *alpha*-position on the side chain. In a disease called alkaptonuria these substances only reach the

[1] Halsey, *Am. J. Physiol.*, **10**, 229 (1904).
[2] Baer and Blum, *Arch. exper. Path. Pharm.*, **4**, 89 (1906).
[3] Neubauer and Falta, *Z. physiol. Chem.*, **42**, 81 (1904).
[4] Neubauer, *Loc. cit.*

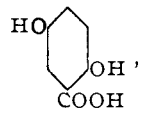
homogentisic acid stage and are then eliminated in the urine. Homogentisic acid, which is destroyed by a normal individual, cannot be oxidized by the alkaptonuric patient any more than glucose can be destroyed by the diabetic. The general transformation is as follows:



Phenylalanine. Homogentisic acid. Tyrosine.

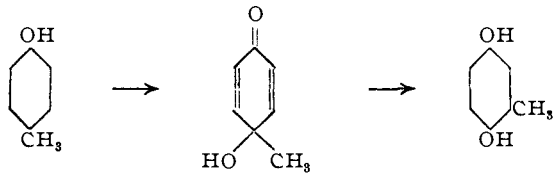
Neubauer states that the hydroxyl group on the benzene ring must be in the para position, or the transformation into homogentisic acid is impossible.

Neubauer finds that the alkaptonuric has lost the power to break the benzene ring. He confirms this by showing that gentisic acid,



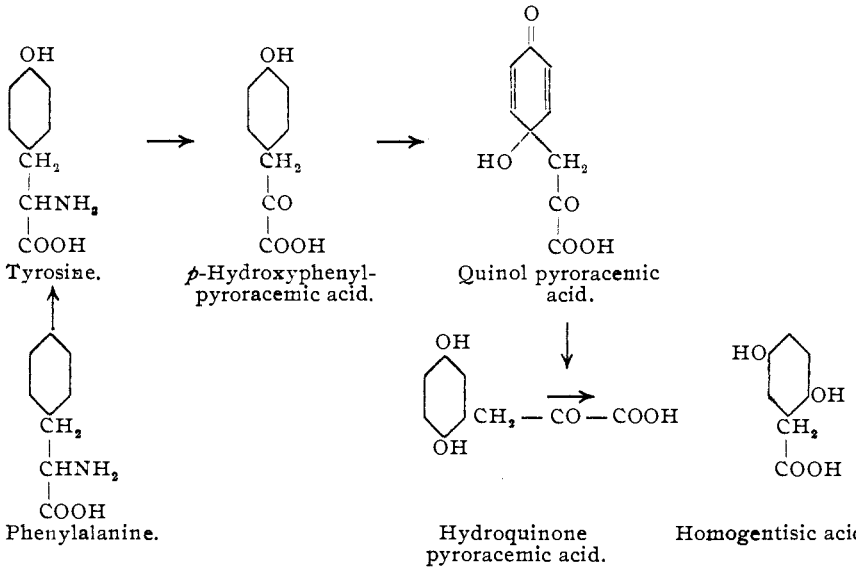
is oxidized by the normal individual but is not attacked by a person with alkaptonuria. He is astonished at the discovery that the alcohol, hydroxyphenyllactic acid, which is formed from tyrosine by hydrolytic deamination, is not oxidized into homogentisic acid by the organism, whereas the ketone, oxyphenylpyroracemic acid is so converted. From this he reaches the important conclusion that in this case there is oxidative deamination and not deamination by simple hydrolysis.

Erich Meyer was the first to call attention to the possibility of the conversion of tyrosine into homogentisic acid through a quinol stage. This is illustrated by the laboratory oxidation of paracresol with persulphuric acid.



p-Cresol. *p*-Toluquinone. Toluhydroquinone.

The formation of homogentisic acid from tyrosine presents so close an analogy to the above that Neubauer assumes a similar process. The complete reaction would then be:



It may be of interest to state in passing that this work on the fate of tyrosine was accomplished by a physician in one of the great medical clinics of Germany where opportunities for such work are liberally afforded.

Regarding the further fate of homogentisic acid, the observation of Jaffé[1] that after feeding benzene to animals, muconic acid may be detected in the urine, is of importance. This indicates that the oxidation of the benzene nucleus takes place as follows:

Should this be the method of breaking the ring, then the straight chain of the dibasic hydroxy acid which would be produced from homogentisic acid would be readily oxidized. It is said that tyrosine, when ingested in diabetes, increases the amount of acetone bodies in the urine (Baer and Blum).

Dr. Ringer and I have investigated the subject of the possibility of its yielding glucose in the body and so far our results have been negative. Since inosite, a hexahydroxybenzene, is completely destroyed by the diabetic, negative results from tyrosine were to have been expected.

In this discussion of the fate of the amino acids in the organism it has been brought out that in their final cleavage products are found either

[1] Jaffé, *Z. physiol. Chem.*, **72**, 58 (1909).

sugar or fatty acid. It is these substances which are the energy givers to the cells. They act as fuel for the machinery of life. In their preparation from protein there are many cleavages, and perhaps oxidations which yield free heat to the organism without ever giving vital energy to the cells. In consequence of this, protein ingestion results in a considerable increase in the amount of heat liberated within the body. This is the *specific dynamic action* of protein in increasing heat production in accordance with the doctrines of Rubner.

As regards the sugar production from meat, for example, one may now in a crude way estimate its source. I have found that in diabetes fifty-eight parts of glucose may arise from one hundred parts of meat protein. Osborne's[1] latest results of the analysis of ox muscle include the following figures:

	Parts in 100.
Glycocoll	2.06
Alanine	3.72
Aspartic acid	4.51
Glutamic acid	15.49

In a paper read in Boston recently, Osborne indicated that owing to the inaccuracy of the Fischer ester method, the figures for alanine and aspartic acid may easily be twice the amounts stated above. Likewise, the work of Parker and Lusk indicates that four grams of glycocoll may arise in the metabolism of flesh within the body of a rabbit, and similar results have been obtained in man. If we substitute these increased quantities for the analytical results obtained by Osborne and then calculate the sugar production from the various acids as obtained in the laboratory, we arrive at the following results:

	Parts in 100 of ox muscle (estimated).	Glucose production (calculated).
Glycocoll	4.0	3.2
Alanine	7.5	7.5
Aspartic acid	9.0	6.1
Glutamic acid	15.5	9.5
	36.0	26.3

From these four acids, therefore, may arise twenty-six, or nearly half of the total of fifty-eight parts, of glucose which may originate from protein in metabolism.

Speaking more strictly, forty-five per cent. of the total sugar production from protein in diabetes may arise from the four acids named, which make up thirty-six per cent. of meat protein. It is only a question of time for the attainment of a complete solution of the problem.

The chemistry of the protein molecule throws new light upon bio-

[1] Osborne and Jones, *Am. J. Physiol.*, **24**, 437 (1909).

logical questions, though it does not explain life itself any more than heredity is explained by the chemistry of nucleic acid.

SOME COLLOID-CHEMICAL ASPECTS OF DIGESTION, WITH ULTRAMICROSCOPIC OBSERVATIONS.

BY JEROME ALEXANDER.

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The changes which occur during digestion, and in fact in almost all physiological processes, are remarkable not only because of their very profound nature, but also because they are produced at comparatively low temperatures and in the presence of extremely dilute reagents. The living organism disintegrates proteins, oxidizes carbohydrates, and with the same apparent ease synthesizes substances of great complexity. Powerful reagents and high temperatures, which would be destructive to life, are necessary to bring about changes of this character under ordinary laboratory conditions.

The digestive process is preliminary to the actual absorption and use of food by the organism, and has for its object the modification or change of the ingested food into such forms or such substances as may be absorbed in the lower part of the digestive tube. To have a correct understanding of the absorption of the products of digestion, we must bear in mind the fact that the walls of the digestive tract act as semipermeable colloidal membranes, and that absorption consists in diffusion into or through these membranes or their constituent cells. Substances in crystalloidal solution, and colloidal sols whose particles are sufficiently small, represent then the two classes of digestion products which are diffusible and therefore absorbable.

Food as ingested consists mainly of substances that may be grouped into two classes:

1. Crystalloids—such as water, sugars, sodium chloride, etc.
2. Colloids—such as starch, proteins, etc.

The crystalloids are usually absorbed directly, although sucrose, for example, undergoes inversion. The colloids, as a rule, are not directly absorbable and for the most part digestion consists in the disintegration[1] of the colloidal complexes of the food, so that they can actually diffuse into the organism and there undergo further changes. Colloidal gels or even sols whose particles are of large size are, practically speaking, non-diffusible, and must therefore be reduced to a more finely dispersed state. So strong is the analogy between digestion and colloidal disintegration that Thomas Graham,[2] the father of colloid chemistry, coined

[1] It must of course be borne in mind that actual chemical changes may and very frequently do accompany changes of this character.

[2] "On the Properties of Silicic Acid and Other Analogous Colloidal Substances," *Proc. Roy. Soc.*, June 16, 1864.