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THE PRESENT SIGNIFICANCE OF THE AMINO-ACIDS IN PHYSIOLOGY AND PATHOLOGY *

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CHEMICAL NATURE OF THE AMINO-ACIDS

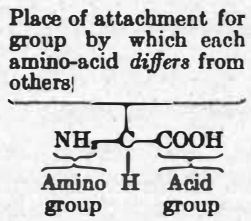
THIS discussion is inserted because it will be necessary, for the ready understanding of the later parts, that speaker and audience shall have in mind from the same viewpoint two or three significant chemical characters of the amino-acids as a class.

We know, chiefly as the result of the researches of Fischer,[1] Kossel, and their collaborators, that the amino-acids are the units or building stones out of which the protein molecule is constructed. They are the final products obtained when proteins are hydrolyzed by strong acids, or by the action of pepsin, trypsin, and erepsin in the alimentary canal. In the characteristic points of their structure, the amino-acids are all alike. That is, they belong to a type, and we have only to understand the type in order to become fairly well acquainted with them all. We have placed on chart I what may be designated as a decapitated amino-acid. It is the portion of the molecule which is common to all the amino-acids, and its formula expresses the chemical properties which are characteristic of them as a class. Of these properties, the most striking are due to the occurrence in the same molecule of an amino group, with a basicity like that of ammonia, and an acid group with an acidity like that of acetic acid. Hence, from these two groups the name, amino-acid. The amino and acid groups are joined by a single carbon atom which serves as a bridge between them. This structure occurs in every amino-acid. The central carbon atom is the centre of the entire molecule. It is flanked on one side by the amino group, on the other by the acid group, a third valence is occupied by an insignificant hydrogen atom, while to the fourth, which in the decapitated formula is left pointing upwards and unattached, is fastened what we may term

* Delivered January 15, 1916.

the head of the molecule. This is different in every amino-acid. It is the source of the individuality of each. There are eighteen varieties of such heads, as may be seen by glancing at Chart 3, and, corresponding to them, eighteen distinct amino-acids, each possessing the common group characteristics indicated by the body, and, in addition, another set of chemical characters entirely belonging to itself, and indicated by the structure of the head.

CHART I

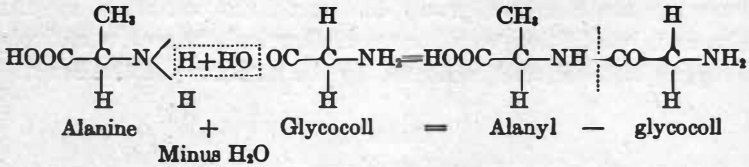


Formula of a decapitated amino-acid.

Our interest centres mainly, however, on the family body and its characteristics. Of these we have already mentioned the basic and acid properties combined in the single substance. I would call your attention to one other, also dependent upon the simultaneous presence of amino and carboxyl acid groups, and this is the ability of the amino-acids to dovetail themselves together and form molecular chains of infinite length. It is this ability which makes possible the existence of such complex substances as proteins and protoplasm. We have indicated in Chart 2 the mechanism by which the linking of the units in the chain is accomplished. It represents the coupling of two of the amino-acids, alanine and glycocoll. We see that the amino group of the alanine condenses with the acid group of the glycocoll, with elimination of a molecule of water. The result is that the two amino-acids are linked together and form a peptide, alanyl-glycocoll, called a dipeptide because it contains two amino-acids. This peptide, however, like the original amino-acid, still contains one free amino group at one end and an acid group at the other. It can, therefore, couple on another amino-acid at either end. These could still condense with two more, and so on *ad infinitum*.

Protein molecules are chains composed of scores or hundreds of amino-acids joined together in this way.

CHART II



Coupling of the amino-acids, alanine and glycocoll, to form the di-peptide, alanyl-glycocoll.

When proteins are hydrolyzed or digested, by trypsin for example, the links of the chain are broken apart and we have first somewhat shorter chains, the albumoses, then still shorter chains, the peptones, which are mixtures of peptides, and finally the separate amino-acids. This successive breaking down of the long protein chain into shorter chains and finally into the separate links constitutes the process of digestion. The building up of new protein consists of the reverse, namely, the linking together of the amino-acids into new chains.

To show at the same time the nature of the protein structure and of the different amino-acids which take part in it, I have placed on Chart 3 the structural formula of an imaginary protein containing one molecule of each of the eighteen known amino-acids. We see along the bottom of the row the repetition of the familiar family body, the central carbon atom flanked in each case by the accompanying amino and acid groups. Above, however, we have the individual heads of the different units in great variety. We might liken the protein chain to a long train of autos, all with small, black, uniform bodies, but with tops of eighteen different shapes, and of three different colors according to whether the properties they carry are acid, basic, or neutral. It will be noted that among the amino-acids the neutral party is in the great majority, which fact accounts in part for the approximately neutral reaction of most proteins.

Whenever a peptide linking in the protein chain is broken by hydrolysis we have at once one amino group and one acid group

CHART III

FORMULA OF AN IMAGINARY PROTEIN CONTAINING ONE MOLECULE OF EACH KNOWN AMINO - ACID.

BASIC AMINO-ACIDS. Two or more basic groups to one acid COOH group. The three "hexone bases." Resemble ammonia in alkalinity.			NEUTRAL AMINO-ACIDS. CONTAIN ONE AMINO GROUP AND ONE CARBOXYL GROUP WHICH NEUTRALIZE EACH OTHER.												ACID AMINO-ACIDS Two acid COOH groups to one NH_2 group. Resemble acetic acid in acidity.	
			Aromatic, containing benzene rings.			Containing pyrrolidine rings.		Containing only aliphatic chains straight or branched.								
ARGININE	LYSINE	HISTIDINE	PHENYLALANINE	TYROSINE	TRYPTOPHANE	PROLINE	OXYPROLINE	NORLEUCINE	LEUCINE	ISOLEUCINE	VALINE	ALANINE	SERINE	GLYCOCOLL	CYSTEINE	GLUTAMIC ACID / AMIDE	ASPARTIC ACID
FORM GLUCOSE +	—	—	—	—	—	+	—	?	—	—	—	+	+	+	+	—	+
" ACETONE -	—	+	+	+	—	—	?	?	+	?	—	—	?	—	?	—	—

set free. Chemically stated, the hydrolysis or digestion of a protein consists in the splitting of some or all of the peptide linkings between its amino-acids, with the formation of new acid and amino groups in exact proportion to the extent of the digestion. In order to determine the occurrence and extent of digestion, with exactness, therefore, we must determine either the amino groups or the acid carboxyl groups that are formed by the process. Only by such means can we obtain results capable of exact chemical interpretation. The various physical methods of colloid coagulation, viscosity determinations, precipitation, etc., useful though they have been, are only rough and indirect measures of the chemical process which constitutes digestion. For a direct measure we must determine either the amino or the acid groups which are set free. Furthermore, our only chemical means for estimating the complexity of any intermediate product, such as a peptone or albumose, lies in determining the ratio between the free amino or acid groups which it possesses and those which are found after it has been completely hydrolyzed. Thus the amino nitrogen of a dipeptide, composed of two amino-acids, is doubled by hydrolysis, that of a tripeptide is tripled, of a tetrapeptide quadrupled, etc.

All of the above facts concerning the relationship between the progress of digestion and the uncovering of amino and carboxyl groups were recognized over ten years ago as soon as Emil Fischer[1] had demonstrated the peptide nature of the protein molecule. As the result of this knowledge, the desirability for quantitative methods for the determination of either amino or carboxyl groups became evident. As generally occurs, when the need became clear the methods were invented. Since the use of these methods is most intimately connected with the experimental work of which I shall speak, I shall stop here for a moment to discuss the two which have found most general application.

The first was published by Soerensen in 1908.[2] It was based on the fact, discovered by Schiff,[3] that formaldehyde added to the water solution of an amino-acid combines with the amino group, and that in consequence the amino group loses its alkalinity. As the formaldehyde itself is neutral, the effect of the reaction is

to reduce the amount of titratable alkali, or increase the titratable acid, by an amount equivalent to the amino nitrogen present. Soerensen tested this method with practically all the known amino-acids, and worked out the details necessary for attainment of the most accurate results. In brief the formol titration of Soerensen is performed by rendering the solution of amino-acid neutral to litmus, adding formaldehyde, and then titrating against phenolphthalein the acid which has been set free by the removal of the alkaline capacity of the amino groups. The ingenuity and simplicity of this method led to its immediate adoption by biological chemists, and many investigations of value have already been conducted with it.

The second method was published by myself in 1909.[4] It rested on the well-known reaction of amines with nitrous acid, as the result of which the nitrogen of the amino group is transformed into nitrogen gas. In order to determine the amount of amino nitrogen present, therefore, one has merely to add nitrous acid and measure the volume of nitrogen gas which is set free by the reaction. The principle is similar to that of urea determination by the hypobromite method. We were able so to fix conditions that the reaction is complete in three minutes. A considerable amount of nitric oxide gas is evolved by spontaneous decomposition of the nitrous acid, and this gas is used to drive the air out of the apparatus before the amino-acid solution is admitted. At the end of the reaction the nitric oxide is absorbed by permanganate solution, and the pure nitrogen gas given off by the amino group is measured.

In the apparatus which finally proved most convenient the entire process can be carried out in a few minutes and results obtained with a high degree of accuracy. As compared with the formol titration, the nitrous acid method has the disadvantage that it requires a special apparatus. It has several advantages, however, in that the readings can be made with a higher degree of accuracy, that the determination is not interfered with by the presence of colored substances in the solution, and that accurate results can be obtained with extremely small amounts of material. With a micro-apparatus readings significant to .001 mg. of amino

nitrogen can be obtained, while a quarter of a milligram is as small an amount as can be determined by the formol method. Because of these advantages, which were important in the conditions under which we worked, we have used the nitrous acid method in our own investigations on the fate of protein digestion products in the body.

The table below gives an idea of the nature of the results obtained in following the course of a protein digestion. It will be noted that there is a slight amount of amino nitrogen present before any digestion has occurred. This is due to the fact that one of the two amino groups of the lysine is free in the protein molecule. This was demonstrated in our laboratory by Birchard, who showed that in a representative series of proteins an amount of free amino nitrogen equal in all cases to half the lysine nitrogen could be demonstrated by the nitrous acid method. It will be noted from Chart III that one amino group in the guanidine nucleus of arginine is also free. This guanidine NH_2, for an unexplainable reason, however, fails to give some of the characteristic reactions of amino groups in general. It does not react with nitrous acid, nor with formol in the Soerensen titration, and therefore is responsible for none of the free amino nitrogen that is noted in the protein even before digestion has begun.

TABLE I.—INCREASE OF AMINO N DURING TRYPTIC DIGESTION OF 4 PER CENT. EDESTIN SOLUTION

Hours	C.c. of N gas from 10 c.c. solution	Per cent. of Hydrolysis
0	1.20	0.0
2	7.62	14.8
4	8.92	18.2
20	12.52	27.4
80	19.56	47.3
Complete hydrolysis with HCl	40.25	100.0

The above finishes our discussion of the organic chemistry of the amino acids, and the methods used for their determination. We shall now turn our attention to a study of the fate of protein

digestion products in the body. This study has been guided by the conception of the relationship between proteins and amino-acids which I have just outlined, and was carried out to a large extent with the aid of the nitrous acid method for the experimental investigation of that relationship.

PHYSIOLOGY OF THE AMINO-ACIDS

Before entering upon an account of these researches I must acknowledge the debt to my collaborators, without whom a large part of the work would have been impossible. I refer to Dr. Gustave Meyer, who collaborated in all the work thus far published, and to Cullen and McLean, members of the Rockefeller Hospital staff, to whose efforts are due results that will be reported for the first time this evening.

It is furthermore a pleasure, as well as a duty, to acknowledge my indebtedness to Dr. Levene, for six years my chief at the Rockefeller Institute. The work detailed this evening is a direct outgrowth of Levene's own researches on the proteins, was carried out with the constant inspiration of his enthusiasm, the help of his counsel, and of his generosity in making available every facility which the laboratory afforded, even at times to the delay of his own immediate work, the ultimate sacrifice that can be taken from a spirit such as his.

At the time these investigations were begun the old Liebig theory of protein metabolism had already long been abandoned, and in place of it there was considerable confusion. Liebig's belief was very simple. He thought that the food proteins were incorporated directly into the tissues of the animal. The only necessary preparation was that of putting the proteins into solution in order that they might be absorbed, and this purely physical change was the sole object of digestion. The better understanding of gastric digestion, Kühne's discovery of trypsin,[6] and finally Cohnheim's [7] demonstration of the action of erepsin in reducing proteoses to amino-acids, led inevitably to the conclusion that food proteins undergo not only physical, but chemical change in the alimentary canal, namely, that digestion is a

hydrolysis, and that the hydrolysis proceeds partially, if not entirely, to the stage of amino-acids before the products are absorbed.

The results of a century of laborious research by many keen investigators from Spallanzani and Beaumont to Cohnheim may be summarized as follows: The proteins enter the stomach and are digested to the stage of albumoses; that is, the long protein chain of amino-acids is broken into somewhat shorter, but still very long, chains, and thereby the protein, which is usually insoluble, is transformed into soluble albumoses. The latter are not absorbed, however. London, working in Petrograd, has shown conclusively that no absorption takes place from the stomach during normal digestion.[8] The albumoses all pass down into the intestine, where they meet the pancreatic juice and are split, partly into short chains of a few amino-acids each, and partly entirely to free amino-acids. That the free amino-acids constitute a considerable part of the products of intestinal digestion was demonstrated by Abderhalden,[9] who isolated most of the known amino-acids from intestinal contents. That the entire mass of products, aside from the free amino-acids, consists of short chain peptides was shown by White and myself [10] with the nitrous acid method in the case of one of the lower animals, the dog fish. This work was done in 1910 at Woods Hole. Shortly after, London, by means of the formol titration, obtained results of the same nature with dogs.[11] Finally, either before or after entering the intestinal wall, the products encounter a third hydrolytic enzyme, erepsin, which is capable of completing the hydrolysis to the stage of amino-acids, in which form it appears that at least the greater part of the protein nitrogen is absorbed.

You will note that the above summary, which indicates the stage of our knowledge five years ago concerning the mechanism of protein nutrition, stops short against the intestinal wall. This was, as a matter of fact, the place where facts ceased and theories began. What happens to the amino-acids and peptides after they are absorbed from the intestine was not known. Neither amino-acids nor peptides could be detected in the blood. As the veteran Pflüger pointed out, the failure to detect either amino-

acids or peptides in the circulation might well be due to a lack of sufficiently delicate methods, for the flow of portal blood is so fast that even a maximum absorption of nitrogen might cause but a very small concentration in the blood at any given moment. Folin, in his classic paper on the theory of protein metabolism,[12] published eleven years ago, took the same stand. In order to fill the gap in experimental results, however, other authors proposed two theories. (1) The amino-acids are decomposed into ammonia and non-nitrogenous residues while passing the intestinal wall. (2) They are synthesized into blood protein. The latter theory, it will be noted, was particularly convenient, because it not only explained the failure to find amino-acids in the blood, but also gave the source of the blood proteins. It was especially championed by Abderhalden.

The development of adequate methods, however, showed that Pflüger and Folin were right, and both of the above explanatory theories became unnecessary. The first theory received its death blow at the hands of Folin. With the extremely delicate calorimetric method for the determination of ammonia which he devised, he was able to show that absorption of amino-acids from the intestine is accompanied by no increase whatever in the ammonia of the portal blood. When put to the rigid test of quantitative experiment, the deaminizing ability of the intestine vanished into thin air.

The fate of the resynthesis theory was similar. The sole foundation on which it rested was the negative results of attempts to find in the blood digestion products, either peptones or amino-acids. As soon as quantitative methods, namely, the formol titration and the nitrous acid method, were applied, however, it was shown by investigators working independently with each that the blood does contain amino-acids, and that they increase markedly during digestion. This was shown by Delaunay, working in Bordeaux, with the formol method, and with the nitrous acid method by Meyer and myself in the Rockefeller Institute. The normal amino-acid concentration in the blood of both dogs and men is near that of sugar, about 0.1 per cent., and it may be nearly doubled in the portal blood as a result of a heavy protein meal.

The force of these results was further strengthened by Abel, Rowntree, and Turner in their remarkable experiments with vividiffusion.[16] These experimenters passed the blood of living dogs through collodion tubes immersed in salt solution into which the non-colloid substances of the blood diffused. From the diffusing substances thus obtained they were able to separate in pure condition and identify several of the individual amino-acids. Abderhalden then also applied dialysis to blood and was able to obtain most of the amino-acids in sufficient amounts to identify them.

After entering the circulation, amino-acids disappear from it again very quickly. Within five minutes after 12 grams of alanine had been injected into the vein of a dog, 90 per cent. had disappeared from the circulation. A similarly rapid removal must occur during digestion, otherwise amino-acids would accumulate in much larger amounts in the blood than we observe. The question then naturally raised itself: What becomes of the amino-acids when they vanish from the circulation? Are they decomposed in the blood; are they at once somewhere synthesized into new protein; are they chemically incorporated into the complex molecules of the tissue protein; or are they merely absorbed by the tissues in general or by certain tissues in particular without undergoing any immediate change?

Analysis of the tissues of dogs which had received intravenous injections of known amounts of amino-acids answered these questions in favor of physical absorption.[17] In one experiment, which will serve as an example, the amount of amino nitrogen injected in the form of hydrolyzed casein was sufficient, if distributed evenly throughout the body, to raise the average amino nitrogen content of the tissues 40 mg. per 100 grams of tissue. The increases actually noted were: Muscles, 27 mg.; liver, 60 mg.; kidney, 60 mg.; intestinal wall, 50 mg. That the absorbed amino-acids could not have been in even loose chemical combination in the tissues was shown by the fact that they could be extracted by such mild agents as water, hot or cold, or dilute alcohol. They must have been held merely by physical forces.

The tissues, despite the great rapidity with which they absorbed amino-acids from the blood, never removed them from

it completely. An equilibrium is reached when, stated very roughly, the tissues contain about ten times the percentage of amino-acid present in the blood. From the fact that they are so much more concentrated in the tissues than in blood it is evident that the process by which they are picked out of the circulation is not a mere diffusion. If it were, we should find approximately equal concentration in both tissues and blood. The physical process, by which the exchange between blood and tissues is carried out, has not yet been definitely classified with any of the physical or physicochemical phenomena with which we are familiar. Until it is explained by such classification we cover our ignorance of the real nature of the process by giving it the general name of "absorption."

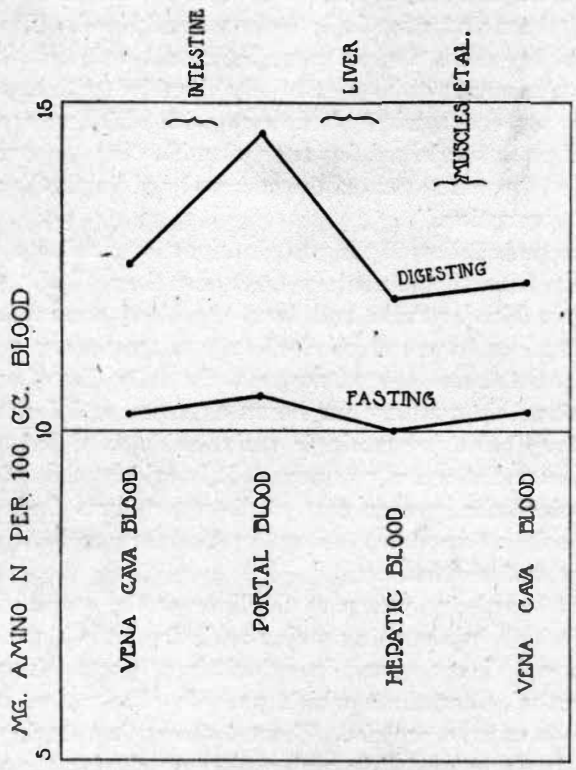
We have now followed the protein digestion products, that is, amino-acids, from the alimentary tract past the wall of the intestine into the blood stream and from the blood stream into the tissues. But we have yet reached only a temporary stopping place. Most of the protein nitrogen in the daily diet of an adult is excreted within twenty-four hours as urea; and Levene and Kober [18] found that when single amino-acids were fed to dogs they were excreted entirely as urea. It is evident that whatever stopping place the greater part of these products finds in the tissues is only a temporary refuge preliminary to their speedy destruction and elimination.

Present knowledge points to the liver as the organ which is most active both in absorbing amino-acids from the blood stream during normal digestion and in submitting them to the preliminary chemical alterations which precede elimination as urea or storage as reserve protein. Chart 4 shows that during digestion there is a greater fall in amino nitrogen during the passage of the liver (difference between portal and hepatic blood) than during passage through the entire remainder of the body (difference between arterial and vena cava blood). The liver is the organ to which the portal blood comes with its newly-acquired amino-acids and it is the liver that takes the lion's share of them. It follows as a necessary corollary that the liver must either store immense amounts of them after a heavy protein meal, or must

quickly transform them, either into urea for elimination, or into reserve protein for storage.

Further experiments have shown that the liver does not store amino-acids as such to an appreciable extent. Chemical trans-

CHART IV



The amino-acid content of the blood during fasting and protein digestion. Average of results from six fasting and six digesting dogs.

formation follows very quickly after absorption. This was shown in three different ways.

First, the tissues of dogs in fasting condition were compared in respect to their amino-acid content with those of dogs which were digesting or had digested large amounts of protein. It

was found that neither the livers nor other tissues of the fed animals contained a definitely greater store of amino-acids than did the tissues of the fasting animals. The digesting dogs must have either destroyed or condensed into protein all the amino-acids which they absorbed, and have done so at a rate which was practically parallel with that of absorption.

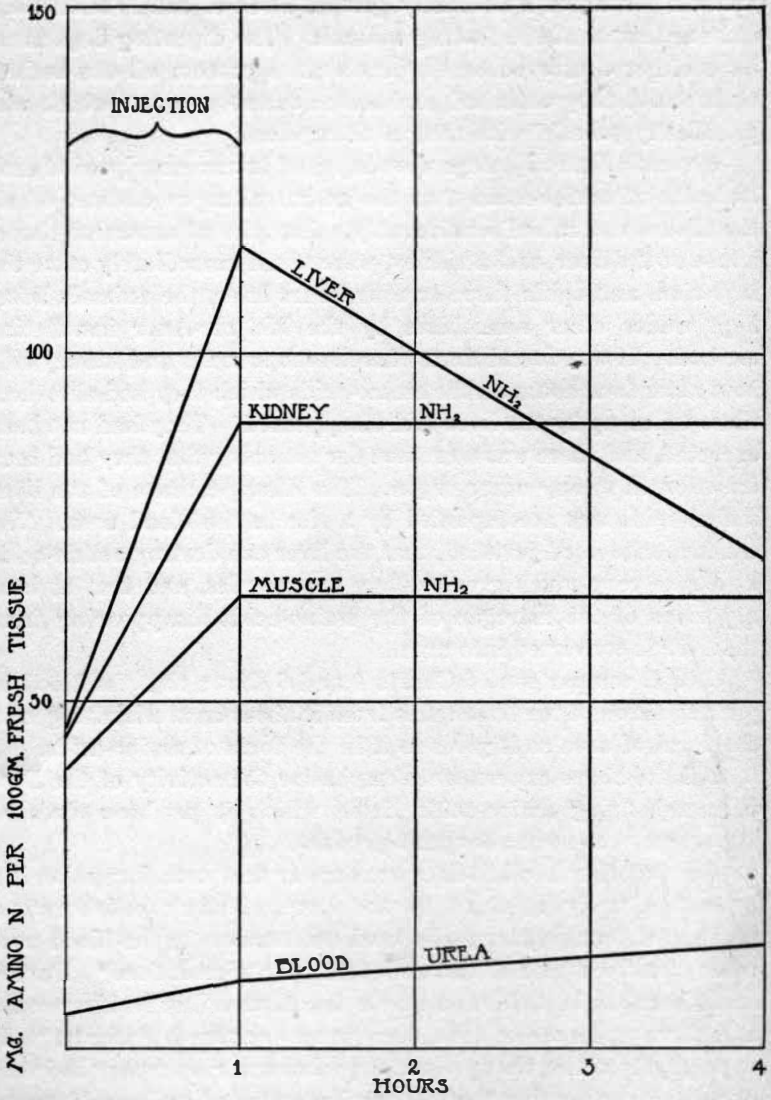
Second (Chart 5), dogs were injected intravenously with such amounts of amino-acids that the amino nitrogen content of all the tissues was raised considerably. Samples of muscular tissue, a lobe of the liver, and a kidney, were taken immediately after the injection, and again three or four hours later, the animals being kept under ether anæsthesia by the Meltzer-Auer insufflation method. It was found that, whereas the muscles and kidney still held after four hours all the amino-acids which they had absorbed, those taken up by the liver had disappeared. They had not been excreted, and there was no reason for assuming that they had been transferred to any other organ. The disappearance of the liver amino-acids was accompanied by a rise in the blood urea. The conclusion seemed justified, that the liver can destroy amino-acids at a rate very much greater than the muscles, and that at least a portion of the nitrogen of the amino-acids disappearing from the liver is converted into urea.

Third, comparison of Charts 4 and 6 shows that the blood in passing through the liver takes from it about as much nitrogen in the form of urea as it gives to it in the form of amino-acids.

All the above experiments emphasize the activity of the liver in metabolizing amino-acids, from which it produces urea as apparently the most abundant product.

On the other hand, it does not appear that urea formation is a process entirely confined to the liver. Folin's collaborators, Fiske and Sumner, have observed an increase in the blood urea when the liver was eliminated from the circulation.[19] Pavlov and Nencki in 1893 showed that a dog deprived of its liver could still form and excrete urea, though in decreased amounts.[20] It appears, therefore, that present experimental results may be interpreted by stating that the most active centre of amino-acid transformation, and of urea formation, appears to be the liver, but

CHART V

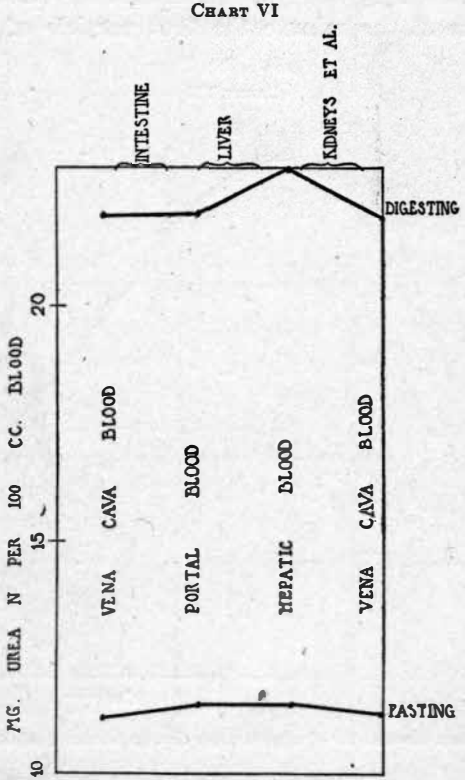


The absorption and retention, by different tissues, of amino-acids injected intravenously.

that the localization of the function is not absolute, and these processes also occur to some extent in other organs.

The next question to be raised is: Does the liver, during the digestion of a protein meal, wait till the other tissues are saturated with amino-acids, and then begin to destroy the unnecessary

CHART VI

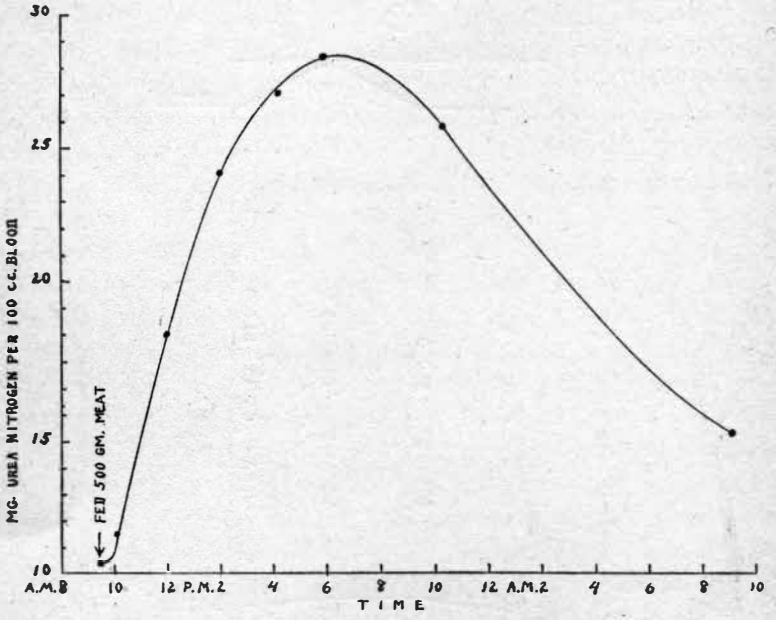


The blood urea during fasting and protein digestion. Average of results from six fasting and six digesting dogs.

excess, which is not needed by the organism, or does it begin to destroy the first that reach it in the portal blood? Unreasonable as it may seem, the latter behavior is what we observed. In order to test this point urea determinations were made at short intervals on blood from dogs which, after a two days' fast, had received

heavy meals of meat. It was found in all cases that the urea began to rise almost immediately after the meat was consumed. There was no interval of waiting commensurate with what might be expected if urea formation were delayed until after the tissues in general had replenished their store of amino-acids. It was furthermore shown by X-ray pictures that the blood urea began to rise at almost the minute the first particle of food passed

CHART VII



Time curve of blood urea changes during protein digestion.

from the stomach into the duodenum. Since London has shown that no absorption occurs until the chyme enters the intestine, our results indicate that the very advance guard of amino-acids entering the blood after a protein meal is, in part at least, immediately turned into urea. The interval between feeding and the beginning of urea formation is so short that this conclusion would really be forced upon us, even without the X-ray evidence. Un-

reasonable as it appears, the organism does not wait until it has absorbed sufficient protein digestion products to meet its immediate requirements, and thereafter begin to turn the surplus into urea. The very beginning of absorption stimulates the urea forming function into activity. This behavior explains the fact that no matter how depleted by disease or hunger the tissues of an individual may be, the greater part of the protein nitrogen which he may subsequently consume is excreted as urea, only a small portion being retained to rebuild the wasted tissues.

That urea is the form taken by all of the amino-acid nitrogen which disappears in the liver does not absolutely follow from our results. Pflüger was of the opinion that the liver cells store reserve protein from the food just as they store reserve carbohydrate in the form of glycogen. Our results do not at present exclude this possibility. In order to do so, we should have to prove that the liver gives out as urea an amount of nitrogen exactly equal to that which it absorbs as amino-acids, and our experimental technic does not yet enable us to say whether or not this is the case. The analytical methods are adequate, but the fact that the amino-acids are held for a certain time before they are destroyed, and that the urea also may not pass instantly from the liver tissue to the hepatic vein, make the striking of an exact balance between amino-acid intake and urea outgo a matter of experimental difficulty which has not yet been overcome. The possibility therefore remains open, though certainly not proven, that some of the amino-acids may be converted by the liver into a form of reserve protein which is stored like glycogen.

A word as to the significance of the free amino-acids which are stored as such by the tissues. They normally amount to from 2 to 4 per cent. of the dry weight of the various organs and might be regarded as a form of reserve food. That they are so in the same sense as fat and glycogen is, however, not the case. Reserve food supplies disappear during a prolonged fast. This occurs with glycogen and fat. It does not occur, even to a slight extent, with the amino-acids. If anything, they are slightly more abundant in the tissues of a fasting animal than in those of

one in a state of normal nutrition.[21] I believe that the explanation is that the free amino-acids, in the tissues as in the blood, are merely transitory bodies in the building up and the breaking down by body proteins. That all the aminoacids in the muscles of a well-nourished animal may have been immediately derived from food proteins would be believable. When, however, after a fortnight's fasting we find an equal or greater supply of free amino-acids in the muscles, we must attribute the source in this case to autolysis of the visibly disappearing tissues themselves. Consequently, it does not appear that the store of free amino-acid in the body functions to a significant extent as a food reserve, since it can neither be increased by feeding nor depleted by fasting. The free amino-acids are there both in blood and tissues, because they are intermediate steps in the never-ending processes of the building up and the breaking down of living protein.

We may, perhaps, most easily summarize the facts which have been brought out by tracing an amino-acid through the body as follows: Entering the alimentary tract as part of a protein molecule, it is set free by digestive hydrolysis and passes into the portal blood stream. It may be at once picked up by the liver and decomposed into urea, or perhaps synthesized into reserve protein. It may, however, pass by the liver and be absorbed from the blood by one of the other tissues. Here it may remain for a time before being incorporated into the tissue protein. The fact that a considerable store of amino-acids is always found in the tissues is proof that chemical incorporation does not instantly follow absorption from the blood stream. After a period of time, concerning the length of which we are absolutely ignorant, the tissue protein autolyzes, and the amino-acid returns to the depot of free amino-acids held by the tissue. From this depot it may pass back into the blood, be taken out by the liver, and destroyed. Or it may in some tissue be reincarnated into a new protein.

We have hitherto dealt with the physiology of the amino-acids without any recognition of the differences between the individual members of the family. Whether we are concerned with their

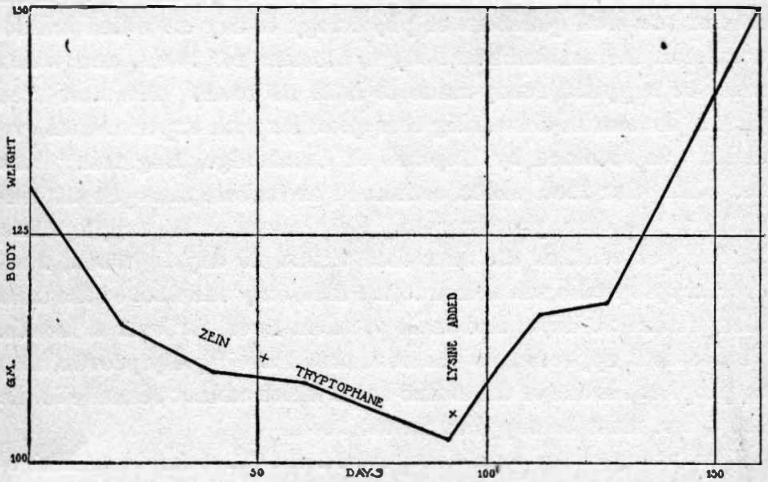
condensation into body proteins, or the manner in which, not being so condensed, they are destroyed, the individuality of the different amino-acids plays a most important rôle.

Let us consider briefly the indispensability of the different amino-acids for the nutrition of the body. All of the amino-acids which are known to occur in the native proteins enter into the structure of living protoplasm. The bacterium can synthesize them all from ammonia and sugar. Loeb has recently presented evidence that even lower animals, such as the banana fly, can also synthesize all of their amino-acids.[22] The higher animals can synthesize some, but must be supplied with others. One of the vital questions of physiology to-day is: which amino-acids can the mammalian body synthesize for itself, and which must be supplied ready-made to it in its food? The first even partial success in answering this question with experimental evidence was obtained by Hopkins of Cambridge, England.[23] He fed mice with food which contained as its sole nitrogenous constituent the corn protein zein. Zein contains no tryptophane. Eighty per cent. of the mice died within 20 days. When, however, tryptophane was added to the diet, only one-fifth of the mice died within 20 days, and most of them lived for over a month. Therefore it appeared, as has since been more rigidly proven, that tryptophane is one of the amino-acids which cannot be made in the body, but must be supplied in the food.

The study of the nutritional function of the individual amino-acids opened by Hopkins' pioneer investigation has been developed by our own chemists Osborne and Mendel who have studied the problem with a monumental attention to detail in the care, control, and even breeding of the rats used as experimental animals, in the accuracy with which the chemical composition of the food utilized was controlled, and in the wealth of experimental evidence with which point after point in the field has been settled. Professor Mendel himself has recently discussed the work before the Harvey Society, and it is mentioned here only because a paper on the physiology of the amino-acids would be incomplete without it. I have reproduced one of the hundreds of curves of

growth which Osborne and Mendel have published. This curve forms, in a way, a connecting link between Hopkins' work and theirs. It shows why the mice which Hopkins fed with zein plus tryptophane lived only a little longer than those which received zein alone. The rat whose weight curve is shown in Chart 8 received at first, like Hopkins' mice, a diet containing zein plus tryptophane as the sole nitrogenous constituents. During this period, although the animal was immature and should have been growing, his weight fell steadily. After 90 days another amino-

CHART VIII



Effect of adding lysine and tryptophane to diet deficient in these amino-acids.

acid, lysine, was added to the diet. Zein is lacking in lysine as well as tryptophane. The effect of making good both these deficits is shown by the immediate resumption of practically normal growth. Osborne and Mendel have proven beyond a doubt by such experiments that both lysine and tryptophane must be supplied to the higher animals in their food, since neither is synthesized in the animal body. A third amino-acid in the indispensable class is cystine, and metabolism experiments by Abderhalden indicate that tyrosine is a fourth.[25] That future

work will answer the question concerning the synthetic power of the body for other amino-acids may be expected with confidence.

That even the higher animals still retain the ability to synthesize the simplest of the amino-acids, glycocoll, is certain. The excretion of glycocoll can be stimulated by feeding benzoic acid. Instead of neutralizing it with ammonia, as it does with most other acids, the body condenses benzoic with glycocoll to form hippuric acid, in which form it is excreted. Magnus-Levy [26] found that by feeding rabbits large amounts of benzoic acid he could make them excrete more glycocoll in the form of hippuric acid than they possessed, either free or combined, in their entire bodies. This proved that they were able to manufacture glycocoll out of other nitrogenous substances. Osborne and Mendel have also found in their feeding experiments that glycocoll does not need to be fed in order to maintain growth, the rat being able to synthesize the amounts necessary for its growing tissue out of other nitrogenous substances. Whether any of the other amino-acids can be synthesized like glycocoll is uncertain. This field, the importance of which from both the practical and scientific standpoints is self-evident may be said to be still 13/18 virgin, since concerning 13 of the 18 amino-acids we have no conclusive knowledge as to whether we can synthesize them in our bodies or must depend upon plants to furnish them for us.

A discussion of the physiology of the amino-acids would not be complete without a word also concerning the manner in which those not incorporated into the body are broken down, yielding not only urea, but non-nitrogenous residuals which are burned or stored like fat or carbo-hydrate for their energy. The three men whose researches entitle them to speak with most authority in this field are Lusk, Dakin and Knoop. Knoop came from Freiburg three years ago to deliver a Harvey lecture on this subject, and, as our city is fortunate in claiming both Dakin and Lusk among its men of science, we either have heard or may reasonably hope to hear from them both the stories of their own researches. I will, therefore, attempt to indicate only in the most general way the manner in which the body is believed to dispose of its unincorpor-

ated amino-acids. The first step is the splitting off of the amino group, which yields ammonia and a hydroxy-acid, a hydroxyl group replacing the amino group of the amino-acid. The ammonia is turned into urea. The non-nitrogenous substance left after the amino group is split off is a fatty acid, and is dealt with accordingly. Varying with their structure, some of the amino-acids yield fatty acids which can be converted into glucose by the body, while others do not. Nearly the entire series of amino-acids has been tested in this respect by either Lusk or Dakin. The substances were either fed to phloridzinized dogs, whose urine was then analyzed for glucose, into which they turn everything that is physiologically capable of being turned into glucose; or the amino-acids were perfused through surviving livers, and the perfusion fluid was analyzed for glucose. The results are indicated by the plus and minus signs on the line at the bottom of Chart 3. The fact that half the amino-acids are glucose formers explains why diabetics can form sugar from protein as well as from carbohydrate. The fact that acetone bodies are formed from several amino-acids explains why diabetics may develop acidosis on a protein diet, or even when living on the proteins of their own tissues.

The nature of the fatty acid radicals left when the amino groups are removed from the amino-acids is also used by Lusk to explain the specific dynamic action of the proteins, their ability so to stimulate the metabolism that the rate of heat formation in the body is accelerated.[27] The amino-acids themselves cannot be responsible for this effect, because their concentration in the body is so well regulated, presumably by the liver, that no great fluctuations ordinarily occur, even after heavy consumption of protein. The stimulated heat production which Lusk and DuBois have discovered after the feeding of either protein or of amino-acids must therefore be due to their decomposition products, presumably the fatty acids that are formed by deamination; and the differences in the heat-stimulating effects of the different amino-acids are due to their individual differences, indicated by the varying shapes of their structural heads (see Chart 3).

PATHOLOGY OF THE AMINO-ACIDS

We now come to the significance of the amino-acids in pathology. The blood and urine have been investigated in regard to their amino-acid contents for the purpose of diagnosing or explaining pathological conditions which may be divided into two classes.

1. Those in which the normal function of catabolizing the amino-acids is injured. From the view that the liver is especially responsible for the conversion of the excess products of protein digestion into urea, it would logically follow that serious injury to this organ should result in a higher amino-acid content of the blood, and perhaps the urine. Consequently, amino-acids have been sought for by a number of investigators both as diagnostic indications and as toxic agents in liver atrophy, in conditions which involve visible injury to the liver, such as toxæmia of pregnancy, and in conditions which are presumably accompanied or caused by decreased liver function, of which diabetes is an example.

2. In the second type of abnormal condition in which amino-acid determinations have been called to the aid of the diagnostician specific ferments are supposed to be formed within the body which are capable of hydrolyzing tissues of an abnormal or pathological nature, thereby forming, either *in vivo* or *in vitro,* amino-acids from such tissues. The action of such specific ferments *in vitro* on the particular tissues towards which their activity is directed constitutes the Abderhalden reaction, which has been of late so largely in the public eye. Dr. Losee, of the Lying-In Hospital, Miss Vinograd, and I have devoted nearly a year of time to this reaction, and I shall consequently devote a moment to it here.

The Abderhalden reaction is based on the belief that when foreign proteins enter the blood stream the body cells elaborate and pour into the circulation enzymes which are capable of hydrolyzing the invading protein and none other. This idea was extended to include the proteins of abnormal tissues produced within the body itself. Thus, the epithelial cells of the placenta

of a pregnant woman are supposed to wander into the blood stream, and thereby stimulate the production of enzymes which can hydrolyze only the proteins of placenta tissue. Similarly, cancer cells are supposed to cause the production of enzymes capable of hydrolyzing only cancer tissue. The idea has been extended by various investigators to such an extent that, to judge from the claims made for the Abderhalden reaction, all that is necessary in order to settle a difficult diagnosis is to mix a little of the patient's serum with samples of tissue from all the suspected organs of the body, and the serum will infallibly pick out and digest the tissue from the affected part, leaving the other tissues unaltered. In justice to Abderhalden, it must be stated that his claims have never been so sweeping as those of some of his satellites. A great controversy arose over the Abderhalden reaction, some investigators reporting their results with enthusiasm, while others failed entirely, and still others took a middle course and utilized the customary "safety-first" formula, to the effect that there was evidently something in the reaction, but that results must be accepted with caution.

It appeared to us that the matter might be settled decisively if, instead of the rather uncertain color reaction with ninhydrin to detect the amino-acids resulting from digestion of the specific tissue, an accurate quantitative method were applied. And the nitrous acid reaction, because of its combined accuracy and specificity for amino groups, seemed to offer such a method. After preliminary experiments to ascertain the most satisfactory way in which to apply it, we finally settled on the following simple technic: Two c.c. of serum are incubated with placenta, as described by Abderhalden, and the undigested proteins are then removed by precipitation with colloidal iron. A control portion of serum is incubated and precipitated in the same way, but without placenta. The amino nitrogen is then determined in both filtrates. The increase in the nitrogen gas from the serum plus placenta over the nitrogen from serum alone indicates the extent of digestion that has taken place. The results could be obtained with great accuracy, and the increases observed were many times larger than the experimental

error. Consequently we believe that we were successful in excluding such error as a factor in interpreting the results. In order to give the reaction the fairest test possible we utilized it only as a test for pregnancy, and the non-pregnant controls were normal men and women, hospital patients. We made several hundred analyses. Both normal and pregnant sera showed a measurable amount of digestive activity, and the results with both varied over practically the same range. A slightly higher average obtained with pregnant sera may explain the fact that some honest investigators have been led to believe that if they could eliminate their own errors they would find the reaction all that had been claimed for it. But even the difference in averages was not significant, and the individual results from perfectly normal subjects covered practically the same range as those from pregnant women.[28] Entirely similar results were obtained by Isaac Levin and myself in attempting to apply the reaction to cancer diagnosis.[29]

We come finally to attempts to detect by amino-acid determination conditions involving impaired liver function. That tyrosine may be found in the urine in acute yellow atrophy is the classical fact in this field. The more the problem is studied with quantitative methods, the more it appears, however, that the liver injury must be extreme before it can cause unusual accumulation or excretion of amino-acids. Soon after the nitrous acid method was perfected I determined the amino nitrogen in the urines of dogs which Drs. Dochez and Opie had treated with chloroform and phosphorus, and was greatly surprised to find no increase in the percentage of amino nitrogen, despite the fact that autopsy showed extreme liver degeneration. More recently Marshall and Rowntree have found that the urine of such dogs, if taken immediately before death occurs, does show an increase in amino nitrogen, and that a still greater increase occurs at this time in the blood.[30] It is evident, however, that, in dogs at least, the liver injury must be most severe in order to affect the amino-acid content of the blood or urine. That it must be equally severe in man does not necessarily follow. Chesney, Marshall and Rown-

tree report that a considerable proportion of patients with impaired liver function showed abnormally high amino-acid nitrogen in the blood.[31] Consequently, although it cannot be said that amino nitrogen of either blood or urine offers at present much assistance to the diagnostician of diseased livers, it may be possible that the very constancy of the amino nitrogen figure under most conditions will enhance its diagnostic value for such conditions as do affect it.

That advanced diabetes is such a condition has been claimed by Cammidge.[32] According to his view in the milder stages of diabetes the body partially loses its ability to burn glucose, but it can still transform amino-acids into glucose. In the most severe stage, however, it cannot even accomplish the prelimniary transformation of amino-acids into glucose, but excretes large amounts of them unchanged. We have performed determinations of amino nitrogen in both blood and urine on a considerable number of patients in Dr. Allen's diabetic clinic at the Rockefeller Hospital, and have found that urines from certain of the patients do show figures distinctly higher than normal. That the high figures indicate diabetes of a special gravity, however, we are not yet prepared to state.

We finally come to the toxæmias of pregnancy. Ewing and Wolf [33] some years ago showed that the urines of such cases had a decreased proportion of urea nitrogen, and an increase in the undetermined nitrogen. From this, and from the gross injuries which the liver suffers during the toxæmia, Ewing and Wolf suggested that the intoxication might be due to protein digestion products which the degenerated liver could not metabolize, and which caused both the toxic symptoms and the increase in the undetermined nitrogen of the urine. The methods used for urine analysis were the most complete available at the time, and the hypothesis put forward was certainly reasonable. However, Dr. Losee and I, in examining both urine and blood from a considerable number of patients with toxæmia of pregnancy, have found in no instance that either blood or urine showed an abnormal concentration of amino-acids or of intermediate protein digestion products. Consequently, the responsibility for the toxæmias of pregnancy cannot be left with the amino-acids. I may add that

we have also tested the hypothesis that acidosis is to blame, with essentially negative results. We must frankly face the fact that we are entirely ignorant concerning the chemical nature of the substances which cause these toxæmias.

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