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PHYSIOLOGY OF THE AMINO ACIDS[1]

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Amino Acid Structure of the Proteins. The tissues of our bodies, skin, muscle, tendon, are chiefly protein substances. The number of proteins in the animal and vegetable world appears to be infinite. Yet they are all constructed of about twenty-one units, called the amino acids. These have an extraordinary ability to link together in chains in numbers up to thousands. One definition of infinity might be the possible number of different protein molecules that could be built by permutations and combinations of the amino acids. The extraordinary thing, in fact, is that nature ever succeeds in duplicating a protein molecule. Perhaps she never does exactly. But she comes so close to it that so far as we can tell the casein of cow's milk is always the same, the proteins of muscle seem to be constant in their properties, and so on through the list of proteins that make up the familiar animal and vegetable structures of which we are constructed and on which we live.

The common structure which all the amino acids possess, and which permits this chain-making, may be formulated as:

$$\overset{\text{I}}{H_2N\cdot\underset{\underset{H}{|}}{\overset{R}{\dot{C}}}\cdot COOH} \quad \text{or} \quad \overset{\text{II}}{\cdot CH_2\cdot HN\cdot\underset{\underset{H}{|}}{\overset{R}{C}}\cdot COOH}$$

All the amino acids except proline and hydroxypro-

[1] From an address given at the Centennial Celebration of the University of Chicago, September, 1941.

line have Structure I, while these two amino acids have II. Each amino acid has an amino group, NH_2 or $NH \cdot CH_2$, which has an alkalinity about equal to that of ammonia; and each has a carboxyl group, COOH, which has the acidity of an unusually strong organic acid. The R represents a chemical group which is different in each amino acid, and gives it its character as an individual.

In proteins Emil Fischer demonstrated that the amino acids are joined, by what he termed peptide linkings, each NH_2 group condensing with the COOH of another amino acid, with elimination of the elements of water. Simple chains of a few amino acids, Fischer termed peptides.

The proteins are peptides of tremendously long chains. These protein chains seem usually to be rolled or folded into balls or otherwise made to take a globular or ellipsoid or sausage shape. Their rates of diffusion were found by Northrop and Anson[1] to approximate the rates that would be calculated for spheres, and the asymmetries calculated from ultra centrifugation are not great. Exceptions are the fibrous proteins, such as silk and wool, in which the molecules appear to be extended into straight wavy chains, long bundles of which make the visible fibers.

Path of Amino Acids through the Body. Except for the transient supply of proteins with which we are born, all those in our bodies are obtained from the proteins of other animals and vegetables, which we eat and digest into their constituent amino acids or simple peptides, and then build into our own tissues. However, only a fraction of the amino acids that we invite into our bodies really find acceptance there as naturalized citizens, integral units of our own structures. Many other fates beset the immigrant amino acid; it may be disintegrated to make some entirely different product; or it may simply be burned for fuel. We shall try to follow some of the paths in the body that are taken by amino acids after digestion and absorption in the alimentary tract.

Methods for Determination of Amino Acids. In studies of protein digestion and of the nature and fate of the digestion products, methods for measuring the amounts of amino acids present in blood and other parts of the body are indispensable tools. Two such methods developed in our laboratory have contributed part of the physiological information that will be discussed.

The first was the "nitrous acid method"[2] which depends on the reaction:

$$R \cdot CH(NH_2) \cdot COOH + HNO_2 = R \cdot CH(OH) \cdot COOH + H_2O + N_2.$$

The N_2 gas is a measure of the amount of amino acid present. The reaction is not entirely specific for amino acids, because other amines with NH_2 groups also react; but such amines are usually not present in important amounts or can be removed.

A later and more specific method[3, 4] depends on reaction with a mild oxidizing agent called ninhydrin. Its effect is indicated by the equation:

$$R \cdot CH(NH_2) \cdot COOH + O = RCHO + NH_3 + CO_2.$$

The analysis consists merely of heating the mixture for a few minutes and measuring the CO_2 evolved. This reaction is so specific for free amino acids that it serves to pick out and measure them in the most diverse mixtures of other amines, organic acids, peptides and other biological products.

Digestion and Absorption. In the human stomach the chief visible change, as noted a century ago by Beaumont[5] through the bullet hole in the stomach of Alexis St. Martin, is that food proteins which enter as insoluble matter, such as meat or coagulated egg white, are dissolved. Chemical studies show that the long protein chains are unrolled and broken into relatively short peptide chains, which still, however, are fairly long. No absorption of the products occurs in the stomach; absorption begins only after the chyme enters the intestine.

In the intestine the chyme meets the enzymes secreted by the pancreas and the intestinal wall. These enzymes hydrolyze the long peptides of the chyme to short peptides containing only 2 or 3 amino acids in the molecule, and to free amino acids. Also, any unchanged protein particles that have escaped the gastric juice are digested. The ability of the intestine to digest, not only gastric peptides, but also intact proteins, makes possible the nutrition of people with achylia gastrica and even of persons who have had the stomach completely removed.

Interchange of Amino Acids between Blood and Tissues. Van Slyke, Cullen and McLean[6] found that in dogs during digestion the amino acid content of the blood rose about 20 per cent, as the blood perfused the intestines, and that the greater part of the absorbed amino acids was removed by the liver. In return, the liver poured into the blood of the hepatic vein an amount of urea nitrogen which almost balanced the amino acid nitrogen that had been taken up. One could watch the work of the liver in taking up the amino acids and destroying them, turning their

[1] J. Northrop and M. L. Anson, *Jour. Gen. Physiol.*, 12: 543, 1928–29.

[2] D. D. Van Slyke, *Jour. Biol. Chem.*, 9: 185, 1911; 12: 275, 1912; 16: 121, 1913; 83: 425, 1929.

[3] D. D. Van Slyke and R. T. Dillon, *Compt. rend. lab. Carlsberg*, 22: 480, 1938.

[4] D. D. Van Slyke, R. T. Dillon, D. A. MacFayden and P. Hamilton, *Jour. Biol. Chem.*, 141: 627, 1941

[5] W. Beaumont, "Experiments and Observations on the Gastric Juice and the Physiology of Digestion," 1833. 1929 edition. Boston.

[6] D. D. Van Slyke, *Arch. Int. Med.*, 19: 56, 1917.

nitrogenous parts into urea for excretion by the kidneys. Unreasonable and wasteful though it seems, a large part of the amino acids absorbed from the intestine appears to be captured and destroyed by the liver, and never to have a chance to reach and nourish the other tissues.

Other experiments, performed with Dr. Gustav Meyer,[7] showed that the liver did not get all the absorbed amino acids, but that some escaped, and could be absorbed by other tissues. It was found that even in the fasting animal the amino acid concentration in the tissues was about 10 times as great as in the blood plasma, *viz.*, about 40 to 60 mg of amino acid nitrogen per 100 grams of tissue, compared with 5 mg per 100 grams of plasma. When amino acids were injected into the circulation they were quickly taken from the blood by the tissues, where the amino acid contents might be increased to 2 or 3-fold their former values. In one experiment the amino nitrogen of the liver rose to 150 mg per 100 grams; in the muscles the increase was never so great. During the next three hours the amino acids in the muscles and kidneys remained practically unchanged, but the amino acids in the liver fell almost back to their original level, and an equivalent of urea nitrogen appeared in the circulation.

Fate of Amino Acids in the Liver. The evidence in these experiments, that the liver is the organ where *urea formation* takes place, supported an old but much contested hypothesis that the liver is the only organ that forms urea. Its unique distinction in this power was confirmed by Bollman, Mann and Magath,[8] of the Mayo Clinic, who showed that removal of the livers from dogs led to an accumulation of amino acids in the blood, and entirely stopped the formation of urea.

Another vicissitude of the amino acids which the work of Mann and his colleagues located in the liver is *transformation into glucose.* Graham Lusk[9] in the early part of the century showed that when protein was catabolized by dogs made totally diabetic by phloridizin poisoning, about 60 grams of glucose were formed and excreted from each 100 grams of protein catabolized. Lusk and his collaborators also showed that when certain amino acids were fed their carbon was partly or entirely turned into glucose when their nitrogen was turned into urea. Mann[8] and his colleagues showed that no glucose formation from proteins or amino acids occurred when the liver was excluded.

[7] D. D. Van Slyke and G. Meyer, *Jour. Biol. Chem.*, 12: 399, 1912; 16: 187, 197, 213 and 231, 1913.

[8] J. L. Bollman, F. C. Mann and T. B. Magath, *Am. Jour. Physiol.*, 69: 371, 1924.

[9] G. Lusk, "The Elements of the Science of Nutrition," Philadelphia, 1928.

Furthermore, the acceleration of the body's heat production that occurs during assimilation of protein digestion products was shown by Mann[8] and his colleagues not to occur when the liver was excluded. This accelerated heat production, called by Rubner the *"specific dynamic action,"* apparently either represents energy produced by the reactions which the amino acids undergo in the liver, or is caused by other reactions in the cells which are stimulated by the presence of products formed in the liver. Such substances must be other than the urea and glucose, for neither of these causes the observed amount of heat acceleration.

Not all the treatment met by the amino acids in the liver is destructive. During periods of heavy protein feeding the body stores considerable amounts of protein in the liver and, in less amounts per gram of tissue, in the other tissues. The *reserve protein* seems to be different from the structural proteins of the tissues. In the liver Berg[10] has shown that it can in fact be differentiated with the microscope by its droplet structure in the cells. Functionally it is characterized by the readiness with which it is metabolized at the onset of starvation, and with which it is used to replace blood proteins depleted by hemorrhage, as found by Whipple[11] and his colleagues.

The liver also appears to be the place where *plasma fibrin and albumin* are formed. It was demonstrated thirty years ago by Whipple[12] that injury of the liver retarded or prevented formation of fibrin. Work by the same author and others[13] has accumulated evidence that the liver is essential also for the formation of the albumin of the plasma. These proteins are presumably formed from free or combined amino acids taken out of the blood by the liver.

Transamination in the Tissues. Some of the amino acids can be synthesized in the body. One of the reactions by which the synthesis occurs has recently been discovered by two Russian biochemists, Braunstein and Kritzman.[14] It is called "transamination," and it enables the cells to change keto-acids, $R \cdot CO \cdot COOH$ to amino acids, $R \cdot CH(NH_2) \cdot COOH$ by replacing the oxygen atom of the ketone CO group with the elements of ammonia. This ammonia, however, must be transferred to the ketone acid from the $CH(NH_2)$ group of one of the dicarboxylic amino acids, aspartic or glutamic. Since at least some of

[10] W. Berg, *Biochem. Z.*, 61: 429, 1914.

[11] F. Robscheit-Robbins and G. H. Whipple, *Am. Jour. Physiol.*, 112: 27, 1935.

[12] G. H. Whipple and S. H. Hurwitz, *Jour. Exp. Med.*, 13: 136, 1911.

[13] S. C. Madden and G. H. Whipple, *Physiol. Rev.*, 20: 194, 1940.

[14] A. E. Braunstein and M. G. Kritzman, *Nature*, 140: 503, 1937; *Idem*, 144: 669, 1939. Also various papers in Biokhimia (Russian), 1937 and later.

the keto acids can be produced by partial oxidation of carbohydrates, transamination appears to be one of the processes by which the body can construct a portion of its own amino acids. The reaction of transamination is caused by an enzyme that occurs in all the tissues, and is rich in the muscles.

Transmethylation. The discovery of du Vigneaud[15] that methyl groups can be transferred from methionine, $CH_3 \cdot S \cdot CH_2 \cdot CH(NH_2) \cdot COOH$, to other substances in the body has opened a field rivaling in interest that of transamination. Du Vigneaud proved that choline could be formed with the aid of methyl groups from methionine. He administered methionine in which the methyl of the $CH_3 \cdot S$ group was marked by hydrogen in the form of deuterium. When this labelled methionine was fed to rats on a choline-free diet choline could be isolated from their tissues with part of its methyl groups containing deuterium. This observation showed that methyl groups had been taken from the methionine to make choline, presumably by methylating ethanolamine. It was found that this marked methyl group could be further transferred from the choline to creatine. The methyl group of the amino acid, methionine, could therefore be used by the body in synthesizing two of its essential non-amino acid constituents, choline and creatine.

The Continual Replacement of Amino Acids in Living Tissue Proteins. The extent to which nitrogen fed in the form of amino acids is synthesized into tissue proteins, both in the form of the fed amino acids and of other amino acids formed in the body from the fed material by transamination or other reactions, has been studied brilliantly by Schoenheimer[16] and his colleagues. They have synthesized amino acids with heavy nitrogen, N^{15}, have fed the amino acids to rats and mice, and have hydrolyzed the proteins in their bodies and isolated various amino acids from the hydrolysates. Finally, they have analyzed the isolated amino acids for N^{15} to determine the amounts of the ingested nitrogen that were built into the body proteins, both in the form of the administered amino acid and in the form of other amino acids derived from the administered one by transamination or other processes. They have found that incorporation into tissue proteins began almost immediately, and that, while more of the marked N^{15} was incorporated in the form of amino acid with which it was administered, than in any other amino acid, nevertheless a considerable proportion of the marked nitrogen was found distributed among other amino acids in the proteins.

The results of Schoenheimer and his colleagues have dispelled the view that the tissue proteins when once laid down remained as unchanging structural blocks until eventually destroyed by the wear and tear of metabolism. It appears that every protein molecule in the living body is itself alive in the sense that it is continually changing and renewing its structure.

Essential and Non-essential Amino Acids for Animal Nutrition. From the fact that by the transaminase action the body can synthesize some of its own amino acids, it would follow that not all the amino acids in the body proteins must be provided ready-made in the food proteins, but that some can be made in the body. The task of finding which of the 21 amino acids are indispensable parts of the diet, and which ones the animal body can build for itself, has occupied some of the leading biochemists since the time of Magnus-Levy's[17] demonstration that the rabbit could make glycine. The names of F. Gowland Hopkins in England and of Lafayette Mendel, T. B. Osborne and W. C. Rose in this country have been especially brilliant in the list of those who have unravelled this problem step by step. As the result of work by all the investigators in the field Rose[18] finally was able to divide the amino acids into the two groups listed in table 1, one which must be supplied in the food of growing rats, while the other group can be made by the rat and need not be supplied in the food. The list does not apply to all animals; for example, glycine is indispensable for the chicken. However, it appears probable, although not yet proven, that the necessities of man and the rat are the same or nearly so.

TABLE I

Essential amino acids must be supplied ready-made in the diet of rats	Non-essential amino acids need not be supplied in the diet of rats
Lysine	Glycine
Tryptophane	Alanine
Histidine	Serine
Phenylalanine	Norleucine
Leucine	Aspartic acid
Isoleucine	Glumatic acid
Threonine	Proline
Methionine	Hydroxyproline
Valine	Tyrosine
Arginine	Cystine
	Hydroxylysine

Detoxifying Effects of Amino Acids. It has long been known that various herbivora and man use glycine to combine with and detoxify benzoic acid by forming hippuric acid:

$$\underset{\text{Glycine}}{CH_2(NH_2) \cdot COOH} + \underset{\text{Benzoic acid}}{C_6H_5 \cdot COOH} = \underset{\text{Hippuric acid}}{CH_2(NH \cdot CO \cdot C_6H_5) \cdot COOH} + H_2O$$

The synthesis appears to occur in the liver, and the

[15] V. du Vigneaud, M. Cohn, J. P. Chandler, J. H. Schenck and S. Simmonds, *Jour. Biol. Chem.*, 140: 625. 1941.

[16] R. Schoenheimer, *Physiol. Rev.*, 20: 218, 1940.

[17] A. Magnus-Levy, *Biochem. Z.*, 6: 523, 1907.

[18] W. C. Rose, SCIENCE, 86: 298, 1937.

ability to form hippuric acid after glycine feeding is used as a test of liver function.[19]

In some much less obvious way cystine and methionine protect the liver from intoxication by chloroform. This peculiar effect of the two sulfur-containing amino acids was discovered by Miller, Ross and Whipple.[20] They had observed that a heavy feeding of meat would protect a dog from the effects of a dose of chloroform that would have led to fatal liver degeneration if administered in the fasting state. Investigation of the different types of amino acids yielded by protein digestion proved that only two containing sulfur had the protective effect.

Nutrition by Intravenously Injected Amino Acids. Henriques and Anderson[21] in Copenhagen demonstrated in 1913 that nitrogen equilibrium could be maintained with intravenously injected amino acids as the sole nitrogen intake. They placed a cannula in the neck vein of a goat kept in a stall, and maintained the animal in nitrogen equilibrium for several weeks by giving the necessary nitrogen in the form of a protein digest hydrolyzed completely to amino acids and injected in a slow stream through the cannula. The therapeutic application did not follow till 25 years later, when Elman[22] began the regular use of intravenous injections of predigested protein. Farr and MacFadyen[23] showed that the injected amino acids were assimilated fully as well as the nitrogen from proteins digested in the alimentary tract. A large part of the nitrogen required can be given in this form for a period of weeks. Whipple[24] and his colleagues and Elman[25] found that when the reserve tissue proteins of dogs had been depleted by fasting and the plasma proteins had also been depleted by bleeding, the proteins could be rapidly restored in tissues and plasma by administering a mixture of amino acids containing all those essential for nutrition, but not if essential amino acids were omitted. It appears that intravenous amino acid administration can be used to nourish the tissues at times when feeding by mouth is impossible or inadvisable.

Formation of Non-Protein Nitrogenous Constituents of the Body from the Amino Acids. Some of the amino acids in the tissues are condensed into peptides. Of these glutathione, which is glutamyl-cysteinyl-glycine,[26] is active in oxidation-reduction reactions. Carnosine, or beta-alanyl-histidine, occurs in mammalian muscle, and presumably has a physiological function. Another compound of beta-alanine is the vitamin, pantothenic acid.[27, 28] Serine combines with phosphoglycerides to form a constituent of nerve tissue.[29] Furthermore, animals have the power to transform the elements of amino acids in more profound ways than by condensation with their amino or carboxyl groups. Animals can be reared with no other nitrogenous foods than proteins and slight amounts of certain nitrogenous vitamins. Hence it appears that all the nitrogenous constituents that occur in large amounts in the animal organism can be formed from the amino acids yielded by the digestion of proteins. Among such constituents are the purines which form part of the nucleic acids of cell nuclei and the creatine of muscle tissue. The hormones thyroxine and adrenalin, from their organic structures, are presumably derived from tyrosine or phenyl alanine.

SUMMARY

We have followed the amino acids from their entrance into the alimentary tract in the form of food proteins through the successive steps of digestion, absorption into the blood stream and passage from the blood stream into the tissues, where they are concentrated by some unknown mechanism to many times their concentration in the blood plasma. We have seen something of the way in which certain of the amino acids can be transformed into one another in the body or synthesized from ammonia and keto acids. However, we have had to admit that our bodies can form in such ways only about half of the different amino acids that are required, and that the other half must be made for us by plants, bacteria or other organisms which have greater synthetic powers than we. And finally we have seen something of the manifold fates of the amino acids after they have entered our tissues; how they may be destroyed and their nitrogenous parts turned into urea in the liver before it is possible to put them to their more specialized uses, how their carbon fractions can be used to form glucose, how they may sacrifice themselves to protect us from toxic products, how they can serve as source material for certain vitamins, hormones and other compounds with physiological functions still to be identified, and how finally those amino acids which are not deflected to these various fates may enter into the proteins of the tissues and become for a time parts of our living structures.

19 A. J. Quick, *Arch. Int. Med.*, 57: 544, 1936.

20 L. L. Miller, J. F. Ross and G. H. Whipple, *Am. Jour. Med. Sci.*, 200: 739, 1940.

21 V. Henriques and A. C. Anderson, *Z. physiol. Chem.*, 88: 357, 1913.

22 R. Elman, *Proc. Soc. Exp. Biol. Med.*, 36: 867, 1937; *Ann. Surg.*, 112: 594, 1940.

23 L. E. Farr and D. A. MacFadyen, *Am. Jour. Dis. Child.*, 59: 782, 1940.

24 S. C. Madden, W. A. Noehren, G. H. Waraich and G. J. Whipple, *Jour. Exp. Med.*, 69: 721, 1939.

25 R. Elman, *Ann. Surg.*, 112: 594, 1940.

26 F. G. Hopkins, *Biochem. Jour.*, 15: 287, 1921; *Jour. Biol. Chem.*, 84: 269, 1929.

27 R. J. Williams and R. T. Major, SCIENCE, 91: 246, 1940.

28 D. W. Woolley, SCIENCE, 91: 245, 1940.

29 J. Folch and H. A. Schneider, *Jour. Biol. Chem.*, **137**: 51, 1941; 139: 973, 1941.