THE AMINO ACIDS IN NUTRITION*

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The past thirty years have witnessed a remarkable change in knowledge regarding the importance of the nitrogenous portion of the ration. At the beginning of the twentieth century comparatively little *quantitative* information was available concerning the chemical make-up of proteins. The majority of the amino acids had been discovered prior to 1900, but very limited data existed concerning their relative distribution. Consequently, there appears to have been little appreciation of the fact that the nutritive value of a protein depends upon the kind and quantities of its components. Under the circumstances, it is not surprising that emphasis should have been placed upon the *amount* of protein ingested without much reference to possible differences in nutritive *quality*.

The advent of the Kossel and Kutscher[34] procedure for the isolation of the diamino acids, and the Fischer[20] ester method for the separation of the monoamino acids marked the beginning of a new era in knowledge not only of the chemistry of proteins, but indirectly of their physiological value as well. It was soon seen that proteins of different sources vary enormously as regards the proportions in which their constituent amino acids occur. Frequently, one or more amino acid was found to be missing entirely. Thus gliadin of wheat proved to be deficient in lysine; zein of corn was shown to be practically devoid of lysine and tryptophane; and gelatin was seen to be lacking in tryptophane, tyrosine, cystine, valine, isoleucine, and hydroxyglutamic acid (cf. Dakin[14]).

The recognition of these facts naturally raised the question as to the biochemical importance of the individual amino acids. Inasmuch as all of the generally recognized amino acids occur as components of tissue proteins, obviously each must be made available, either preformed in the diet, or by synthesis in the organism from other materials. Thus protein metabolism immediately became a much more complex phenomenon than was originally supposed. Instead of being concerned with a single dietary factor, it was now seen to involve each of the so-called "Bausteine" of proteins of which eighteen (including norleucine) had been discovered

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by 1912. As stated by Osborne and Mendel[44], "Obviously the relative values of the different proteins in nutrition are based upon their content of those special amino-acids which cannot be synthesized in the animal body and which are indispensable for certain distinct, as yet not clearly defined processes which we express as maintenance or repair." As a result of this new view-point attention was directed in several laboratories toward determining which amino acids are necessary dietary components.

The Indispensable Amino Acids. Among the earlier investigations regarding the rôle of the individual amino acids in nutrition

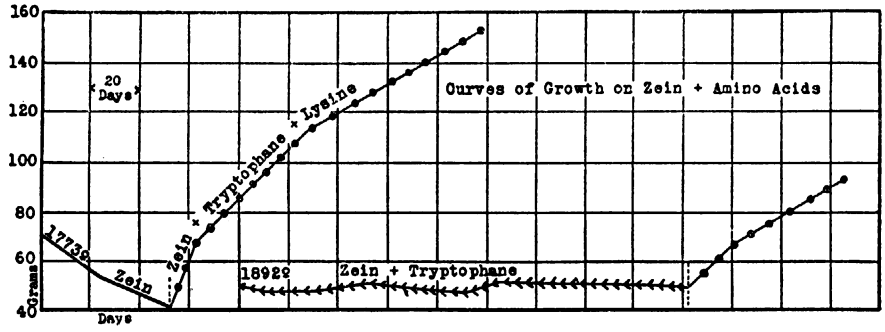


CHART I. Showing the effect of adding tryptophane, or tryptophane and lysine to a zein ration.

those of Osborne and Mendel are of particular interest and yielded results of extraordinarily great importance. Willcock and Hopkins had shown in 1906 that the protein zein "has no power whatever of maintaining growth in the young animal." They report also that the addition of tryptophane to a zein ration is insufficient "to convert such loss (in weight) into equilibrium or gain", although the duration of life is thereby prolonged. By the use of young rats upon diets containing zein as the sole protein, Osborne and Mendel[44] succeeded in demonstrating in a beautiful fashion the indispensable nature of both tryptophane and lysine. They observed that rats receiving zein rations not only fail to grow but rapidly lose weight. The addition of tryptophane to the food leads to maintenance but no growth, while the inclusion of both tryptophane and lysine is followed by rapid growth. In like manner, when gliadin of wheat serves as the sole protein of the diet growth does not occur until lysine is incorporated in the ration (cf. Osborne and Mendel[43, 44]). Typical results of this sort are reproduced in Chart I (Mendel[40]).

Thus was provided the first conclusive demonstration that lysine is indispensable for the functions of growth.

Abderhalden[1, 2], and Wheeler[69] in Mendel's laboratory had already secured evidence for the unique importance of tryptophane. Abderhalden demonstrated that dogs upon a diet of casein freed of tryptophane lose nitrogen and decline in weight. The substitution of a ration containing the missing amino acid is followed by nitrogen equilibrium and the recovery of body weight. Wheeler observed that mice upon zein diets lose weight less rapidly and survive longer when tryptophane is incorporated in the food. The results obtained in the growth studies of Osborne and Mendel[44] confirmed and extended the earlier investigations, and left no doubt as to the dietary importance of tryptophane. In such investigations, as was pointed out by the latter authors, "growth sets a standard decidedly higher than that of maintenance." Furthermore, it is not necessary to supply the missing amino acids in the free state. The supplementation of a zein diet with some other protein containing adequate quantities of lysine and tryptophane results immediately in growth (cf. Osborne and Mendel[45]). Unquestionably, both lysine and tryptophane are indispensable dietary components. In the absence of either, nutrition fails and eventually death results.

In a similar fashion Osborne and Mendel[46] showed that cystine is essential. When 18 per cent of casein is incorporated in an otherwise adequate ration young rats receiving such a food mixture grow at normal rates. When, however, the proportion of casein is progressively diminished cystine becomes the limiting factor. At a 9 per cent level, casein is incapable of inducing normal growth; but the addition of cystine renders the diet adequate, and growth ensues. Data of this sort are shown in Chart II (Mendel[40]). Confirmatory evidence for the indispensable nature of cystine has been reported from several laboratories. Johns and Finks[29] found that the addition of cystine to diets containing phaseolin markedly improves the nutritive quality of the food. Like results were secured by Sherman and Merrill[57] in the use of a diet of whole milk powder overdiluted with starch. Indeed, so pronounced is the growth response of rats to the inclusion of cystine in an otherwise adequate ration that the phenomenon actually may be employed as a method for the quantitative determination of the amino acid, as has been done by Sherman and Woods[58].

A fourth indispensable amino acid is histidine. In 1916, Ackroyd and Hopkins[8] observed that when arginine and histidine are removed from acid-hydrolyzed casein, the resulting material is inadequate for maintenance or growth. The authors state that if either arginine or histidine is included in the ration, no loss in weight occurs, and growth may be resumed. From these results they con-

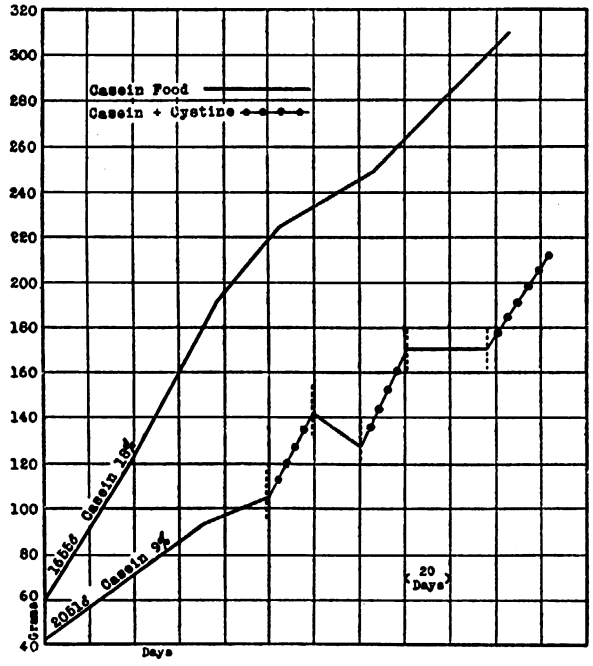


CHART II. Showing that cystine is the limiting factor in a diet containing 9 per cent of casein.

clude that the two amino acids are interchangeable in metabolism, but that at least one must be present in the diet. In so far as the indispensable nature of histidine is concerned, the experiments of Rose and Cox[51, 52] completely confirmed the findings of Ackroyd and Hopkins. On the other hand, the former were unable to demonstrate an interchangeable relationship between the two amino acids. The addition of arginine to the deficient diet exerted no influence upon growth even when the quantity added was more than equivalent to the sum of the arginine and histidine present in casein. In

Chart III are reproduced the growth curves of two of the experimental animals of Rose and Cox. The upper curve shows the effect of histidine when added to the deficient ration. The lower curve demonstrates the inability of arginine to supplement the histidine-deficient food. Confirmatory evidence in support of the essential nature of histidine was furnished by the later publications of Cox and Rose[11, 12], and by Harrow and Sherwin[26].

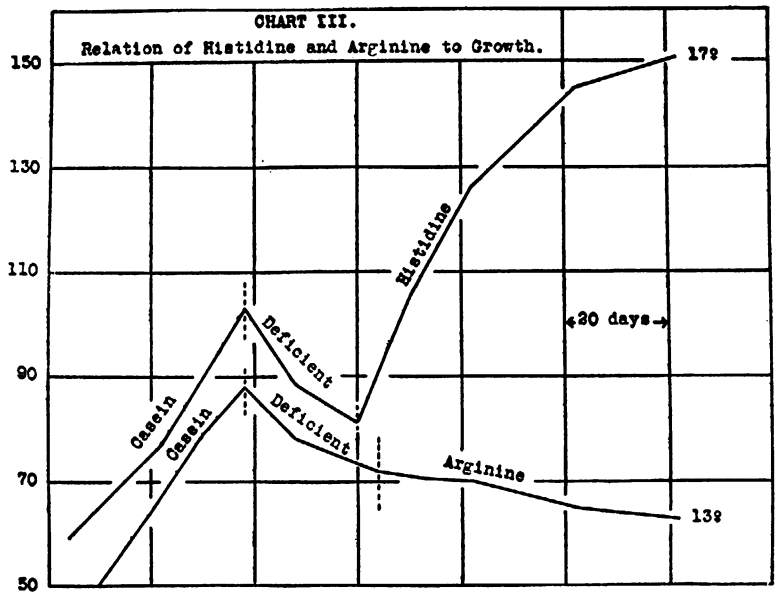


CHART III. The upper curve shows the growth-stimulating effect of histidine when added to a diet deficient in histidine and arginine. The lower curve demonstrates the ineffectiveness of arginine.

Evidence for the Dispensable Nature of Certain Amino Acids. In contrast to lysine, tryptophane, cystine, and histidine, which are now generally recognized as essential dietary components, evidence is available indicating that certain amino acids may not be necessary. It is well known that when benzoic acid is administered to man or to most animals it is conjugated with glycine, and is eliminated in the urine as hippuric acid. By measuring the maximum production of hippuric acid in rabbits and goats Wiechowski[70], Ringer[49] and others report that the output of hippuric acid may carry more glycine than is found preformed in the proteins metabolized. The origin of the

glycine is unknown, but McCollum and Hoagland[39], Lewis[36], and Shiple and Sherwin[60] have shown that a considerable portion of the nitrogen which in the normal metabolic processes is converted into urea, may, after excessive doses of benzoic acid, be diverted to the synthesis of hippuric acid. These findings led to the general impression that glycine may be synthesized by the organism out of ammonia and non-nitrogenous materials, or from other amino acids. As further evidence in this direction the fact has sometimes been emphasized that casein, though low in glycine, serves admirably for purposes of growth in both man and animals (cf. Abderhalden[1, 3]). In like manner gliadin and zein, both of which are believed to be devoid of glycine, are made satisfactory for growth by suitable supplementation without the addition of glycine.

The evidence, however, is not all in favor of the dietary dispensability of this amino acid. Thus Griffith and Lewis[24, 25] have demonstrated that the *rate* of synthesis of hippuric acid in rabbits is closely dependent upon the supply of preformed glycine. The administration of the amino acid, or of proteins containing it, greatly accelerates the rate of detoxication of benzoic acid. On the other hand, the administration of amino acids other than glycine, or of proteins which are quite low in the latter, does not increase the rate of hippuric acid synthesis. More recently, Griffith[22, 23] has shown that the growth of young rats may be inhibited by the inclusion of benzoate in the diet unless glycine as such, or in the form of protein, is supplied in amounts sufficient to detoxicate the benzoate, and meet the needs of tissue synthesis. While the author believes that his data "support the idea that glycine is synthesized by animal tissues", evidently the synthesis is limited in extent. Apparently, the use of glycine for detoxication purposes may create a deficiency for the growth function unless an increased supply of the amino acid is provided. In the light of these investigations one must conclude that the prevailing idea that glycine may be formed practically *ad libitum* by the animal organism is at the present time scarcely warranted.

The relation of arginine to maintenance and growth has been the subject of several investigations. Reference has already been made to the papers of Ackroyd and Hopkins[8] and of Rose and Cox[51, 52] involving the feeding of casein digests from which both arginine and histidine had been precipitated by silver sulfate and barium hydroxide. The latter authors pointed out that the ability of animals to grow upon diets in which the nitrogenous requirements were met by

an arginine-low mixture of amino acids "must not be interpreted as indicating that arginine is an unnecessary component" of the ration, inasmuch as no definite information was available as to the completeness of its removal by the method employed. According to Abderhalden[4] arginine is probably indispensable in nutrition. His investigation, involving the use of mixtures of purified amino acids, is in some respects remarkable; but owing to the difficulties experienced in the synthesis of the dietary components, the available materials were necessarily limited, and the feeding trials were few in number and of short duration. His results appear to be open to the further criticism that frequently his animals were provided with inadequate supplies of vitamins. Using an entirely different procedure, Crowdle and Sherwin[13] report that fowls are capable of synthesizing ornithine for the detoxication of benzoic acid. Birds, in contrast to most other animals, conjugate benzoic acid with ornithine and excrete the resulting ornithuric acid. Since ornithine is a component of arginine, the observation of Crowdle and Sherwin suggests that arginine also may be a synthetic product, at least in the species in question.

Additional evidence for the unnecessary nature of arginine was supplied by Bunney and Rose[10] in experiments involving the use of hydrolyzed casein from which the amino acid has been precipitated by flavianic acid (Kossel and Gross[33], and Kossel and Staudt[35]). This reagent appears to effect a more nearly quantitative removal of arginine than does the older silver sulfate-barium hydroxide procedure. Despite this fact, the experimental animals which received the amino acid mixture at a 12 per cent level grew at practically normal rates. When the nitrogenous portion of the ration was decreased to 9 per cent, the increase in body weight occurred at about half the normal rate. The less rapid growth under the latter circumstance was not due to a deficiency of arginine, since the addition to the diet of the amino acid in question failed to accelerate growth. The authors state that the above results "are believed to point very strongly to the conclusion that arginine is not indispensable for normal nutrition." It must be admitted, however, that the traces of arginine which failed to be precipitated from the hydrolyzed protein, together with the amounts administered unavoidably in the vitamin supplement, may have been sufficient to meet the growth needs of the animals.

In view of the uncertainties inherent in the above experiments the arginine problem was attacked by a different method (Scull and

Rose[55]). This involved a comparison of the arginine intake of growing rats on an arginine-low diet and the increments in tissue arginine, in order to determine whether the latter may be accounted for by the amounts of the amino acid in the basal ration and vitamin supplement. For this purpose, hydrolyzed casein was rendered as nearly devoid of arginine as possible, and was incorporated in a diet which was administered *ad libitum*. At the beginning of the experiments, litter mates of the animals employed in the growth studies were killed and subjected to analysis *in toto* for arginine. The other members of each litter were killed and analyzed after they had received the experimental diet for a period of 64 days. In the meantime they had gained 73 to 113 gm. each. The results are summarized in Table I reproduced from the paper of Scull and Rose. The data show that without exception the increase in tissue arginine

TABLE I

APPARENT ARGININE SYNTHESIS

Rat No. and sex	*Arginine increment in tissues*	*Total arginine intake*	*"Arginine synthesis"*
	mg.	*mg.*	*mg.*
1198 ♂	1536	462	1074
1199 ♂	1377	443	934
1200 ♀	1580	458	1122
1201 ♀	1636	466	1170
1202 ♀	1374	451	923
1203 ♀	1611	473	1138
1220 ♂	1411	443	968
1221 ♂	1610	449	1161
1222 ♂	1460	474	986
1223 ♀	1414	467	947
1224 ♀	1355	490	865
1225 ♀	1405	481	924
1226 ♀	1159	466	693
1227 ♀	2057	506	1551
1228 ♀	1578	466	1112

was 2 to 3 times as large as could be accounted for by the total arginine content of the food. While one would not be justified, perhaps, in regarding the figures as representing *absolute amounts*, in view of the complicated nature of the analytic procedure necessary for the determination of arginine, nevertheless, the occurrence of

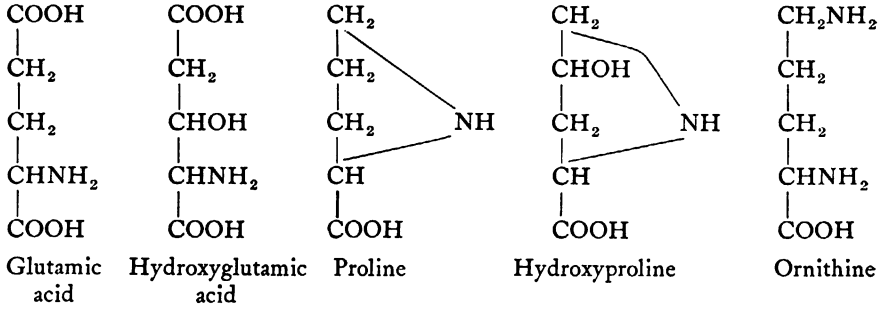
errors sufficiently large to invalidate the results appears very improbable. Therefore, the findings "seem to warrant the conclusion that arginine may be synthesized by the organism of the rat, and in this species at least is not an indispensable dietary component" (Scull and Rose[55]). Certainly, the evidence for the non-essential nature of this amino acid is more convincing than in the case of any other.

Investigations concerning the relation of certain other amino acids to nutrition have been made, but at the present time the results scarcely warrant positive conclusions. A number of years ago Abderhalden[1] suggested that proline might not be an indispensable amino acid. On the other hand, Sure[62] is of the opinion that it is necessary. His data, however, are not very convincing. St. Julian and Rose (unpublished data) have removed proline as completely as possible from hydrolyzed proteins by 40 extractions with hot absolute alcohol, without impairing the growth-promoting value of the resulting material. The same investigators, making use of the method of Kingston and Schryver[32], have precipitated the dicarboxylic amino acids, glutamic, hydroxyglutamic, and aspartic acids, without diminishing the nutritive properties of the residue. Hopkins[27] is of the opinion that neither glutamic nor aspartic acid is indispensable. Sherwin, Wolf, and Wolf[59] report that in the human subject glutamine may be synthesized for the purpose of detoxicating phenylacetic acid, which in man is excreted in the urine as phenacetylglutamine. This observation has been confirmed by Shiple and Sherwin[60], who state that the synthesis is accomplished at the expense of nitrogen which otherwise would appear in the excreta as urea. Furthermore, the latter investigators find that both glycine and glutamine may be synthesized simultaneously in man following the administration of a mixture of benzoic and phenylacetic acids. Hydroxyglutamic acid is usually listed as non-essential inasmuch as edestin, which presumably is devoid of this amino acid, supports normal growth (cf. Osborne and Mendel[48, 46] and Osborne, Leavenworth, and Nolan[42]).

Very little information is available concerning the possible nutritive importance of hydroxyproline. Spörer and Kapfhammer[61] report that several vegetable products, notably soy-bean flour, do not contain detectable amounts of this amino acid. The authors suggest that in view of the quantitative importance of soy-beans in the dietaries of oriental peoples, hydroxyproline may not be an essential constituent. Recently West and Howe[67] have described the isolation from liver extract of an acid which yields hydroxyproline and

hydroxyglutamic acid on hydrolysis (cf. also Dakin and West[16]). At first this material, which appears to be of the nature of a dipeptid or diketopiperazine, was thought to be the active agent in the liver causing the reticulocyte response in pernicious anemia. Later investigations indicate that this is probably not the case (West and Howe[68]). Under the circumstances it is not known whether the product plays a dominant rôle in nutrition or not.

The interpretation of the results of feeding experiments in which proline, hydroxyproline, and the dicarboxylic acids are supposedly absent from the diet is rendered difficult by the lack of delicate tests for these amino acids. In such investigations one must recall that even traces of a life essential may suffice to meet the growth requirements of the organism. It is possible that the prolines, glutamic acids, and the ornithine part of arginine may all be interchangeable in metabolism, or be capable of yielding a single essential. Thus if proline were necessary but absent from the food, one or more of the other four might be transformed into the missing amino acid, and thus prevent a dietary deficiency. The similarity in structure of the five compounds is readily seen from the accompanying formulas.



Indeed, Abderhalden[1, 4] has frequently suggested that glutamic acid and proline may be capable of replacing each other. We have attempted to secure evidence for such a substitution by removing all five amino acids from hydrolyzed casein (St. Julian and Rose, unpublished data). For this purpose, the dicarboxylic acids were precipitated with barium hydroxide and alcohol (5 volumes) according to the procedure of Kingston and Schryver[32]. Jones and Moeller[30] are of the opinion that this accomplishes a practically quantitative separation of the dibasic acids from the remaining materials. After removal of the excess barium and alcohol, arginine

and histidine were precipitated according to the Vickery and Leavenworth[66] modification of the Kossel and Kutscher[34] method. Finally, the residue previously freed of excess silver and thoroughly dried, was extracted 40 times with hot absolute alcohol for the removal of the proline and hydroxyproline. It is well known that proline is rather readily soluble in alcohol, but pure hydroxyproline is only slightly soluble in this reagent. However, Kapfhammer and Eck[31] have shown that a solution of proline in absolute alcohol dissolves appreciable amounts of hydroxyproline. Inasmuch as casein contains relatively little hydroxyproline (supposedly 0.23 per cent), it seems likely that 40 extractions with alcohol would effectively remove both compounds.

The amino acid mixture prepared as outlined above was supplemented with cystine, tryptophane, and histidine, and was incorporated in the diet at a level of 11.5 per cent (including the supplements). The rats which received this ration each gained at a rate of approximately 1 gm. per day. The addition to the food of arginine, glutamic acid, aspartic acid, and proline failed to accelerate the increase in body weight. In view of the low level at which the hydrolysate was fed, it is difficult to interpret the results on any basis other than that the amino acids in question are not necessary dietary components. The final solution of the problem must await the use of a diet containing a synthetic mixture of amino acids known to be entirely devoid of the compounds in question. Preliminary experiments of this nature have already been made in this laboratory (Rose and S. M. Jackson, unpublished data), and will be reported elsewhere later.*

Finally, Abderhalden[3, 4] is of the opinion that either tyrosine or phenylalanine must be included in the food, but that the two amino acids are mutually interchangeable in metabolism, at least in part. This opinion is in line with the observation of Embden and Baldes[19] that the perfusion of the surviving liver with blood containing phenylalanine leads to the production of small amounts of tyrosine.

* In a recent paper Mary Adeline (Ztschr. f. physiol. Chem., 1931, *199*, 184), reports that rats upon diets containing 6 per cent of edestin cease growing after approximately 12 weeks. The addition of either proline or hydroxyproline is said to remedy the nutritive deficiency, and to induce growth. She concludes, therefore, that the two compounds are interchangeable in metabolism. Unfortunately, the data are not so extensive or clear-cut as one would wish, in view of the conflicting evidence regarding the subject.

However, evidence in conflict with the latter conception has been reported by Dakin[15], and more recently by Shambaugh, Lewis, and Tourtellotte[56]. If phenylalanine is transformed into tyrosine as the first step in its metabolism, as Embden and Baldes believe, then both amino acids should yield the same intermediates in the body. According to Lewis and his associates this is not the case. But assuming that the two amino acids are normally *catabolized* by different paths, this fact does not exclude the possibility that in the absence of tyrosine from the diet phenylalanine might undergo oxidation in the benzene ring, and thereby yield sufficient tyrosine to meet the *anabolic* requirements. However, the problem of the interchangeability of the two amino acids must await convincing proof that the absence of either or both leads to nutritive failure. Totani[64], and Lightbody and Kenyon[37] were unable to demonstrate any relationship between the growth of rats and the tyrosine content of the diet. The problem is complicated by the fact that no method exists for the complete removal of phenylalanine from hydrolyzed proteins.

The present status of knowledge regarding the relation of the amino acids to nutrition is summarized in Table II. Of the twenty

TABLE II

TENTATIVE CLASSIFICATION OF AMINO ACIDS WITH RESPECT TO THEIR NUTRITIVE IMPORTANCE

Indispensable amino acids	*Amino acids which have not been definitely placed, but which appear to be dispensable*	*Amino acids of unknown nutritive importance*
Lysine	Glycine	Alanine
Tryptophane	Arginine	Serine
Cystine	Proline	Valine
Histidine	Hydroxyproline	Leucine
	Glutamic acid	Isoleucine
	Hydroxyglutamic acid	Norleucine
	Aspartic acid	Phenylalanine
	Tyrosine	Methionine

generally recognized protein components, the indispensable nature of only four has been positively established. The importance of eight others is at the present time uncertain. Concerning the

remaining eight, available information does not warrant their classification with respect to maintenance and growth. Abderhalden[4] has suggested that alanine may be dispensable, and that norleucine and isoleucine may be unnecessary provided leucine is present in the food. But in our judgment the experimental evidence is much too meager to justify one in hazarding a guess as to the nutritive importance of these compounds. For this reason they are placed tentatively in the third group (Table II). From a theoretical point of view it would be very remarkable if the leucines proved to be interchangeable. The fact that they all contain six carbon atoms is much less significant than is their structural dissimilarity in other respects.

In a preliminary paper Jackson and Block[28] have recently recorded the interesting observation that methionine may stimulate the growth of animals upon a cystine-deficient ration. However, the authors are not yet prepared to state that methionine is capable of satisfying the cystine requirements of the organism, but prefer to await the results of further experiments before drawing final conclusions.

The fundamental nature of investigations regarding the nutritive rôle of the individual amino acids need scarcely be emphasized. Obviously, adequate interpretation of the facts of protein metabolism, especially with respect to the growth process, will remain impossible until the importance of each amino acid has been determined. The problem of the protein requirements of man and of animals depends in ultimate analysis upon the demonstration of which amino acids are necessary, and in what quantities (cf. Osborne and Mendel[47, 48]). It appears that if further information of this sort is to be secured, either more adequate methods must be devised for the quantitative removal of single components of proteins, or else one must resort to the use of synthetic mixtures of highly purified amino acids. The latter procedure seems to be the more promising, and has already yielded results of considerable biochemical interest.

Feeding Experiments with Mixtures of Purified Amino Acids. Among the earlier attempts to substitute synthetic mixtures of amino acids in place of the protein of the diet is that of Abderhalden[1]. Dogs were employed as the experimental animals, and sixteen amino acids were incorporated in the food. Unfortunately, the experiments were complicated by the refusal of the animals to eat, or by vomiting and diarrhea. But for brief periods (6 to 8 days) the dogs are said to have been maintained in approximate nitrogen equilibrium.

Hopkins[27] reports that rats which received a mixture of cystine, tyrosine, lysine, tryptophane, and histidine as the only nitrogen supply experienced a "remarkably slow loss of weight, and long maintenance of apparent health." When, however, leucine, valine, alanine, glycine, and glutamic acid were fed in place of the previous five, the losses in weight were rapid, and the animals soon succumbed. At about the same time Osborne and Mendel[48] described very briefly the results of similar experiments. They say (p. 2, foot-note):

"We have attempted to learn whether it would be possible to maintain rats on a non-protein diet with additions of tryptophane alone, or together with cystine, histidine, tyrosine, phenylalanine, proline, and ammonium citrate, or urea. All such attempts failed, even when the supply of energy in the form of non-protein substances was liberal and the food contained all of the necessary inorganic salts and 'food accessories', and in addition at least 0.5 per cent of protein, present in the 'protein-free' milk. On such diets the rats declined just as rapidly as when the amino-acid additions were not made."

In experiments involving the alternate feeding of (a) diets containing six to fifteen amino acids and (b) a nitrogen-free diet (except for the nitrogen present in 28 per cent of "protein-free" milk), Mitchell[41] succeeded in keeping mice alive for 70 to 98 days. During these periods the animals showed pronounced losses in weight. The author states that the alternate feeding induced a better total food consumption than did the administration of the amino acid ration alone. "However," he adds, "it is probable that in no case was the amino-acid intake sufficiently large to assure a fair test of its adequacy."

Reference has already been made to the extensive studies of Abderhalden[4] in which were employed diets containing mixtures of amino acids, glucose, fatty acids, glycerol, and inorganic salts, with and without the addition of yeast. In the absence of the latter the animals rapidly lost weight. With the addition of 0.1 gm. of yeast daily the rats are said to have gained. The author concludes (p. 225): "Bei wachsenden Tieren ergab sich, dass die Bausteinnahrung das Wachstum nicht unterhalten kann. Erst bei Zusatz von ganz geringen Mengen von Hefe bzw. von Butter usw. kam das Wachstum in Gang." The amino acid mixture contained nineteen amino acids including α-aminobutyric acid. Hydroxyglutamic acid

was not present; and methionine, at the time of publication of the paper, had not yet been identified as a component of proteins.*

More recently Suzuki, Matsuyama, and Hashimoto[68] attempted to maintain rats upon a ration of purified amino acids, protein-free milk, butter, starch, and calcium lactate and phosphate. Fourteen amino acids and ammonium carbonate were employed as the source of nitrogen. Serine, isoleucine, norleucine, hydroxyglutamic acid, hydroxyproline, and methionine were not included. The animals rapidly lost weight, and died in the course of the experiments. McClendon[38] has suggested that peptids may be necessary for normal nutrition, and that the failure of animals to grow on synthetic mixtures of amino acids may be due to the absence of such complexes from the food.

For about two years feeding experiments with amino acids have been under way in this laboratory. In formulating our amino acid mixture we imitated the composition of casein in so far as available information permitted. Nineteen amino acids were used. The rats receiving the diets rapidly lost weight during the first 12 days, and then declined gradually or maintained weight to the end of the experiments. Hydroxyglutamic acid was the only recognized protein component not incorporated in the food. That its absence was not the limiting factor was shown by supplementing the ration with a crude fraction of protein containing the dicarboxylic acids. The results were interpreted as indicating that growth-promoting proteins contain at least one essential component other than the twenty known amino acids (Rose[50]). In line with this conclusion, it was found that when 5 per cent of casein, gliadin, or gelatin was substituted in the diet for an equivalent quantity of the amino acid mixture, the animals after 4 days slowly gained (Ellis and Rose[18]). Evidently the supplements furnished something which was lacking in the amino acid mixture.

*In discussing the paper of Abderhalden[4] elsewhere, the statement was made (Rose[50]) that "the make-up of the amino acid mixture is not clear inasmuch as the author reports that it contained two parts of 'Pyrrolidinkarbonsäure' and five parts of 'Prolin'. The identity of these compounds introduces an element of uncertainty which prevents repetition of the experiments." In a personal communication, Professor Abderhalden states that the confusion in terms arises through a typographical error which had escaped his attention until he saw the criticism in question. "Pyrrolidinkarbonsäure" should read "Pyrrolidonkarbonsäure". The writer is very glad indeed to take this opportunity of correcting the unfortunate error in Professor Abderhalden's paper.

Inasmuch as casein, in the above experiments, proved to be more effective in stimulating growth than did either gliadin or gelatin, an attempt was made to concentrate the active material by fractionating hydrolyzed casein. The results show that the fractions which contained respectively the less soluble amino acids, the dicarboxylic acids, the diamino acids, and the alcohol-soluble material (proline) were almost or completely devoid of activity. On the other hand, the monoamino acids supplied the growth essential in much greater concentration than did whole casein. Indeed, by a second fractionation of part of the monoamino acids a material was obtained which in 5 per cent concentration induced normal growth. The results are believed to provide conclusive proof for the presence in casein of an hitherto unrecognized dietary essential (Windus, Catherwood, and Rose[72]).

Since the publication of the above results we have successfully concentrated the active substance by other methods of casein fractionation. By the use of the Town[65] copper salts procedure, we find the growth essential is present in the amino acids whose copper salts are soluble both in water and in anhydrous methyl alcohol (Caldwell and Rose, unpublished data). The best information available (cf. Brazier[9], and Damodaran[17]) indicates that the only known amino acids present in abundance in this fraction are valine, isoleucine, proline, and hydroxyproline. Of course, traces of others are undoubtedly present as contaminants since the procedure does not lead to an absolutely quantitative separation. The proline and most of the hydroxyproline have been removed by extraction of the free amino acids with absolute alcohol. Therefore, at the present time we believe that valine and isoleucine are the chief obstacles in the way of obtaining the growth essential in pure condition. The difficulty of separating valine and isoleucine from each other is well known to students of protein chemistry. But by the use of methods now being employed in this laboratory we hope to be able to remove both, and leave the desired product behind. If we are successful, it then will be possible to determine with certainty which of the known amino acids are essential for life.

In conclusion, it should be pointed out that our unknown essential is not identical with aminobutyric acid (Foreman[21], Abderhalden and Weil[1]), the amino acids described by Schryver and his associates[53, 54], or norvaline (Abderhalden and Bahn[5], Abderhalden and Reich[6]). Furthermore, feeding trials have shown that it is not α-methyl-α-amino-n-butyric acid, nor α-methyl-α-amino-n-valerianic acid, the other possible isomers of valine and leucine respectively.

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