equal importance of modern technology for the advance of science, I shall close, acknowledging my obligation to these modern philosophers by quoting from one who was a contemporary of Galileo—for it was Sir Francis Bacon who said, "Nature to be commanded must first be obeyed. Knowledge is power."

THE NUTRITIVE SIGNIFICANCE OF THE AMINO ACIDS AND CERTAIN RELATED COMPOUNDS[1,2]

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THE relation of the amino acids to growth has been the subject of numerous investigations during the past thirty years. Until comparatively recently, however, only three of these compounds, namely, tryptophane, lysine and histidine, had been shown definitely to be indispensable components of the food. Evidence which appeared to demonstrate the essential nature of cystine was presented by Osborne and Mendel; while certain other protein components, notably the dibasic amino acids and the prolines, seemed to be dispensable.[3]

Progress in such investigations was hampered by reason of the fact that methods were not available for the quantitative removal of single protein components. It appeared that if further information was to be obtained, one must resort to the use of diets in which the proteins were replaced entirely by mixtures of highly purified amino acids. Such investigations have been under way in this laboratory since 1930.

It soon became apparent that the *known* amino acids, when incorporated in otherwise adequate diets, were incapable of supporting growth. Evidently, proteins supply something which was not present in our original mixtures. This led eventually to the isolation and identification of a new indispensable constituent, namely, α-amino-β-hydroxy-n-butyric acid.[4] This compound is widely distributed in nature, but appears to occur in greatest abundance in the blood proteins. It has been named d-threonine, inasmuch as its spatial configuration is exactly analogous to that of the sugar d-threose.[5] When threonine is added to suitable amino acid mixtures the resulting preparation is an excellent substitute for dietary proteins.

In all our investigations young albino rats from our own colony have served as the experimental animals. The diets have carried the usual components—carbohydrates, fats, inorganic salts and vitamins—and differed from the ordinary so-called "synthetic" rations only in that proteins were replaced by amino acids. Food and water were kept in the cages at all times, so that the animals ate and drank *ad libitum*. By such a procedure, the importance of the individual amino acids has been determined by omitting them from the food one or more at a time.

Data have already been presented elsewhere demonstrating the indispensable nature of phenylalanine,[6] leucine and isoleucine.[7] It has also been shown that hydroxyglutamic acid,[8] citrulline,[9] tyrosine,[6] norleucine,[7] glycine and serine[10] are non-essential. During the past eighteen months all the generally recognized amino acids present in proteins have been classified with respect to their growth importance. It is the purpose of this note to present these findings, together with an outline of the physiological behavior of certain optically isomeric amino acids and related compounds. The evidence upon which the following statements are based will be described in detail elsewhere.

Contrary to the usual belief, cystine is not an indispensable component of the food. On the other hand, methionine is indispensable. In the absence of the latter from the ration, animals rapidly lose weight and eventually die, even though an abundant amount of cystine is supplied. Of particular interest in this connection is the fact that if methionine is administered at a level which permits maintenance or slow increase in weight, the addition of cystine greatly improves the quality of the diet. Thus, cystine stimu-

1 Aided by a grant from the Rockefeller Foundation.

2 This paper is a summary of one presented before the joint session of the Federation of American Societies for Experimental Biology at Memphis, Tenn., April 22, 1937. The author is indebted to Drs. Madelyn Womack, S. H. Eppstein, C. E. Meyer and a number of graduate students for the actual conduct of the experiments.

3 The earlier literature is reviewed by W. C. Rose, *Yale Jour. Biol. and Med.*, 4: 519, 1932.

4 R. H. McCoy, C. E. Meyer and W. C. Rose, *Jour. Biol. Chem.*, 112: 283, 1935–36.

5 C. E. Meyer and W. C. Rose, *Jour. Biol. Chem.*, 115: 721, 1936.

6 M. Womack and W. C. Rose, *Jour. Biol. Chem.*, 107: 449, 1934.

7 M. Womack and W. C. Rose, *Jour. Biol. Chem.*, 116: 381, 1936.

8 W. Windus, F. L. Catherwood and W. C. Rose, *Jour. Biol. Chem.*, 94: 173, 1931.

9 C. T. Caldwell and W. C. Rose, *Jour. Biol. Chem.*, 107: 57, 1934.

10 R. H. McCoy and W. C. Rose, *Jour. Biol. Chem.*, 117: 581, 1937.

lates growth *only when methionine is supplied in sub-optimal quantities.*

Valine also is an essential component of the food. Despite its structural simplicity, it can not be synthesized by the organism. Its absence from the diet induces a profound failure in growth accompanied by the development of symptoms which are unlike any we have encountered in other types of deficiencies. Animals deprived of valine become sensitive to touch and manifest a severe lack of coordination in movement. Whether the symptoms are associated with specific lesions of the nervous system remains to be determined. As would be expected, the condition is readily cured by the administration of the amino acid.

Several years ago Scull and Rose[11] demonstrated that arginine can be synthesized by the animal organism. It seemed unlikely, therefore, that this amino acid would prove to be an essential. However, following its exclusion from the ration the experimental animals increase in weight at only about three fourths of the normal rate. The addition of arginine to the food remedies the dietary defect. Evidently, the synthesis of this amino acid *in vivo* does not keep pace with the demands of *normal* growth. Thus arginine is unique in that it alone of the essential group may be excluded from the food without occasioning a *loss* in weight.

In contrast to the indispensable amino acids, a number of others have been shown to be dispensable. Alanine, aspartic acid, glutamic acid, proline and hydroxyproline belong to this group. The exclusion of any one of these from the food fails to inhibit growth, or to exert any other detrimental effect under the conditions of our experiments.

The above observations are summarized in Table 1. As a more severe test of the correctness of the above classification, experiments were conducted in which animals received a diet devoid of all the non-essential acids. Under such extraordinary conditions, necessitating the synthesis of twelve tissue components simultaneously, a failure in growth would not have been surprising. As a matter of fact, excellent growth occurred, thereby providing the final proof that only ten of the twenty-two components of proteins need be administered preformed. However, attention should be called to the fact that these findings do not exclude the possibility that functions other than growth, notably reproduction and the detoxication mechanism, may necessitate the administration of other amino acids. The results of such studies will be reported at a later date.

TABLE 1

FINAL CLASSIFICATION OF THE AMINO ACIDS WITH RESPECT TO THEIR GROWTH EFFECTS

Essential	Non-essential
Lysine	Glycine
Tryptophane	Alanine
Histidine	Serine
Penylalanine	Norleucine
Leucine	Aspartic acid
Isoleucine	Glutamic acid
Threonine	Hydroxyglutamic acid
Methionine	Proline
Valine	Hydroxyproline
*Arginine	Citrulline
	Tyrosine
	Cystine

* Arginine can be synthesized by the animal organism, but not at a sufficiently rapid rate to meet the demands of *normal* growth.

Incidental to the above investigations, tests have been made of the nutritive value of the optical antipodes of the essential amino acids. Data already in the literature show that d-tryptophane[12] and d-histidine[13] can serve in place of their enantiomorphs. On the other hand, l-lysine is said to be incapable of replacing d-lysine,[14] an observation which we have confirmed by the use of diets of purified amino acids. Recently, it has been shown in this laboratory that both forms of phenylalanine and of methionine[15] promote growth. The unnatural isomers may not be quite so efficient as the natural, although the differences in this respect are slight. But only the natural forms of valine, leucine and isoleucine are effective. Furthermore, d- and l-alloisoleucine are completely incapable of replacing d-isoleucine.

Experiments were also undertaken to establish how much of each indispensable amino acid is necessary to induce optimum growth. This was accomplished by varying the proportions of one while maintaining all others at constant levels. The results are summarized in Table 2 and are to be regarded as *strictly tentative.* The values represent the *percentages* of the natural amino acids which must be present in the

TABLE 2

MINIMUM AMOUNT OF EACH ESSENTIAL AMINO ACID NECESSARY TO SUPPORT NORMAL GROWTH WHEN THE NON-ESSENTIALS ARE INCLUDED IN THE FOOD

Amino acid	Per cent.
Lysine	1.0
Tryptophane	0.2
Histidine	0.4
Phenylalanine	0.7
Leucine	0.9
Isoleucine	0.5
Threonine	0.6
Methionine	0.6
Valine	0.7
Arginine	0.2
	5.8

[11] C. W. Scull and W. C. Rose, *Jour. Biol. Chem.,* 89: 109, 1930.

[12] V. du Vigneaud, R. R. Sealock and C. Van Etten, *Jour. Biol. Chem.,* 98: 565, 1932; C. P. Berg, *Jour. Biol. Chem.,* 104: 373, 1934.

[13] G. J. Cox and C. P. Berg, *Jour. Biol. Chem.,* 107: 497, 1934.

[14] C. P. Berg, *Jour. Nutrition,* 12: 671, 1936.

[15] See also R. W. Jackson and R. J. Block, *Proc. Soc. Exp. Biol. and Med.,* 30: 587, 1933.

rations. From the food intakes, which are not included in this note, one can calculate the milligrams of each amino acid which were consumed by the animals daily. This may prove to be a more accurate method of expressing the data. It should be emphasized that factors such as the proportion of fat and carbohydrate in the ration, and the age, weight and sex of the subjects may play important rôles in determining the minimum level of a given component. Furthermore, we have not yet investigated the possibility of inducing growth when the animals receive only ten amino acids *each at the minimum level.* If normal increases in weight occur on restricted diets of this nature, the amino acid mixture will prove to be the most efficient source of nitrogen ever devised for the purpose. On theoretical ground it appears more likely that growth will not occur. As indicated above, the values in Table 2 were obtained when liberal quantities of the non-essential amino acids were furnished. In the absence of the non-essential group, the nitrogen intake probably would be inadequate to meet the synthetic demands. In this event, a method will become available for the study of the origin of each dispensable amino acid and of the chemical transformations involved in its formation.

Finally, additional information has been obtained on the replacement of amino acids for growth purposes by the corresponding α-hydroxy and α-keto acids. The results of unpublished experiments demonstrate that phenylalanine, leucine and isoleucine can be replaced by both the hydroxy and keto compounds. Valine is replaceable by α-hydroxyisovaleric acid. Tests with the corresponding α-ketoisovaleric acid have not yet been completed.

OBITUARY

NATHANIEL LYON GARDNER

NATHANIEL LYON GARDNER was born in Keokuk, Iowa, on February 26, 1864. He passed away at his home in Berkeley on August 15, 1937. Graduating from the high school at Iowa Falls, he, after passing the necessary examinations, began teaching school in Iowa, but soon removed to Tacoma, Washington, to enter business with a relative. After the panic of 1893 he returned to teaching, having graduated from the normal school at Ellensburg, Washington. He received the degree of B.S. from the University of Washington in 1900, that of M.S. from the University of California in 1903 and that of Ph.D. in 1906.

As a teacher in the schools, he was soon called to Island County, in the State of Washington. Here he continued studies in natural history which had intrigued him even in his earlier years in Iowa, collecting specimens, of most excellent character, of various groups of plants and sending them to eminent authorities for determination and advice. In 1897, while teaching school at Coupeville, he addressed a letter to the writer at the University of California, asking for instruction in preparing, and assistance in determining, specimens of marine algae, of which he estimated there were about sixty-five species in his neighborhood. There began at that time a cooperation between Gardner and Setchell which increased in scope and lasted until the death of the former. Due to their combined efforts the knowledge of the marine algae of the Pacific coasts of North America was tremendously advanced.

Professor Gardner was an extraordinary collector, with keen insight, careful selection and skilful manipulation, so that his specimens and his knowledge increased side by side. When it came to publicaton, he was most meticulous. Some thirty or more papers were published under his name, while many more than that were published as joint papers with the writer.

He was an authority on blue-green algae of the world, as well as those of the Pacific coasts of North America. His knowledge of the green algae, while more limited to the marine species, was nevertheless extensive and he made very valuable contributions towards a better knowledge of the morphology and development of many species of this group. When it comes to the brown algae, his researches in various groups, from the minute forms to the more gigantic, were characterized not only by the distinguishing of many new species, but by substantial additions to our knowledge of their morphology and development. He was engaged particularly in the study of various polymorphous genera of the red algae of our Pacific coasts at the time of his death.

Professor Gardner, through his wonderful patience in collecting, culturing and studying his specimens, was rapidly bringing order out of the chaos of our knowledge as it previously existed. His work along these lines was cut short, much to the detriment of all those who are interested.

He served the University of California as assistant in botany from 1900 to 1906, as acting assistant professor of botany during the academic year 1909–1910, as assistant professor of botany from 1913 to 1923, as associate professor of botany from 1923 to 1934, and was retired in 1934 as associate professor of botany, emeritus. In 1920 he was also appointed curator of the herbarium of the University of California, which position he held until his retirement in 1934, when he was made curator, emeritus. He was head of the department of biology in the Los Angeles Poly-