Lysine

AN INDISPENSABLE AMINO ACID

ADVANCEMENTS IN AMINO ACID RESEARCH

this review on Lysine

is the third in a series of publications which in due time will cover all of the essential amino acids. It is our firm belief that through the information contained herein other research workers and laboratories will be encouraged to further studies leading to a more complete comprehension of this important field.

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN

AN INDISPENSABLE

AMINO ACID

Amino Acid Research

The discovery of a lysine deficiency in many proteins of vegetable origin has raised questions concerning the nutritional significance of this amino acid. Basic concepts have been developed using diets supplemented with purified lysine, but progress of such fundamental work has been governed by availability of the materials for experimentation.

Today more economical methods of synthesis are helping to expand the horizons of amino acid research. Clinical research with these important compounds is now possible. Furthermore, amino acids are destined to assume an important role in the investigation of livestock and poultry feeding.

The Dow Chemical Company has undertaken leadership in making the essential amino acids available for research in this special field.

BIBLIOGRAPHY

Part I

1. *Drechsel, E., J. prakt. Chem.*, **39,** *425, 1889.*

2. *Vickery, H. B., and Schmidt, C. L. A., Chem. Rev.*, **9,** *169, 1931.*

3. *Hlasiwetz, H., and Habermann, J., Ann.*, **169,** *150, 1873.*

4. *Schützenberger, P., Ann. chem. phys.*, (5) **16,** *289, 1879.*

5. *Schulze, E., and Steiger, E., Ber.*, **19,** *1177, 1886.*

6. *Drechsel, E., Ber.*, **23,** *3096, 1890.*

7. *Hedin, S. G., Z. physiol. Chem.*, **20,** *186, 1895.*

8. *Hedin, S. G., Z. physiol. Chem.*, **22,** *191, 1896.*

9. *Drechsel, E., Archiv. Physiol., 248, 1891.*

10. *Drechsel, E., and Kruger, T. R., Ber.*, **25,** *2454, 1892.*

11. *Ellinger, A., Ber.*, **32,** *3542, 1899; Z. physiol. Chem.* **29,** *334, 1900.*

12. *Fischer, E., and Weigert, F., Z. physiol. Chem.*, **35,** *3772, 1902.*

13. *Vickery, H. B., and Leavenworth, C. S., J. Biol. Chem.*, **76,** *437, 1928.*

Part II

1. *Kossel, A., and Kutscher, F., Z. physiol. Chem.*, **31,** *165, 1900-01.*

2. *Vickery, H. B., and Leavenworth, C. S., J. Biol. Chem.*, **76,** *707, 1928; see Vickery, H. B., and Block, R. J., Ibid*, **93,** *105, 1931, for additional references.*

3. *Cox, G. J., King, H., and Berg, C. P., Biol. Chem.*, **81,** *755, 1929.*

4. *Albanese, A. A., J. Biol. Chem.*, **134,** *467, 1940.*

5. *Rice, E. E., J. Biol. Chem.*, **131,** *1, 1939.*

6. *Kurtz, A. C., J. Biol. Chem.*, **140,** *705, 1941.*

7. *Van Slyke, D. D., J. Biol. Chem.*, **10,** *15, 1911-12.*

8. *Block, R. J., and Bolling, D., The Amino Acid Composition of Proteins and Foods, Chas. C. Thomas, Springfield, Illinois, 1947.*

9. *Van Slyke, D. D., Dillon, R. T., MacFadyen, D. A., and Hamilton, P., J. Biol. Chem.*, **141,** *627, 1941. See p. 658.*

10. *Van Slyke, D. D., Hiller, A., and Dillon, R. T., J. Biol. Chem.*, **146,** *137, 1942. See p. 156.*

11. *Van Slyke, D. D., and Birchard, F. J., J. Biol. Chem.* **16,** *539, 1913-14.*

12. *Dunn, M. S., and Lewis, H. B., J. Biol. Chem.*, **49,** *327, 343, 1921; Wiley, F. H., and Lewis, H. B., Ibid*, **86,** *511, 1930.*

13. *Hopkins, S. J., and Wormall, A., Biochem. J.*, **27,** *740, 1706, 1933;* **28,** *228, 1934.*

14. *Gurin, S., and Clarke, H. T., J. Biol. Chem.*, **107,** *395, 1934.*

15. *Herriott, R. M., Advances in Protein Chemistry*, **3,** *169, 1947. See p. 173.*

16. *Lieben, F., and Lee, Y. C., J. Biol. Chem.*, **145,** *223, 1942.*

17. *Boulet, M., Nelson, J. A., and MacFarlane, W. D., Can. J. Research*, **25B,** *540, 1947.*

18. *Kibrick, A. C., Arch. Biochem.*, **20,** *22, 1949.*

19. *Gale, E. F., and Epps, H. M. R., Biochem. J.*, **38,** *232, 1944.*

20. *Zittle, C. A., and Eldred, N. R., J. Biol. Chem.*, **156,** *401, 1944.*

21. *Gale, E. F., Biochem. J.*, **39,** *46, 1945.*

22. *Dunn, M. S., Camien, M. N., Shankman, S., Franke, W., and Rockland, L. B., J. Biol. Chem.*, **156,** *715, 1944.*

23. *Brand, E., Saidel, L. J., Goldwater, W. H., Kassel, B., and Ryan, F. J., J. Am. Chem. Soc.*, **67,** *1524, 1945.*

24. *Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C., J. Biol. Chem.*, **160,** *35, 1945.*

25. *Shemin, D., J. Biol. Chem.*, **159,** *439, 1945.*

26. *Doermann, A. H., J. Biol. Chem.*, **160,** *95, 1945.*

27. *Rittenberg, D., and Foster, G. L., J. Biol. Chem.*, **133,** *737, 1940.*

28. *Foster, G. L., J. Biol. Chem.*, **159,** *431, 1945.*

29. *Whitehorn, J. C., J. Biol. Chem.*, **56,** *751, 1923.*

30. *Turba, F., Ber.*, **74,** *1829, 1941.*

31. *Wieland, T., Ber.*, **77,** *539, 1944.*

32. *Block, R. J., Arch. Biochem.*, **11,** *235, 1946.*

33. *Consden, R., Gordon, A. H., and Martin, A. J. P., Biochem. J.*, **38,** *224, 1944.*

34. *Williams, R. J., and Kirby, H. Science*, **107,** *481, 1948.*

35. *Albanese, A. A., and Lein, M., Science*, **110,** *163, 1949.*

PART I

DISCOVERY AND IDENTIFICATION

TIME AND AGAIN new discoveries have grown out of the development of new techniques, the use of new reagents, or attempts to reconcile divergent experimental observations. The isolation of lysine, one of the three basic amino acids, by Drechsel in 1889 (1), is an exemplification of this in the amino acid field.

Prior to 1889 only eleven of the nineteen amino acids now commonly associated with proteins had been isolated and only nine were known to be components of proteins (2). Drechsel had become anxious to learn why the hydrolysis of protein with hydrochloric acid in the presence of stannous chloride, first used by Hlasiwetz and Habermann in 1873 (3), and the digestion of proteins with barium hydroxide, as outlined by Schutzenberger in 1879 (4), should both yield ammonia and amino acids. The acid hydrolysis should liberate little or no carbon dioxide, the alkaline hydrolysis a considerable amount. He was also struck with the realization that the amino acids which had been isolated had failed to account for more than a small portion of the total nitrogen in the protein. He therefore hydrolyzed casein and isolated the readily crystallizable amino acids (glutamic acid, aspartic acid, tyrosine and leucine), as Hlasiwetz and Habermann had done, then concentrated the mother liquors to a syrupy consistency and added phosphotungstic acid. He obtained a very bulky precipitate. This he suspended in water and decomposed with barium hydroxide. He removed the barium phosphotungstate, freed the filtrate of excess barium by precipitating the latter as the sulfate, added hydrochloric acid, and concentrated the solution to thick syrupy residue. Upon standing over sulfuric acid, crystals formed. These were dissolved in a minimum of water and absolute alcohol was then added. Addition of ether produced an oily precipitate which became crystalline upon standing. Drechsel recognized the crystals as the chloride of a strong base which could be liberated by adding silver carbonate. When the solution of the free base was heated, a weak, sperm-like odor was evolved.

Phosphotungstic acid had long been used to precipitate alkaloids, and had only three years before (1886) been applied by Schulze and Steiger (5) to a water extract of etiolated lupine seedlings, as part of a procedure which had led to the isolation of arginine. It is quite likely that the experience of Schulze and Steiger had prompted Drechsel to apply the reagent for the first time to residues obtained from an acid hydrolysate of a protein, a procedure still in common use for the isolation of the basic amino acids, arginine, histidine, and lysine.

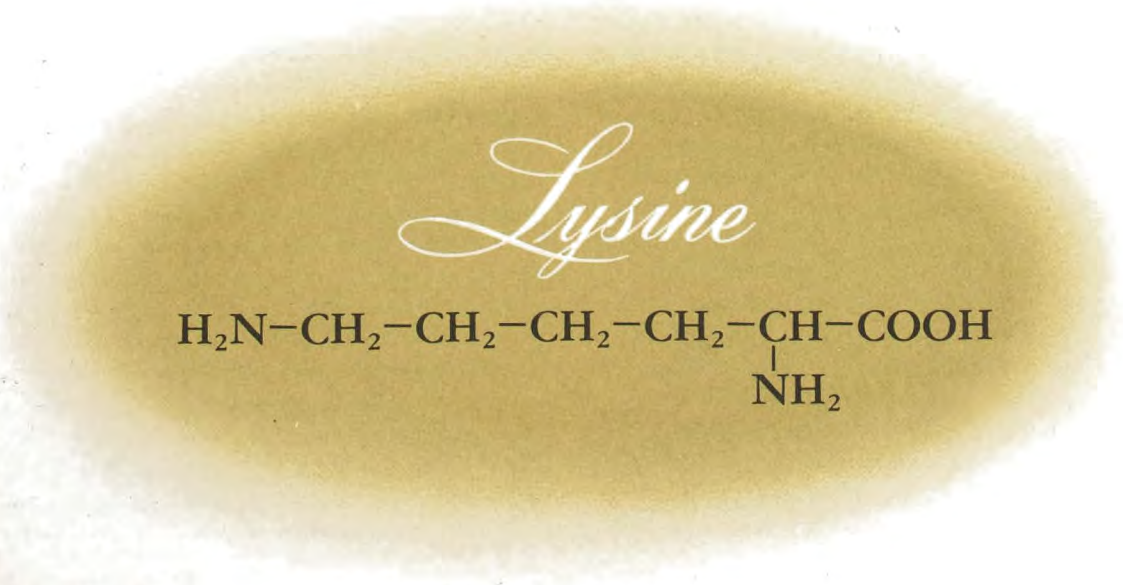
From the crystalline product which Drechsel had obtained and from its mother liquors, he proceeded to prepare chloroplatinate derivatives. He obtained two products differing in color and composition. He noted that the crude mixture of the chlorides could be heated with concentrated hydrochloric acid to 150° without decomposition, but that when this or the crystalline chloride was heated with barium hydroxide at 120°-130°, degradation occurred, with the production of barium carbonate. He assumed that the base was the source of the carbon dioxide which Schutzenberger's method of protein hydrolysis had produced.

In attempting to evaluate Drechsel's early work, it is important to note that arginine had not yet been isolated from protein hydrolysates and that histidine had not been recognized. It is therefore not surprising that he did not at once recognize that some of his products were mixtures. In 1890, he applied a new reagent, silver nitrate, and obtained a double salt of a base which yielded urea upon hydrolysis with barium hydroxide (6). From the analysis of the double salt plus the assumption that it contained a readily removable molecule of water, he concluded that the base was a homologue of creatine which could lose water to form its corresponding anhydride. Presumably be-

cause the base underwent hydrolysis, he called it *lysatine* and its anhydride *lysatinine*. The probability that lysatine was not a pure substance was not recognized until five years later when Hedin (7), a former student of Drechsel, applied the methods which Drechsel had used to a hydrolysate of horn and was able to isolate both lysine and arginine from a compound having the composition of Drechsel's double salt of lysatine and silver nitrate. Hedin thus discovered for the first time that arginine was present in a protein hydrolysate. Later he isolated the third hexone base, histidine, from an amorphous precipitate produced when silver nitrate was added to a solution of the free bases obtained from protein hydrolysates (8). Thus, the confusion first caused by Drechsel's use of silver nitrate, now recognized as an invaluable reagent, was resolved as experience was gained in its application.

The name lysine first appears in 1891 in a long paper by Drechsel (9) on the decomposition of proteins. Of the six sections in the paper, the first three appear under Drechsel's own name, each of the last three under the name of one of his students, Fischer, Siegfried, and Hedin. In the second section, Drechsel first gives the correct formula for lysine chloroplatinate and recognizes the base as a diaminocaproic acid and a homologue of ornithine. In the fourth section, Fischer first applies the name lysine, without explanation, to the same base. He had obtained it from a hydrolysate of gelatin by first preparing Drechsel's so-called lysatine (or lysatinine) in crude form, decomposing this with barium hydroxide, and isolating the lysine from the resulting hydrolysate as the chloroplatinate.

The deductions of Drechsel and Kruger (10) that lysine was a derivative of pentamethylenediamine were confirmed by Ellinger's demonstration that this diamine could be obtained by incubating lysine anaerobically with putrefactive bacteria (11). That lysine was indeed α, ε-diaminocaproic acid was established by Fischer and Weigert (12), who synthesized it by using nitrous acid to convert γ-cyanopropylmalonic ester to the ethyl ester of a-oximino-δ-cyanovalerianic acid, reducing and hydrolyzing the latter in absolute alcohol solution with sodium, and isolating it as the dihydrochloride. He found it identical with the DL-lysine dihydrochloride which he prepared by heating natural lysine dihydrochloride with 20 parts of 20 per cent hydrochloric acid in a closed tube for 15 hours. It may be pertinent here to add that free lysine, as Drechsel and Kruger noted, is so alkaline that it readily combines with carbon dioxide. Special care must therefore be taken to prepare crystalline lysine. This was first accomplished in 1928 by Vickery and Leavenworth (13).



PART II

DETERMINATION

THE CHEMICAL METHODS which have thus far been most commonly employed in estimating lysine have involved either its isolation or its determination by nitrogen distribution analysis. In the former type of procedure, the basic amino acids must usually be isolated and separated; in the latter, histidine nitrogen and arginine nitrogen must be determined in order to calculate lysine nitrogen. Most of the variations in the isolation procedure have been concerned with the method of hydrolysis, the fractionation of the hydrolysate to yield the basic amino acids, and the method of precipitating or otherwise removing the histidine and arginine, rather than with the precipitation of the lysine.

A. Isolation Methods

The procedure of Kossel and Kutscher (1) may be considered more or less basic. The protein is hydrolyzed with sulfuric acid, then freed of humin, excess acid and ammonia. The histidine and arginine are precipitated as the silver salts, the solution being made strongly alkaline with barium hydroxide. The silver salts are filtered off and the filtrate and washings are used for the isolation of lysine which is precipitated as the phosphotungstate, after first removing excess barium and silver. The phosphotungstate is next decomposed and the lysine converted to the picrate. Usually the lysine is estimated gravimetrically as the picrate. The Kossel and Kutscher procedure has been modified in various ways by various investigators. It has undoubtedly been studied most critically by Vickery and Leavenworth (2) whose revisions have made it a more precise analytical tool.

Since histidine, arginine and lysine are more basic than the other amino acids, and the isoelectric points of histidine and arginine are respectively almost two pH units less and two greater than that of lysine, the three may first be separated from the other amino acids by electrophoresis, then the arginine and lysine separated from the histidine—also by electrophoresis, the arginine precipitated as the flavianate, and the lysine as the picrate, without previous precipitation as the phosphotungstate (3). Although this method has been used more extensively as a preparative method, it has also been recommended, with modifications, as an analytical procedure (4).

Direct precipitation of lysine as the picrate has been applied to sulfuric acid hydrolysates of blood corpuscle paste after neutralization with calcium or barium hydroxide (5). According to Kurtz (6), this procedure fails entirely with gelatin. He has devised a method consisting of the conversion of the amino acids to their copper salts, followed by benzoylation. ε-Benzoyllysine copper precipitates and is converted first into ε-benzoyllysine, then into lysine dihydrochloride. The last two methods, at least at their present state of development, should be regarded as preparative, rather than as analytical procedures. Lysine dihydrochloride in alcoholic solution may be converted into the monohydrochloride through the addition of pyridine in absolute alcohol (5).

B. Nitrogen Distribution

In this procedure the basic amino acids and cystine are precipitated with phosphotungstic acid from a protein hydrolysate previously freed of ammonia. Usually the phosphotungstate is decomposed and aliquots of the filtrate are analyzed for sulfur, total nitrogen, amino nitrogen, and arginine nitrogen. Of the four amino acids, cystine and lysine yield all of their nitrogen when allowed to react with nitrous acid for thirty minutes, whereas histidine yields but a third and arginine but a fourth of theirs. The cystine nitrogen can be calculated from the sulfur analysis. When boiled with alkali, arginine undergoes hydrolysis to urea and ornithine, and the urea decomposes to yield ammonia. Half of the nitrogen of arginine is thereby

liberated. Besides arginine, only the histidine contains non-amino nitrogen, two-thirds of its total nitrogen being in this form. Hence, Histidine N = 3/2 [(Total N—Amino N)—¾ Arginine N]. The lysine nitrogen can then be calculated by difference: Lysine N = Total N—(Cystine N—Arginine N—Histidine N).

When only lysine is to be determined, advantage may be taken of the observation that in four minutes at 20° all of its α-amino nitrogen and very little of its ε-amino nitrogen reacts, whereas in thirty minutes both groups react. If this is true, then the ε-amino nitrogen can be determined by difference and the total lysine nitrogen calculated without also determining the cystine, arginine and histidine nitrogen.

The Van Slyke nitrogen distribution method has been modified in various ways since it was first published in 1911 (7). The interested reader may find a review of these and a detailed discussion and evaluation of the various steps of both the Kossel and Kutscher and the Van Slyke methods elsewhere (8).

C. Estimation from "Carboxyl Nitrogen" and Amino Nitrogen

In 1941, Van Slyke *et al.* devised a procedure for determining the carboxyl groups in free amino acids gasometrically (9). Advantage was taken of the observation that when α-amino acids are boiled with an excess of ninhydrin at pH 1 to 5, the CO_2 from their carboxyl groups is evolved in a few minutes. Only the carboxyl group adjacent to the α-amino group responds, except in the case of aspartic acid in which both carboxyl groups release CO_2. In a mixture of the basic amino acids, such as that obtained by precipitation with phosphotungstic acid, each of the amino acids except lysine yields one amino nitrogen by the nitrous acid method and all yield one "carboxyl nitrogen" by reaction with ninhydrin at pH 1. In the case of lysine the ratio is 2:1. Hence, lysine N = 2 (amino N—carboxyl N). Van Slyke recommends this calculation as more exact (10), than the calculation from total nitrogen and arginine, cystine and histidine nitrogen originally used (7).

D. Estimation from "Free" Amino Nitrogen

The ε-amino group of lysine apparently does not participate in the production of the peptide linkages found in native proteins, but exists in a form which will readily react with nitrous acid (11, 12), or with such reagents as phenylisocyanate (13), or benzenesulfonyl chloride (14). Proteins treated with nitrous acid for 25 minutes yield approximately half as much nitrogen as the lysine fraction obtained from hydrolysates of the untreated protein (11), and no lysine can be isolated from hydrolysates that are prepared from the treated protein (12). In some proteins there appear to be some free α-amino groups which would also react (15).

Lieben and Loo (16) have devised a method for the estimation of lysine, based on the reaction of the native protein in the Van Slyke apparatus, with correction for the α-amino groups liberated by the hydrolytic action of the glacial acetic acid on the protein. They determine the amino nitrogen liberated in 30 (a), 60 (b), and 90 (c) minutes and calculate the ε-amino nitrogen from the equation: ε-amino N = c—3 (b—a). When a 100 mg. sample of protein is used and the amino N is expressed in mg., ε-amino N x 146/14 then gives the per cent of lysine.

E. Colorimetric Estimation

Recently a method has been devised for the determination of lysine which is based upon the capacity of chlorinated or brominated lysine to reduce Folin's phenol reagent. Since tryptophan, tyrosine and other amino acids also react with the reagent, the lysine from a protein hydrolysate is first absorbed, along with arginine, by passing the solution through a column of Decalso (17), or it is removed by shaking with permutit, which also adsorbs histidine (18). The eluate containing the lysine is chlorinated (16), or brominated (18), before the color is developed. A correction must be applied for the presence of histidine. The results are said to agree favorably with those obtained by other methods of analysis.

F. Microbiological Decarboxylation

In 1944 Gale and Epps (19), published a study which showed the feasibility of using an L-lysine decarboxylase preparation from *Bacterium cadaveris* as an agent for determining L-lysine. The carbon dioxide liberated is measured manometrically. Zittle and

Eldred (20), and Gale (21), have applied the method to a number of protein hydrolysates and have obtained results in good agreement with analytical data yielded by chemical methods and by other methods of microbiological assay. The procedure is stereochemically specific. Gale and Epps obtained a 45 per cent response upon applying it to DL-lysine.

G. Lactic Acid Production

Leuconostoc mesenteroides P-60 (22, 23), and *Streptococcus faecalis* (24), have been recommended for use in the microbiological assay of L-lysine in protein hydrolysates. Titration of acid production in the medium has usually been employed as the index of growth, but measurement of turbidity has also been used. Higher values have been obtained by these procedures than by isolation and nitrogen partition, as might be anticipated. According to Brand (23), the use of L. *mesenteroides* is preferable to that of the decarboxylase of *B. cadaveris* which seems to yield low results. In the analysis of bovine serum albumin the result obtained with the former was practically identical with the isotopic dilution value reported by Shemin (25).

H. Mycelial Growth

Doermann (26) has employed a "lysine-less" mutant of *Neurospora crassa*, using the weight of the dried mycelium obtained after seven days of growth as the criterion. Since arginine inhibits the growth of this mutant, it must first be precipitated from the hydrolysate of the protein being assayed, or converted to ornithine with arginase. The results obtained for lysine were considered reliable.

I. Isotopic Dilution

The isotopic dilution method originally devised by Rittenberg and Foster (27) has been applied in the determination of lysine by Foster (28) and by Shemin (25). As applied to lysine, the method consists essentially of hydrolyzing a protein, adding a known weight of isotopic lysine of known N^{15} excess, isolating and purifying the lysine from the hydrolysate, and determining the N^{15} excess in the isolated and purified product by the mass spectrograph. When the synthetic form of the isotopic amino acid is added, as is usually the case, the method of recrystallization chosen must be one which will yield the pure L-amino acid. From the amount of isotopic lysine added and the amount of isotope in the final product, the amount of natural lysine with which the isotopic lysine was diluted can be calculated. The method is one of the most accurate, but obviously its application is limited to laboratories having facilities for isotopic analysis. Radioactive, as well as stable, isotopes might be applied.

J. Adsorption

Some years ago, Whitehorn (29) showed that permutit could be used to separate relatively strong bases, among them lysine, from weaker bases (those having dissociation constants below $5x10^{-9}$) and nonbasic substances. Until recently, the principle was not applied quantitatively. In 1941, Turba (30) described the quantitative separation of arginine, histidine and lysine from each other and from the neutral and acid amino acids. Upon passing an amino acid mixture through a "Floridin XXF extra" column, the arginine and lysine were adsorbed, but the other amino acids were washed out with water. The adsorbed amino acids were then eluted with a mixture of pyridine and sulfuric acid. The pyridine and sulfuric acid were removed and the eluate was then concentrated and passed through a "Filtrol-Neutrol" column. The lysine was extracted with M/6 KH_2PO_4. Recovery from known mixtures was 98 per cent complete. The adsorbents indicated above are of the Fuller's earth type. Synthetic resins have been studied by a number of workers. Wieland has observed that Wofatit C, one of the carboxyl resins which bind only the basic amino acids, will retain only lysine and arginine in the presence of potassiums ions, the histidine passing through (31). Block has tested a number of different cation exchange resins (32) for their capacities to remove the basic amino acids from protein hydrolysates. The method appears to be one of great promise, but at present is in the developmental stage.

An exceedingly useful qualitative method of amino acid separation which may eventually develop into a procedure suitable for the quantitative determination of minute amounts of amino acids is that of partition chromatography on paper (33). A one dimensional chromatogram is obtained by placing a drop of solution of amino acids near the top of a filter paper strip

and allowing the strip to hang from a trough containing a solvent in a closed chamber saturated with water and the solvent vapor. The individual amino acids are carried down the paper at rates depending upon the ratio of water to solvent and by the partition coefficient. The order of the amino acids down the strip differs with the solvent. The position of the amino acids may be determined by drying the paper and spraying it with ninhydrin solution, then drying and heating to produce the color reaction. By using an almost square filter paper and placing the spot to be analyzed at one corner, allowing it to develop along one edge with one solvent, then drying the paper and placing the edge along which the chromatogram was first developed at the top, and redeveloping with a second solvent, a two-dimensional chromatogram may be obtained. Williams and Kirby (34) have used capillary ascent rather than capillary descent, and Albanese and Lein (35) have applied the principle of ascent in the detection of lysine in the urine, converting the amino acids first to their copper salts, partitioning 0.02 cc. aliquots of the dried salt solution on paper strips in an aqueous phenol atmosphere for 6 hours. After drying, the strip was painted with ferrocyanide to locate the amino acids, the copper salts reacting to produce the pink copper ferrocyanide.

BIBLIOGRAPHY

Part III

1. *Fischer, E., and Weigert, F., Ber.*, **35**, *3772, 1902.*

2. *Sörensen, S. P. L., Compt. rend. trav. lab. Carlsberg*, **6**, *1, 1903.*

3. *von Braun, J., Ber.*, **42**, *839, 1909.*

4. *Eck, J. C., and Marvel, C. S., J. Biol. Chem.*, **106**, *387, 1943.*

5. *Galat, A., J. Am. Chem. Soc.*, **69**, *86, 1947.*

6. *Adamson, D. W., J. Chem. Soc., 1564, 1939.*

7. *Gaudry, R., Can. J. Research*, **26B**, *387, 1948.*

8. *Rogers, A. O., J. Am. Chem. Soc.*, **71**, *1837, 1949.*

9. *Sugasawa, S., J. Pharm. Soc. Japan*, **550**, *1044, 1927.*

10. *Warner, D. T., and Moe, O. A., J. Am. Chem. Soc.*, **70**, *3918, 1948.*

Part IV

1. *Vickery, H. B., and Leavenworth, C. S., J. Biol. Chem.*, **76**, *437, 1928.*

2. *Fischer, E., and Weigert, F., Ber.*, **35**, *3772, 1902.*

3. *Adamson, D. W., J. Chem. Soc., 1564, 1939.*

4. *Dunn, R., Inouye, K., and Kirk, P. L., Mikrochemie*, **27**, *154, 1939.*

5. *Berg, C. P., J. Biol. Chem.*, **115**, *9, 1936.*

6. *Rice, E. E., Biochemical Preparations*, **1**, *63, 1949.*

7. *Eck, J. C., and Marvel, C. S., Organic Syntheses, Coll. vol. II, 374, 1943.*

8. *Siegfried, M., Ber., 24, 418, 1891; Z. physiol. Chem.*, **43**, *363, 1904-05.*

9. *Neuberger, A., and Sanger, F., Biochem. J.*, **38**, *125, 1944.*

PART III

SYNTHESIS

As INDICATED earlier, lysine was first synthesized by reducing ethyl α-isonitroso-δ-cyanovalerate with sodium and alcohol (1). Several other synthetic procedures have subsequently been employed, among them the reduction and hydrolysis of diethyl (3-cyanopropyl) phthalimidomalonate, produced by condensing γ-chlorobutyronitrile and sodium ethyl phthalimidomalonate (2); the conversion of N-benzoylpiperidine (3) or cyclohexanone oxime (4) to ε-benzamidocaproic acid, thence to the α-bromo acid with phosphorus and bromine (3, 4) or to the α-chloro acid with sulfonyl chloride in the presence of iodine (5), and finally to lysine by amination and hydrolysis (3, 4); reaction of hydrazoic acid with ethyl 2-oxocyclohexanecarboxylic acid and subsequently with the α-aminopimelic acid thus produced (6); hydrolysis of 3, 4-dihydro-2H-pyran with acid to yield the δ-hydroxyvaleraldehyde which is then converted by a modified Strecker synthesis to 5-(4-hydroxybutyl)-hydantoin, followed by hydrohalogenation to the 5-(4-halobutyl) -hydantoin and amination of the latter with concentrated ammonia (7, 8); preparation of N-(3-formylpropyl) benzamide from acrolein via β-cyanopropionaldehyde diethyl acetal, conversion to ε-benzamidocaproic acid, thence to the α-bromo derivative and to lysine (9); conversion of diethyl acetamido (2-formylethyl) malonate via the cyanohydrin to diethyl acetamido (4-acetamidobutyl) malonate which is hydrolyzed with concentrated hydrochloric acid (10).

PART IV

PROPERTIES

THE ISOLATION of lysine from hydrolysates has been most commonly via the monopicrate, a derivative which has also been used for purposes of identification, or verification. In a capillary tube, the pure L-lysine monopicrate darkens at 240°, becomes very dark around 250° and shows no obvious melting, but rather a slight explosion at 265-266°. The decomposition temperature is little altered by rate of heating (1). According to Fischer and Weigert (2), the monopicrate of DL-lysine darkens and decomposes at 230°. The DL-lysine dipicrate sinters at 169° and melts at 188-190° C. (3). Photomicrographs of the phosphotungstates, picrates, and picrolonates and their crystallographic characteristics have been published (4).

As indicated earlier, free lysine is so alkaline that its solutions absorb carbon dioxide from the air, but with proper precautions it can be prepared (1). Free L-lysine crystallizes as fine transparent needles from water which usually aggregate into irregular striated, plate-like forms and in radiating groups. From aqueous alcoholic solutions either fine needles or hexagonal plates may form. Free lysine darkens above 210° and melts with decomposition at 224-225°. $[a]_D^{20} = +14.6°$.

L-Lysine dihydrochloride crystallizes in elongated prisms which melt at 201-202°; its $[a]_D^{20}$, using 3 gm. per 100 cc. of solution, in water, is +15.63° (5). DL-lysine dihydrochloride melts at 188-190° (5). Aqueous solutions of the dihydrochloride are acid to litmus. The dihydrochloride may be converted to the monohydrochloride, which is neutral to litmus, by dissolving it in 95 per cent alcohol and adding an alcoholic solution of pyridine. The anhydrous L-lysine monohydrochloride melts at 263-264° (6), as does also the DL form (7). The L-lysine monohydrochloride shows $[a]_D^{26} = 20\text{-}21°$, when its solution in normal hydrochloric acid contains 1 gm. per 100 cc.

Racemic lysine may be resolved with camphoric acid (5). Natural lysine may be racemized by heating at an elevated temperature for several hours with barium hydroxide solution (8) or with concentrated hydrochloric acid (2, 8). Neuberger and Sanger (9) have prepared D-lysine by Walden inversion. Action of nitrosyl bromide on ε-N-benzoyl-L-lysine produces levorotatory a-bromo-ε-benzamidocaproic acid which on aminolysis yields ε-N-benzoyl-D-lysine having a specific rotation opposite in direction, but equal in absolute value, to the starting material.

BIBLIOGRAPHY

Part V

1. *Drechsel, E., J. prakt. Chem.*, **39**, *425, 1889.*

2. *Hausmann, W., Z. physiol. Chem.*, **27**, *95, 1899.*

3. *Kossel, A., and Kutscher, F., Z. physiol. Chem.*, **31**, *165, 1900-01.*

4. *Fischer, E., Z. physiol. Chem.*, **33**, *151, 1901.*

5. *Hopkins, F. G., and Cole, S. W., J. Physiol.*, **27**, *418, 1901.*

6. *Osborne, T. B., and Harris, I. F., J. Am. Chem. Soc.*, **25**, *853, 1903.*

7. *Osborne, T. B., and Harris, I. F., J. Am. Chem. Soc.*, **25**, *323, 1903.*

8. *Willcock, E. G., and Hopkins, F. G., J. Physiol.*, **35**, *88, 1906.*

9. *Osborne, T. B., and Mendel, L. B., J. Biol. Chem.*, **12**, *473, 1912.*

10. *Osborne, T. B., and Mendel, L. B., J. Biol. Chem.*, **17**, *325, 1914.*

11. *Totter, J. R., and Berg, C. P., J. Biol. Chem.*, **127**, *375, 1939.*

12. *Osborne, T. B., and Mendel, L. B., J. Biol. Chem.*, **26**, *293, 1916.*

13. *Grau, C. R., Kratzer, F. H., and Asmundson, V. S., Poultry Sci.*, **25**, *529, 1946.*

14. *Rose, W. C., and Rice, E. E., Science*, **90**, *186, 1939.*

15. *Albanese, A. A., Holt, L. E., Jr., Brumback, J. E., Jr., Hayes, M., Kajdi, C., and Wangerin, D. M., Proc. Soc. Exp. Biol. and Med.*, **48**, *728, 1941.*

16. *Rose, W. C., Proc. Am. Phil. Soc.*, **91**, *112, 1947.*

17. *Osborne, T. B., and Mendel, L. B., J. Biol. Chem.*, **25**, *1, 1916.*

18. *Rose, W. C., Science*, **86**, *298, 1937.*

19. *Wretlind, K. A. J., Acta Physiologica Scandinavica*, **17**, *Suppl.* **59**, *1, 1949.*

20. *Almquist, H. J., and Mecchi, E., Proc. Soc. Exp. Biol. and Med.*, **49**, *174, 1942.*

21. *Rose, W. C., Federation Proc.*, **8**, *546, 1949.*

22. *McGinty, D. A., Lewis, H. B., and Marvel, C. S., J. Biol. Chem.*, **62**, *75; 1924-25.*

23. *Berg, C. P., and Dalton, J. L., Proc. Soc. Exp. Biol. and Med.*, **31**, *709, 1934.*

24. *Berg, C. P., J. Nutrition*, **12**, *671, 1936.*

25. *Albanese, A. A., Advances in Protein Chemistry*, **3**, *227, 1947.*

26. *Albanese, A. A., and Lein, M., Science*, **110**, *163, 1949.*

27. *Van Pilsum, J. F., and Berg, C. P. unpublished data.*

28. *Wolf, P. A., and Corley, R. C., Am. J. Physiol.*, **127**, *589, 1939.*

29. *Neuberger, A., and Webster, T. A., Biochem. J.*, **39**, *200, 1945.*

30. *Burroughs, E. W., Burroughs, H. S., and Mitchell, H. H., J. Nutrition*, **19**, *363, 1940.*

31. *Mitchell, H. H. Arch. Biochem.*, **12**, *293, 1947.*

32. *Wissler, R. W., Steffee, C. H., Frazier, L. E., Woolridge, R. L., and Benditt, E. P., J. Nutrition*, **36**, *245, 1948.*

33. *Geiling, E. M. K., J. Biol. Chem.*, **31**, *173, 1917.*

34. *Frazier, L. E., Wissler, R. W., Steffee, C. H., Woolridge, R. L., and Cannon, P. R., J. Nutrition*, **33**, *65, 1947.*

35. *Winters, J. C., Smith, A. H., and Mendel, L. B., Am. J. Physiol.*, **80**, *577, 1927.*

36. *Lafon, M., Ann. Physiol. et Physicochim. Biol.*, **15**, *1, 1939.*

37. *Pearson, P. B., Am. J. Physiol.*, **118**, *786, 1937.*

38. *Asdell, S. A., and Crowell, M. F., J. Nutrition*, **10**, *13, 1935.*

39. *Hart, E. B., Nelson, V. E., and Pitz, W., J. Biol. Chem.*, **36**, *291, 1918.*

40. *Fowler, A. B., Morris, S., and Wright, N. C., Scottish J. Agr.*, **17**, *261, 1934.*

41. *Harris, H. A., Neuberger, A., and Sanger, F., Biochem. J.*, **37**, *508, 1943.*

42. *Gillespie, M., Neuberger, A., and Webster, T. A., Biochem. J.*, **39,** *203, 1945.*

43. *Sydenstricker, V. P., Hall, W. K., Hock, C. W., and Pund, E. R., Science,* **103,** *194, 1946.*

44. *Fritz, J. C., Hooper, J. H., Halpin, J. L., and Moore, H. P., J. Nutrition,* **31,** *387, 1946.*

45. *Albanese, A. A., Holt, L. E., Jr., Frankston, J. E., Kajdi, N., Brumback, J. E., Jr., and Wangerin, D. M., Proc. Soc. Exp. Biol. and Med.,* **52,** *209, 1943.*

46. *Hogan, A. G., and Ritchie, W. S., J. Biol. Chem.,* **107,** *179, 1934.*

47. *Hogan, A. G., Powell, E. L., and Guerrant, R. E., J. Biol. Chem.,* **137,** *41, 1941.*

48. *Hogan, A. G., Guerrant, R. E., and Ritchie, W. S., J. Biol. Chem.* **115,** *659, 1936.*

49. *Gingras, R., Page, E., and Gaudry, R., Science,* **105,** *621, 1947.*

50. *Page, E., Gaudry, R., and Gingras, R., J. Biol. Chem.,* **171,** *831, 1947.*

51. *Dent, C. E., Biochem. J.,* **43,** *169, 1948.*

52. *Osborné, T. B., and Mendel, L. B., J. Biol. Chem.* **37,** *557, 1919;* **41,** *275, 1920.*

53. *Jones, D. B., Caldwell, A., and Widness, K. D., J. Nutrition,* **35,** *639, 1948.*

54. *Block, R. J., Advances in Protein Chemistry,* **2,** *119, 1945.*

55. *Block, R. J., and Bolling, D., The Amino Acid Composition of Proteins and Foods, C. C. Thomas, Springfield, Illinois, 1945.*

56. *Sherman, H. C., Foods: Their Values and Management, Columbia University Press, New York, 1946.*

57. *Bricker, M., Mitchell, H. H., and Kinsman, G. M., J. Nutrition,* **30,** *269, 1945.*

58. *Kuether, C. A., and Myers, V. C., J. Nutrition,* **35,** *651, 1948.*

59. *Hoffman, W. S., and McNeil, G. C., J. Nutrition,* **38,** *331, 1949.*

60. *Allison, J. B., and Anderson, J. A., J. Nutrition,* **29,** *413, 1945.*

61. *Morgan, A. F., and King, F. B., Proc. Soc. Exp. Biol. and Med.,* **23,** *353, 1926.*

62. *Morgan, A. F., J. Biol. Chem.,* **90,** *771, 1931.*

63. *Murlin, J. R., Nasset, E. S., and Marsh, M. E., J. Nutrition,* **16,** *249, 1938.*

64. *Stewart, R. A., Hensley, G. W., and Peters, F. N., Jr., J. Nutrition,* **26,** *519, 1943.*

65. *Greaves, E. O., and Morgan, A. F., Proc. Soc. Exp. Biol. and Med.,* **31,** *506, 1934; Greaves, E. O., Morgan, A. F., and Loveen, M. K., J. Nutrition,* **16,** *115, 1938.*

66. *Block, R. J., Jones, D. B., and Gersdorff, C. E. F., J. Biol. Chem.,* **105,** *667, 1934.*

67. *Seegers, W. H., and Mattill, H. A., J. Biol. Chem.,* **110,** *531, 1935.*

68. *Eldred, N. R., and Rodney, G., J. Biol. Chem.,* **162,** *261, 1946.*

69. *Schmidt, C. L. A., The Chemistry of Proteins and Amino Acids, C. C. Thomas, Springfield, Ill., p. 285, 1938.*

70. *Harris, R. L., and Mattill, H. A., J. Biol. Chem.,* **132,** *477, 1940.*

71. *Adamson, D. W., J. Chem. Soc.,* **39,** *1943.*

72. *Devlin, H. B., and Zittle, C. A., J. Biol. Chem.,* **156,** *393, 1944.*

73. *Block, R. J., Cannon, P. R., Wissler, R. W., Steffee, C. H., Jr., Straube, R. L., Frazier, L. E., and Woolridge, R. L., Arch. Biochem.,* **10,** *295, 1946.*

74. *Evans, R. J., and Butts, H. E., J. Biol. Chem.,* **175,** *15, 1948.*

PART V

NUTRITIONAL IMPORTANCE

THE PRECIPITATION of the basic amino acids from protein hydrolysates with phosphotungstic acid, which led in 1889 to Drechsel's discovery of lysine (1) was soon adopted as a key step in procedures for differentiating proteins. In 1899, Hausmann used it in formulating his procedure for estimating the amide nitrogen diamino (basic) nitrogen, and monoamino nitrogen fractions of protein hydrolysates (2), since superseded by the Van Slyke nitrogen distribution method. In 1900, Kossel and Kutscher (3) employed it and precipitation with silver in the systematic isolation of arginine, histidine, and lysine. In 1901, Fischer published his method of separating the monoamino acids (4) and Hopkins and Cole announced the isolation of tryptophan, with the help of an improved color test (5).

As a result of the impact of these discoveries, it became clear that proteins varied greatly in their amino acid content. Thus, in 1903, Osborne called attention to the extremely wide variation in response of purified proteins to the Hopkins-Cole test (6). In his application of Hausmann's method to various vegetable proteins, he was struck with the relatively low amounts of basic amino nitrogen in the alcohol soluble proteins of wheat (gliadin), rye (gliadin), barley (hordein), and maize (zein) and noted that from the alcohol soluble proteins of wheat and zein Kossel and Kutscher had been able to isolate no lysine (7). He closed his paper with the comment, "The fact that so many of the vegetable proteins, which serve extensively as food, have been shown, by our present investigation, to yield such different proportions of the various nitrogenous decomposition products, as compared with the animal proteins, makes it a matter of greatest interest and importance to know something more of the processes involved in this synthesis."

Indispensability of Lysine

In one of the first contributions to our present knowledge of the nutritive value of the proteins, Willcock and Hopkins, in 1906, observed that zein failed to maintain mice. Although the addition of tryptophan to the diet induced the animals to consume more food and enabled them to live longer, it did not permit them to grow, "a fact possibly due to other deficiencies in the zein molecule, such as the absence of lysine or the lack of some other amino-acid not yet observed" (8). In 1912, Osborne and Mendel reported that rats could be maintained, but would show only slight growth on diets containing 18 per cent of wheat gliadin, from which they were themselves able to isolate only 0.15 per cent of lysine (9); similar results were obtained on gliadin from rye and hordein from barley. They also showed (10), that normal growth occurred when gliadin was supplemented with lysine; also that the addition of tryptophan to zein led to maintenance, but that the inclusion of lysine was also necessary to promote growth. Thus, for the first time was lysine demonstrated conclusively to be "indispensable for the functions of growth."

CLASSIFICATION OF THE AMINO ACIDS WITH RESPECT TO THEIR GROWTH EFFECTS

DISPENSABLE	INDISPENSABLE
Alanine	Histidine (a)
Aspartic Acid	Isoleucine
Cystine	Leucine
Cysteine	LYSINE
Glutamic Acid	Methionine
Glycine (b)	Phenylalanine
Hydroxyproline	Threonine
Proline	Tryptophan
Serine	Valine
Tyrosine	Arginine (c)

(a) Histidine is indispensable to the rat but is not required for nitrogen balance in the human adult.

(b) Glycine is indispensable to the chick.

(c) Arginine can be synthesized by the rat but not rapidly enough to meet the demands of normal growth. It is indispensable in the chick.

Lysine has subsequently been shown to be necessary for growth in the mouse (11), in the chick (12), and in the turkey poult (13), and for maintenance in the dog (14) and in man (15, 16). The amount of L-lysine required for good growth in the rat was estimated by Osborne and Mendel (17) to be 2 per cent or more of the protein (18 per cent of gliadin) in the food. Using purified amino acid mixtures, Rose tentatively placed the amount needed at 1 per cent of the diet when a complete amino acid mixture is provided (18). On mixtures of the DL modifications of the nine amino acids essential for moderate rat growth, Wretlind (19) obtained maximal weight increments when the diet contained 1-2 per cent of DL-lysine. For the chick 0.9 per cent of L-lysine is considered adequate (20), for the turkey poult, 1.3 per cent (13). Rose has estimated tentatively that, when the diet furnishes sufficient nitrogen for the synthesis of the nonessential amino acids, the human adult subject requires 0.8 gm. of L-lysine per day; he recommends supplying 1.6 gm. per day (21).

The Availability of DL-Lysine

Several years ago McGinty, Lewis and Marvel (22) reported that L-lysine seemed to promote better growth than DL-lysine. This was later confirmed by Berg and Dalton (23). It has since been shown that D-lysine cannot be utilized for growth in the rat (24) or in the mouse (11). No data have been published concerning the availability or unavailability of the D isomer in the human subject. In a review on the amino acid requirements in man published in 1947 (25), Albanese states (p. 256), "Unpublished results based on changes in urinary lysine and amino N output indicate that more than 80% of orally administered unnatural lysine is utilized by man." He also tabulated (p. 253) the D isomer as being utilizable by man, but not by the rat or the mouse. More recently, however, he states (26) that supplementation of a wheat gluten diet with 6 per cent of the D-lysine "caused a tremendous increase in the lysine output, which became normal on changing to 6% L-lysine. On the basis of this observation and the poor N-retention and weight changes manifested by infants maintained on the D-lysine supplemented wheat gluten diet, it would appear that D-lysine is not utilized for growth by the infant." Conclusions which are based on nitrogen retention or weight changes, as well as on urinary excretion, would seem to be much safer than conclusions based on urinary excretion alone.

Although the evidence indicates that DL-lysine is only half as well utilized for tissue synthesis, there is no basis for assuming that the presence of the D isomer may be deleterious. A mixture of the DL-modifications of the essential amino acids in proper proportions will afford as rapid growth as a physiologically equivalent mixture of the same essential amino acids, all in the L form (27). So far as lysine itself is concerned, Wretlind has observed that the consumption by rats of diets containing mixtures of the indispensable amino acids in the DL form is essentially maximal whether the diet contains 0.25 or 4.0 per cent of DL-lysine, and that no toxic effect can be definitely shown until 16 per cent of DL-lysine is incorporated.

Need of Lysine for Maintenance Alone

Whether lysine is required for maintenance in all animals, as it is in man, is a debated question. In the adult rat, nitrogen balance studies by Wolf and Corley with amino acid mixtures (28) and weight change studies by Neuberger and Webster, who used zein fortified with tryptophan (29), have been interpreted to indicate a definite need of lysine for maintenance. On the other hand, nitrogen balance data presented by Burroughs, Burroughs, and Mitchell (30) who used amino acid mixtures and by Mitchell (31) who used proteins are interpreted differently. In most of the tests thus far made, even in the growing rat, it is quite true, as Neuberger and Webster indicate (29), that traces of lysine may have been introduced in the "protein free" milk used to supply minerals or in the yeast or liver preparation used as a source of the vitamins; on the other hand, Mitchell (31) emphasizes that the loss of weight observed by Neuberger and Webster may have been due to the failure of their rats to consume enough diet ad libitum to maintain caloric equilibrium, a possibility prevented in the study of Burroughs *et al.* (30) by tube feeding. Recently Wissler *et al.* (32) have reported that the removal of L-lysine from an amino acid mixture patterned after casein produced only slight interference with food consumption in either the protein-depleted or the normal adult rat. Despite equalization of food intakes by force-feeding, the animals on these diets lost weight

when the lysine was removed. It seems fair to infer that the balance of evidence favors lysine as a requisite even for maintenance.

The adult mouse fed diets ad libitum which contained "protein free" milk is able to maintain weight (33), as is also the young mouse fed diets containing tryptophan and zein and a separate daily allotment of yeast vitamin concentrate (11). It is quite possible, however, that at least the yeast concentrate may have contained some lysine.

In the young rat fed mixtures of purified amino acids, Rose reports a rapid loss of weight and decreased food intake when any of the essential amino acids but arginine is withdrawn (16). On mixtures of only the essential amino acids, all in the DL form, Wretlind obtained maintenance on 0.2 per cent of DL-lysine but noted that when the lysine was withdrawn completely the decline in weight was much smaller than that occasioned by the withdrawal of any of the other essential amino acids (19).

In adult rats placed on an amino acid mixture after a period of protein depletion, the omission of lysine from the mixture also caused less weight loss than observed when any of the other amino acids except arginine was removed (34).

Physiological Consequences of Lysine Deficiency

So far as it is yet known few, if any, specific symptoms follow the withdrawal of lysine from the diet (29, 16, 34). Loss of weight and poor appetite occur in the rat when any essential amino acid, except arginine, is omitted. Retardation in growth of the various organs and tissues is not uniform. The body, tail and long bones increase in length (35), and the kidneys and eyes continue to develop at the expense of the rest of the body (36). Pearson observed cessation of the estrus cycle in rats maintained on an 18 per cent gliadin diet, with resumption after adding 0.6 per cent of L-lysine dihydrochloride (37). A similar effect can be induced, however, by restricting the intake of a complete diet (38).

Failure to rear young had been noted some years earlier in rats fed zein diets fortified only with tryptophan (39), possibly because of poor milk production. Milk cows fed rations deficient in lysine produced less milk and showed an increased loss of nitrogen in the urine, whereas high yields of milk protein ordinarily are accompanied by decreased output of nitrogen in the urine (40).

Carefully-conducted studies in both the weanling rat (41) and in the 110-140 gm. animal (42) indicate that hypoproteinemia develops on lysine-deficient diets. A mild anemia, with slightly greater reduction in hemoglobin than in red cells, also occurs, with loss of liver protein, but little or no change in the total protein content of the body. In the rat fed an adequate diet in amounts so restricted as to produce similar body weight changes, none of these symptoms other than loss of weight were noted (42).

The lysine-deficiency symptoms thus far discussed cannot be regarded as unique. They are qualitatively the same as those produced by any general interfer-

ence with protein synthesis. The same is true of the production of corneal vascularization by diets low in lysine; similar symptoms may be produced by the lack of other amino acids (43). Whether this will prove to be true also in the case of feather depigmentation, which can be induced in bronze turkey poults by lysine-poor diets and corrected by the addition of lysine (44), remains to be seen.

In 1943, Albanese *et al.* reported that several human subjects placed on a lysine-deficient diet "complained of nausea, dizziness and hypersensitivity to metallic sounds" (45). The symptoms were "associated with a rise of non-ketone organic acid output in the urine and varied in intensity with the amount of these acids excreted" (25). This observation has not been confirmed. Unfortunately, the chief source of nitrogen in the lysine-deficient diet used in this study was a hydrolysate prepared from casein which had been treated with nitrous acid to render the lysine nutritionally ineffective by destroying its terminal amino group. Failure to remove the resulting organic acid residues, rather than a lysine deficiency *per se*, might well have been the cause of the "biochemical lesion" described. Studies in the rat indicate that deaminized casein is toxic. Animals receiving it survive for only a few weeks and develop a profound anemia (46). The anomaly can be overcome by adding lysine to the diet, but twice as much is required for growth and recovery as would suffice in the usual diet for satisfactory growth (47). The anemia-producing agent is not destroyed by hydrolyzing the deaminized casein with sulfuric acid, and may or may not be found in the hydrolysate at toxic levels, depending upon how completely it is adsorbed on/or co-precipitated with the barium sulfate produced in removing the excess sulfate (48). Recent tests of synthetic a-amino-ε-hydroxycaproic acid, whose L component would result from the deamination of the ε-amino group of lysine, have shown that it cannot replace lysine in the diet (49) and that it produces anemia (50). The presence of the derivative in hydrolysates of deaminized casein has been confirmed by paper chromatography; no lysine was detected (51).

Lysine Deficiency in Natural Foods

As has already been indicated, the deficiency of lysine in some of the proteins of the cereal grains was noted in even the earliest determinations of their basic amino

acid content (3), as well as of their nutritive value (8, 9). This is true particularly of the alcohol soluble proteins of the endosperm of wheat, rye, barley, and maize. On the other hand, determinations have shown the nutritive values of the total proteins of wheat, rice, oats, rye, barley, and maize to be fairly satisfactory (52), the proteins of oats, rice, and rye seemingly outranking those of wheat and corn, perhaps partly because of their somewhat higher lysine content (53, 54).

It should be made quite clear that the various proteins found in a single cereal may differ rather widely in composition (54-56), more lysine usually being found in the germ and bran protein per unit weight than in the proteins of the endosperm. Hence, milling, as commonly practiced, reduces the lysine content of the flour below that found in the whole grain. Thus, Bricker, Mitchell, and Kinsman (57) were able to convert a negative nitrogen balance of −0.872 gm. per day during a 5-day period on a wheat flour diet into a slightly positive balance during the next 5 days by supplementing the 38.7 gm. daily allotment of protein with 1.37 gm. of lysine (0.263 gm. of nitrogen). Kuether and Myers (58) have noted an improved nitrogen balance on supplementing diets containing manufactured oat cereals (rolled oats and exploded oats) with 5 gm. of DL-lysine monohydrochloride daily (0.38 of nitrogen as L-lysine). In addition to the nitrogen in the L-lysine, 0.8 to 1.02 "extra" nitrogen was retained on the supplemented diets. Hoffman and McNeil (59) have used the nitrogen balance index method of Allison and Anderson (60) to gauge the nutritive value of wheat gluten in 10

hospital patients, most of them with chronic protein deficiency. The addition of "4% purified L-lysine hydrochloride" raised the mean nitrogen index from 0.62 to 0.76, approaching the value for casein.

It should be pointed out that the nutritive values of several proteins have been shown to be influenced by heating them, some having thereby been improved, others impaired. In 1926 Morgan and King (61) showed that the heat treatment used in processing toasted breakfast cereals reduced their nutritive value to the rat. This was verified in an extended study which showed similar damage of dry whole wheat, wheat gluten, and casein after heating them for 30 minutes at 150°C. The lowered biological values were associated with only a slight lowering of digestibility (62). These observations have in general been confirmed in the human subject, particularly with the exploded type of breakfast cereal (63, 64, 58). Subsequently, Greaves and Morgan noted that supplementation of heated casein with lysine compensated for most of the impairment (65) and Block, Jones and Gersdorff reported that the proportion of lysine yielded upon hydrolyzing casein with acid was not appreciably altered by this type of heat treatment (66).

Decrease in biological value occurs also when liver, muscle, and kidney are similarly heated. Thus Seegers and Mattill (67) noted that beef liver which had been carefully dried at temperatures below 60° suffered considerable loss in its value as a source of protein for maintenance and growth in rats when it was heated at 120° for 72 hours, or extracted with boiling 95 per cent alcohol for 130 hours. Yet, acid hydrolysates of the three products, when similarly supplemented to compensate for tryptophan destruction during the hydrolysis, produced approximately equal rates of growth and showed the same biological values, thus confirming the results of the chemical assays (66) on heated casein that the lysine is not destroyed. Digestion of casein with purified proteolytic enzymes was only slightly impaired by the heating process, but appreciably less free lysine was detected in the resulting hydrolysate by the lysine decarboxylase procedure than in the hydrolysate of the unheated casein (68). Presumably the heat treatment caused the free ε-amino group of the lysine in the protein to combine with some other group to form a linkage which is not affected by digestion (nor by metabolism, if absorbed). An anhydride or diketopiperazine type of linkage has been suggested (69, 70). That the ε-amino group reacts readily at elevated temperatures is suggested by the observation that the heating of DL-lysine methyl ester produces aminohomopiperidone by the condensation of the ε-amino with the carboxyl group (71).

The heat treatment to which many of the animal proteins were exposed may be regarded as rather rigorous. Devlin and Zittle have reported that human globin appears to be damaged when dried at 55-85° for 18 hours (72) and Block *et al.* (73) have observed a rapid deterioration in the capacity of a cake mix to engender weight recovery in protein depleted rats after it had been baked and subjected to relatively mild processing procedures.

Apparently there are two types of heat inactivation of lysine which occur when soy bean oil meal is autoclaved for several hours. In a recent study in which the meal was autoclaved at 121° for 4 hours, approximately 40 per cent of the total lysine present was destroyed, as judged by determinations made after acid hydrolysis, and 20 per cent more converted into a form from which it could be liberated by hydrolysis with acid, but not enzymolysis. When the soy bean protein alone was similarly autoclaved, little loss occurred, as judged from the acid hydrolysate, but about a fourth of the lysine could not be liberated by enzymolysis. When the protein was mixed with 25 per cent sucrose by weight, both destruction and inactivation occurred. Samples subjected to dry heat at the same temperature and for the same length of time showed much less inactivation toward enzymatic digestion in vivo and relatively minor destruction (74).

In processing foods for human or animal consumption, inactivation must obviously either be avoided or steps taken to compensate for it, if the foodstuff is to be utilized efficiently.

BIBLIOGRAPHY

Part VI

1. *Berg, C. P., and Rose, W. C., J. Biol. Chem.*, **82,** *479, 1929.*

2. *Geiger, E., J. Nutrition,* **34,** *97, 1947.*

3. *Geiger, E., J. Nutrition,* **36,** *813, 1948.*

4. *Conrad, R. M., and Berg, C. P., J. Nutrition,* **14,** *35, 1937.*

5. *Frazier, L. E., Wissler, R. W., Steffee, C. H., Woolridge, R. L., and Cannon, P. R., J. Nutrition* **33,** *65, 1947.*

5*a*. *Sauberlich, H. E., Pearce, E. L., and Baumann, C. A., J. Biol. Chem.*, **175,** *29, 1948.*

6. *Madden, S. C., and Whipple, G. H., Physiol. Revs.*, **20,** *194, 1940.*

7. *Berg, C. P., in Sahyun, Proteins and Amino Acids in Nutrition, Reinhold, New York, 290, 1948.*

8. *Schoenheimer, R., The Dynamic State of Body Constituents, Cambridge, Mass., Harvard University Press, 1942.*

9. *Foster, G. L., Rittenberg, D., and Schoenheimer, R., J. Biol. Chem.* **125,** *13, 1938.*

10. *Foster, G. L., Schoenheimer, R., and Rittenberg, D., J. Biol. Chem.*, **127,** *319, 1939.*

11. *Schoenheimer, R., Ratner, S., and Rittenberg, D., J. Biol. Chem.*, **127,** *333, 1939.*

12. *Schoenheimer, R., Ratner, S., and Rittenberg, D., J. Biol. Chem.*, **130,** *703, 1939.*

13. *Weissman, N., and Schoenheimer, R., J. Biol. Chem.*, **140,** *779, 1941.*

14. *Ratner, S., Schoenheimer, R., and Rittenberg, D., J. Biol. Chem.*, **134,** *653, 1940.*

15. *Rose, W. C., Physiol. Revs.*, **18,** *109, 1938.*

16. *Bauer, C. D., and Berg, C. P., J. Nutrition,* **26,** *51, 1943.*

17. *Ratner, S. Weissman, N., and Schoenheimer, R., J. Biol. Chem.*, **147,** *549, 1943.*

18. *Berg, C. P., J. Nutrition,* **12,** *671, 1936.*

19. *Totter, J. R., and Berg, C. P., J. Biol. Chem.*, **127,** *375, 1939.*

20. *Albanese, A. A., and Lein, M., Science,* **110,** *163, 1949.*

21. *Doty, J. R., and Eaton, A. G., J. Biol. Chem.*, **122,** *139, 1937-38.*

22. *Doty, J. R., and Eaton, A. G., J. Nutrition,* **17,** *497, 1939.*

23. *Silber, R. H., Seeler, A. O., and Howe, E. E., J. Biol. Chem.*, **164,** *639, 1946.*

24. *Madden, S. C., and Clay, W. A., J. Exp. Med.*, **82,** *65, 1945.*

25. *Wright, L. D., Russo, H. F., Skeggs, H. R., Patch, E. A., and Beyer, K. H., Am. J. Physiol.*, **149,** *130, 1947.*

26. *Dunn, M. S., Camien, M. N., Shankman, S., and Block, H., Arch. Biochem.*, **13,** *207, 1947.*

27. *Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A., J. Nutrition,* **33,** *209, 1947.*

28. *Woodson, H. W., Hier, S. W., Solomon, J. D., and Bergeim, O., J. Biol. Chem.*, **172,** *613, 1948.*

29. *Thompson, R. C., and Kirby, H. M., Arch. Biochem.*, **21,** *210, 1949.*

30. *Neuberger, A., and Sanger, F., Biochem. J.*, **38,** *119, 1944.*

31. *Wohlgemuth, J., Ber.*, **38,** *2064, 1905; see also reference in Berg, C. P., and Potgieter, M., J. Biol. Chem.*, **94,** *661, 1931-32.*

32. *McGinty, D. A., Lewis, H. B., and Marvel, C. S., J. Biol. Chem.*, **62,** *75, 1924-25.*

33. *Page, E., Gaudry, R., and Gingras, R., J. Biol. Chem.*, **171,** *831, 1937.*

34. *Waelsch, H., and Miller, H. K., J. Biol. Chem.*, **145,** *1, 1942.*

35. *Green, D. E., Nocito, V., and Ratner, S., J. Biol. Chem.*, **148,** *461, 1943.*

36. *Cohen, P. P., J. Biol. Chem.*, **136,** *585, 1940.*

37. *Klein, J. R., and Handler, P., J. Biol. Chem.*, **139,** *103, 1941.*

38. *Gordon, W. G., J. Biol. Chem.*, **127,** *487, 1939.*

39. *Neuberger, A., and Sanger, F., Biochem. J.*, **38,** *125, 1944.*

40. *Neuberger, A., and Sanger, F. Biochem. J.*, **37,** *515, 1943.*

LITHO IN U. S.

FORM NO. SP13-S-850

41. *Dakin, H. D., J. Biol. Chem.*, **14**, *321, 1914.*

42. *Butts, J. S., and Sinnhuber, R. O., J. Biol. Chem.*, **140**, *597, 1941.*

43. *Sharp, G. O., and Berg, C. P., J. Biol. Chem.*, **141**, *739, 1941.*

44. *Ringer, A. I., J. Biol. Chem.*, **12**, *223, 1912.*

45. *Ringer, A. I., Frankel, E. M., and Jonas, L., J. Biol. Chem.*, **14**, *539, 1914.*

46. *Corley, R. C., J. Biol. Chem.*, **70**, *99, 1926.*

47. *Corley, R. C., J. Biol. Chem.*, **81**, *545, 1929.*

48. *Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., J. Biol. Chem.*, **176**, *1383, 1948.*

49. *Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., J. Biol. Chem.*, **179**, *689, 1949.*

50. *Borsook, H. Deasy, C. L., Haagen-Smit, A. J., Keighley, G., and Lowy, P. H., J. Biol. Chem.*, **176**, *1395, 1948.*

51. *Borsook, H., and Dubnoff, J. W., J. Biol. Chem.*, **141**, *717, 1943.*

52. *Braunstein, A. E., Advances in Protein Chemistry*, **3**, *40, 1947.*

53. *Dubnoff, J. W., and Borsook, H., J. Biol. Chem.*, **173**, *425, 1948.*

54. *Mitchell, H. K., and Houlahan, M. B., J. Biol. Chem.*, **174**, *883, 1948.*

55. *Geiger, E., and Dunn, M. S., J. Biol. Chem.*, **178**, *877, 1949.*

56. *Udranszky, L. V., and Baumann, E., Z. physiol. Chem.* **13**, *562, 1889.*

57. *Garrod, A. E., Inborn Errors of Metabolism, 2nd Edition, Oxford University Press, London, 1923.*

58. *Werle, E., Biochem. Z.*, **288**, *292, 1936.*

59. *Ackermann, D., and Kutscher, F., Z. Biol.*, **37**, *355, 1912.*

60. *Yeh, H. L., Frankl, W., Dunn, M. S., Parker, P., Hughes, B., and György, P., Am. J., Med. Sci.*, **214**, *507, 1947.*

PART VI

METABOLISM

IN CONSIDERING the anabolic phase of metabolism, it seems important to emphasize that if a protein is to be fabricated all of the units needed must be made available to the tissue simultaneously, whether by synthesis in the organism or through the diet. Synthesis of an essential amino acid, of course, either does not occur at all or takes place at a rate too slow to meet body needs.

The limited capacity of the body to conserve the separately fed essential amino acid has been repeatedly demonstrated. Some twenty years ago Berg and Rose (1) noted that growth on a tryptophan-deficient diet supplemented by the separate feeding of tryptophan was more rapid when the daily allotment of tryptophan was divided into two and four doses, fed 12 and 6 hours apart, than when the daily allowance was provided in a single dose. More recently Geiger (2) has shown that when zein supplemented with tryptophan is fed for 12 hours and lysine alone or zein supplemented with lysine is made available for the next 12 hours, the animals lose weight, instead of gaining slowly as do rats fed zein supplemented with both tryptophan and lysine. That these observations are of practical, as well as of academic, interest is indicated even more clearly by experiments with wheat gluten, which is low in lysine, and blood protein, which is capable of supplementing it. When rats were allowed access to one of these for 10 hours each day, and after a 2-hour interval, to the next for 10 hours, they lost or at best barely maintained weight, whereas, when the two proteins were fed mixed, growth ensued (3).

If the supplement is not allergenic, it need not be fed by mouth. Thus, in the case of lysine, an animal fed a diet containing zein fortified with tryptophan will show a good growth response whether the lysine is fed or is injected subcutaneously or intraperitoneally (4). The fact that somewhat better growth is invariably obtained by mixing the missing essential amino acid intimately with the deficient protein source than by feeding it separately or injecting it is in harmony with the concept that most efficient utilization cannot occur unless all of the amino acids needed for tissue construction are provided simultaneously in adequate proportion and quantity. The administration of one or two relative massive doses of a missing essential amino acid each day would naturally provide a great excess at one time and a shortage at others (4). In protein-depleted rats which were provided the 9 amino acids indispensable for repletion, Frazier *et al.* noted an excellent recovery when lysine was injected subcutaneously and the other 8 amino acids were fed (5). Curiously, injection of the missing component produces an increased consumption of the deficient diet (4, 5), thus suggesting that taste is at least not an overwhelming determinant of appetite.

Confirmation of the better use of complete mixtures is had in tests which indicate that mice and rats fed incomplete proteins actually excrete proportionately more amino acids into the urine than do rats fed adequate proteins, even though they ingest less than do the latter (5a).

It is important also not to overlook the correlative fact that when only one amino acid is supplied, the course of metabolism is limited largely to catabolism and is bound to differ widely from the course that would have been followed, had the amino acid been supplied as a component of a well-balanced mixture of use also for anabolic purposes.

Dynamic Interrelationships

Experimental evidence has shown conclusively that organs and tissues of the body lose protein in varying degrees during starvation and regain it variously

during recuperation. Presumably autolysis of expendable proteins sets in during starvation to provide energy or to insure conservation or replacement of vitally needed proteins (or vitally needed nonprotein nitrogenous constituents). When repletion again becomes possible, some tissues are given priority (6, 7). Incontrovertible proof and extension of this concept of a fluid or "dynamic state" of the body constituents, involving the use of isotopes, has been provided by the work of Schoenheimer and his associates (8, 7). Constant interchange has been shown to occur between tissue nitrogen and dietary nitrogen. What appears to be a static state is actually one of equilibrium, involving the summation of a complex array of interdependent equilibria between opposing dynamic forces.

In this connection, lysine occupies a unique position. Isotopic tracer experiments have shown that lysine, unlike the other amino acids of the tissues, does not exchange any of its hydrogen metabolically for deuterium (9), nor does it exchange its nitrogen for N^{15} fed in the form of ammonia (10) or of amino acids (11, 12). Apparently lysine is not subjected in the body to reversible chemical reactions which involve substituents on the carbon chain, such as deamination and reamination. When L-lysine having its carbon chain substituted with stably bound deuterium and its α-amino group marked with heavy nitrogen, was added to the normal diet of the young rat, the lysine subsequently isolated from its body proteins was found to contain the two isotopes in the same proportion. The smaller isotopic excess in the body proteins indicated that admixture with other dietary lysine had obviously occurred. Some of the isotopic nitrogen was found in other amino acids isolated from the proteins and some in the excreta (all but a small fraction of this in the urine) (13).

Inversion

In contrast with the carbon chain of D-leucine, which can be deaminated and reaminated to produce L-leucine (14), though to an extent obviously too small to promote growth on a leucine-deficient diet (15, 16), the carbon chain of D-lysine is entirely unavailable, at least to the rat, for the synthesis of lysine having the natural configuration (17). In the tests noted (14, 17), the carbon chain of both of these amino acids was substituted with deuterium, the α-amino group with heavy nitrogen. Inability of the rat (18), the mouse (19) or the human subject (20) to utilize unnatural lysine for maintenance and growth has been mentioned in the section on nutrition.

Absorption

Although lysine appears to be more slowly absorbed from the gastrointestinal tract than most of the other amino acids, as judged from tests using the Cori technique (21), the very small amount of isotopic nitrogen recovered in the feces after the L (13) of the D isomer (17) of marked lysine is fed indicates that the absorption of free lysine ordinarily is virtually complete.

Specific Dynamic Effect

The catabolic course taken by the lysine which is not used for tissue synthesis is obscure. In dogs which were injected intraperitoneally with L-lysine monohydrochloride, apparently in about 8-9.5 gm. doses,

about a third of the lysine nitrogen was retained, a fourth converted into urea, and the remainder excreted as amino nitrogen. The metabolism was much slower than that of glycine, and there was no detectable production of specific dynamic action (22).

Urinary Excretion

Among the several studies beside the one above which indicate that lysine is rather readily excreted by the kidneys is one by Silber, Seeler and Howe (23). They used the "Vuj" amino acid mixture (24) and a modification of it. In these 50 and 10 per cent of the amino acids were in the racemic form, respectively. Both mixtures contained 12.3 per cent of L-lysine monohydrochloride. When 220 mg. of nitrogen per kilo were infused into dogs at a rate of 12 mg. of N per kilo per minute, 23 per cent of the amino acids were lost in the urine; when the modified mixture was infused at the same rate, only 13 per cent. Microbiological assays showed that, of the amino acids excreted, from 32 to 45 per cent was lysine; of the lysine infused about 15 per cent was lost in normal dogs, about 6-10 per cent in protein-depleted animals. As two possible reasons for the greater wastage of threonine, histidine, and lysine, the authors suggest that the proportions in the mixture infused may not have approximated closely enough the proportions needed by the dog, or that the amino acids may have been lost because the kidney could not retain them. In the case of lysine, the second factor was probably at least partly involved. A study of the renal clearance of the singly infused essential amino acids (25) in the dog has shown that only two—arginine and lysine—are not readily reabsorbed by the tubules. The average maximal rate of tubular reabsorption of lysine was found to be 13.2 mg. per minute. At postabsorptive levels, less than 0.5 per cent calculated as filtered at the glomerulus was found in the urine, but as the blood levels increased, the clearance decreased sharply.

Assays of the lysine lost in the urine of the human subject have been reported by several investigators (26-29). On the K ration (26) and on uncontrolled diets (26, 28) the total urinary lysine (free and bound) averaged about 73-85 mg. per day and represented from 3 to 8.5 per cent of the total amino acid excretion. Of the total lysine about a third to a half was present in the free form (28, 29). On egg and soy bean diets, only 1.5-3.0 mg. per day of free lysine were found to be excreted (27); the calculated average daily intakes were 1.79 and 1.62 gm. Individual variations in excretion appear to be wide and to show no positive correlation with body weight (29).

Deamination and Urea Formation

As has already been indicated, injection of lysine into dogs produced a rise in the urea content of the blood and urine (22). When L-lysine with N^{15} in the α-amino group was fed to the growing rat, 20 to 25 per cent of the nitrogen was recovered in the urine, apparently chiefly as urea and ammonia (13), 8 to 15 per cent was found in the body as nonprotein nitrogen and part of the remainder in other amino acids of the body protein. When marked D-lysine was similarly tested, about half of it was excreted in the urine unchanged and about 19 per cent of its nitrogen as ammonia and urea; 21 per cent was found in the various amino acids of the body protein, and 5.5 per cent as nonprotein nitrogen (17). Neuberger and Sanger (30) report having fed 0.5 gm. of L-lysine monohydrochloride to rats of unstated age on two consecutive days, or a single dose of 1 gm. on a single day, and having accounted for 67-78 per cent of the nitrogen as extra urea or ammonia. When racemic modification was similarly fed, the increases were only a little smaller, but the non-volatile basic nitrogen excretion was greatly increased. The D form was metabolized still more slowly, and 55 per cent of the single 1 gm. dose was recovered from the urine unchanged. The observation that the D component is less well utilized when fed alone in large amounts is consistent with the results of the many similar tests which have been made on other amino acids since 1905 (31). There seems to be no doubt, however, that appreciable metabolism occurs, presumably with release of energy, whether the L, DL or D form is administered.

The mechanism by which either isomer of lysine is deaminated is not clear. Failure of α-hydroxy-ε-aminocaproic acid (32) to replace L-lysine for purposes of growth is consistent with the observation that reamination does not occur. Failure of α-amino-ε-hydroxycaproic acid to stimulate growth (33) suggests either that oxidation and reamination cannot occur on the ε-carbon or that deamination of the α-amino group takes priority. Waelsch and Miller (34)

have reported that both L- and DL-lysine produce an increase in the α-keto acid output in the urine, when fed to the fasting rat. The keto acid was not identified, and the increase appears to have been very small.

L-lysine is apparently not attacked by L-amino acid oxidase (35), nor does it seem to participate in transamination reactions (36). D-Lysine is not deaminized by reconstituted D-amino acid oxidase (37).

Neither α-N-dimethyl lysine nor α-N-monomethyl lysine will support growth in rats when incorporated into lysine-deficient diets (38). ε-N-Methyl-DL-lysine is available for growth and is about as effective as DL-lysine (39). ε-N-Acetyl-L-lysine is also available for growth on a lysine-deficient diet, but the α-N-acetyl derivative is not (40), nor is ε-N-acetyl-D-lysine (39). Kidney slices produce L-lysine from ε-N-methyl-DL-lysine, but liver slices yield very little; the method used involved estimation of the lysine with L-lysine decarboxylase, hence could not detect D-lysine if such were liberated (30). The process seemed to require oxygen, but formaldehyde production could not be demonstrated D-Amino acid oxidase showed a definite oxygen uptake when the ε-N-acetyl and the ε-N-benzoyl derivatives of DL-lysine served as the substrates, only a small uptake with the ε-N-methyl derivative, and none or only a trace with the DL- or D-lysine monohydrochlorides (30). The L-amino acid oxidase of Green et al. (35) oxidized the ε-acetyl-L-lysine slowly, but not L-lysine (30). Observations such as these have been the basis for suggesting that acetylation of the terminal amino group may be the first step in the oxidation of lysine (30).

Intermediary Metabolism

Some years ago Dakin was unable to show that any appreciable amounts of acetoacetic acid or of extra glucose were produced when 12.69 and 16.43 gm. of lysine were fed to phlorizinized dogs, nor was acetoacetic acid produced in marked amounts when lysine was added to the blood used in perfusing a dog's liver (41). Reinvestigation of this problem with the newer methods now available has failed to show any production from DL-lysine of liver glycogen (42, 43) or any excretion of appreciable extra acetone bodies in the fasted rat (43) or in the rat fed sodium butyrate (42, 43).

From the available evidence lysine appears to be (41) the only straight chained amino acid recognized as a component of proteins which has not been shown to promote increased sugar output in the diabetic animal or glycogen deposition in the fasted rat.

On the basis of Dakin's evidence, Ringer, who had earlier found that glutaric acid did not yield extra glucose or diminish acetone body output (44) in the phlorizinized dog, proposed that lysine might undergo deamination and oxidation to glutaric acid (45). Failure of this acid to produce extra glucose has been substantiated by evidence indicating that glutaric acid fails to promote either glycogen formation or any appreciable change in the acetone body excretion in the fasting rat (43). If α-deamination of lysine occurs, the α-ketonic acid must escape reamination (13), but decarboxylation and oxidation to δ-aminovaleric acid might well follow. The product is not a glucose former in the phlorizinized dog (46) and could conceivably be converted into glutaric acid by deamination and oxidation in the δ-position.

It seems doubtful, but by no means impossible, that lysine might be reductively deaminized to produce ε-aminocaproic acid which neither replaces lysine for growth purposes in the rat (32) nor produces glucose in the diabetic dog (47).

If the terminal amino group is first attacked, as has frequently been suggested (e.g., 13, 30), then a-aminoadipic acid might first be produced. This could then be converted to glutaric acid by deamination, decarboxylation, and oxidation. In a recent series of brilliant tests, Borsook and his associates have been able to prove that this pathway is a feasible one. They have synthesized and resolved DL-lysine containing C^{14} in the ε-position, and incubated the enantiomorphs with guinea pig liver homogenates. The incubated mixture was deproteinized, the filtrate chromotographed on filter paper with phenol and collidine, and the paper treated with ninhydrin. Two of the ninhydrin spots obtained in tests with L-lysine were radioactive, one spot being found in the lysine, the other in the glutamic acid position. The radioactivity of the latter was traced to α-aminoadipic acid, which was synthesized and shown to respond similarly. D-lysine was inactive. The rate of conversion of L-lysine was slow, only 0.336 mg. of the 10 mg. added being converted to the aminoadipic acid in 6 hours (48). Some of the lysine was incorporated into the protein of the homogenate (49). In continuing their study, this research

group has shown that α-aminoadipic acid labeled in the ε-position is deaminized to α-ketoadipic acid, though at a rate slower than that by which it was formed from lysine. The rate at which the α-ketoadipic acid is oxidatively decarboxylated to form glutaric acid exceeds its rate of formation (50).

The conversion of lysine to α-aminoadipic acid and α-ketoadipic acid is assumed to occur also in the kidney. A few years earlier Borsook and Dubnoff had observed that lysine was active in the synthesis of arginine from citrulline in liver slices (51) and had assumed that it was converted to glutamic acid (a glucose former) which then aminated the citrulline. The assumption of Braunstein that α-aminoadipic acid which is active in transamination reactions (52) was instead the dicarboxylic acid involved has now been accepted (53).

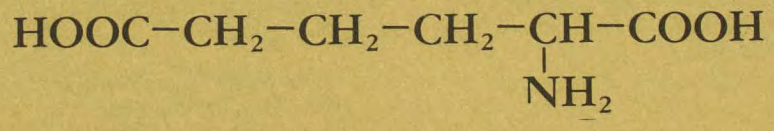
Mitchell and Houlahan have shown that α-aminoadipic acid can function as a precursor of lysine in one of four Neurospora mutants requiring lysine for growth (54). As might have been anticipated, the product cannot be used as a lysine substitute by the rat; nor is it thus utilizable by bacteria commonly employed in microbiological assays (55).

There are no known pathological conditions in which the metabolism of lysine is primarily involved. In cystinuria, however, the excretion of cadaverine may occur (56), though apparently rather spasmodically (57). Whether the diamine is produced by putrefaction in the alimentary tract or arises metabolically is uncertain. Although the existence in animal tissues of decarboxylases capable of acting on other amino acids has been demonstrated (58), there is little basis for assuming that decarboxylation occurs as the initial step in the main catabolic pathway of any one of them. On the other hand, the possibility that some decarboxylation of lysine might normally occur to produce cadaverine has not been ruled out.

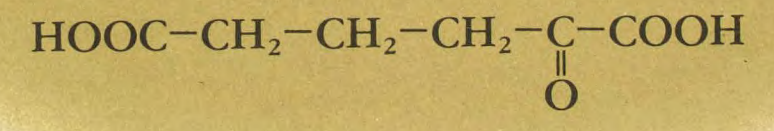
Incidentally, it was also earlier noted (59) that lysine could be isolated from the urine of the cystinuric. In a recent study in which the urine of a cystinuric has been subjected to microbiological assays, it has been shown that the metabolism of several amino acids must be deranged. The urinary excretion of glycine and histidine was diminished, the excretion of arginine and lysine greatly increased. In normal urines tested simultaneously, the lysine accounted for 2.4 per cent of the free amino acids and 5.2 per cent of the total after hydrolysis; in the urine of the cystinuric, the corresponding values were 24.2 and 23.7 per cent. The assay procedures did not respond to cadaverine, hence would not have measured it if it was present. In any event, the data do show that the metabolism of lysine was affected (60).

In compiling this review the aim has been neither to catalog completely the research which has been done on lysine nor to refer to it merely to provide proof of the reviewer's opinion, but rather to afford the reader enough of a glimpse of the experimental evidence to permit him some basis for judging for himself the characteristics of lysine, a rugged individualist among the essential amino acids.

α-AMINOADIPIC ACID



α-KETOADIPIC ACID



PART VII

THE ROLE OF LYSINE IN THE NUTRITION OF POULTRY AND SWINE

A NUMBER of experiments have been conducted which demonstrate that lysine is indispensable in the nutrition of chickens, turkeys and swine. Its significance in the practical feeding of livestock and poultry has not been definitely established but certainly progress made to date indicates a solution in the near future. A bibliography of the published reports is given below:

Mertz, E. T., D. C. Shelton and W. M. Beeson, J. Animal Sci. **8**, *524-531 (1949).*
The amino acid requirements of swine, lysine.

German, H. L., B. S. Schweigert, R. M. Sherwood, and L. E. James, Poultry Sci. **28**, *165-68 (1949).*
Further evidence of the role of lysine in the formation of normal bronze turkey feathers.

Grau, C. R. and Taylor, L. W. Poultry Science **27**, *665 (1948).*
Experiments on the amino acid requirement of laying hens.

Grau, C. R., J. Nutrition **36**, *99-108 (1948).*
Effect of protein level on the lysine requirements of the chick.

McGinnis, James, Peng Tung Hsu and J. S. Carver, Poultry Sci. **27**, *389-93 (1948).*
Nutritional deficiencies of sunflower seed oil meal for chicks.

de Man Th. J. and K Bos, Mededel. Inst. Moderne Veevoed "De Schothorts" Hoogland bij Amersfoort Dec. 1947; Chem. Abst. **42**, *7848 (1948).*
The influence of some important amino acids on the growing rate of chickens.

Schweigert, B. S., Poultry Sci. **27**, *223-7 (1948).*
The value of various feeds as sources of arginine, histidine, lysine and threonine for poultry.

Evans, R. J. and Helan A. Butts. J. Biol. Chem. **175**, *15-20 (1948).*
Studies on the heat inactivation of lysine in soybean-oil meal.

Evans, R. J. and McGinnis, James. J. Nutrition **35**, *477-88 (1948).*
Cystine and methionine metabolism by chicks receiving raw or autoclaved soybean-oil meal.

Weast, Elsie O, Mary Grody and Agnes Fay Morgan Am. J. Physiol **152**, *286-301 (1948)*
Utilization by dogs of the nitrogen of heated casein.

McGinnis, James and R. J. Evans J. Nutrition **34**, *725-32 (1947).*
Amino acid deficiencies of raw and overheated soybean-oil meal for chicks.

Clandinin, D. R., W. W. Cravens, C. A. Elvehjem and J. G. Halpin, Poultry Sci. **26**, *150-6 (1947).*
Deficiencies in overheated soybean-oil meal.

Riesen, W. H., D. R. Clandinin, C. A. Elvehjem and W. W. Cravens J. Biol. Chem. **167**, *143-50 (1947).*
Liberation of essential amino acids from raw, properly heated and overheated soybean-oil meal.

Grau, C. R., F. H. Kratzer and V. S. Asmundson Poultry Sci. **25**, *529-30 (1946).*
The lysine requirements of poults and chicks.

Fritz, J. C., J. H. Hooper, J. L. Halpin and H. P. Moore, J. Nutrition **31**, *387-96 (1946).*
Failure of feather pigmentation in bronze poults due to lysine deficiency.

Almquist, H. J. and E. Macchi Proc. Soc. Exptl. Biol. Med. **49**, *174-6 (1942).*
Lysine requirement of the chick.

Buckner, G. D., A. M. Peter, R. H. Wilkins and J. J. Hooper, Kentucky Agr. Exp. Sta. Bul. **220**, *1919.*

NOTES

NOTES

This review is published with the desire to further the study of the known and unexplored problems involving amino acids. Such studies and research hold great future promise for the improvement of human and animal nutrition and therapy.

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN

LITHO IN U.S.A.

FORM NO. SP13-S-1050

TRYPTOPHAN

an important essential Amino Acid

ADVANCEMENTS IN AMINO ACID RESEARCH

this review on

TRYPTOPHAN

is the first in a series of publications which in due time will cover all of the essential amino acids. It is our firm belief that through the information contained herein other research workers and laboratories will be encouraged to further studies leading to a more complete comprehension of this important field.

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN

BIBLIOGRAPHY

1. *Albanese, A. A., Holt, L. E., Jr., Kajdi, C. N., and Frankston, J. E., J. Biol. Chem.*, **148,** *299, 1943.*

2. *Elman, R., Ann. Surg.*, **112,** *594, 1940.*

3. *Holt, L. E., Jr., Albanese, A. A., Brumback, J. E., Jr., Kajdi, C., and Wangerin, D. M., Proc. Soc. Exp. Biol. Med.*, **48,** *726, 1941.*

4. *Albanese, A. A., and Buschke, W., Science*, **95,** *584, 1942.*

5. *Curtis, P. B., Hauge, S. M., and Kraybill, H. R., J. Nutrition*, **5,** *503, 1932.*

6. *Berg, C. P., and Rose, W. C., J. Biol. Chem.*, **82,** *479, 1929.*

7. *Berg, C. P., and Potgieter, M., J. Biol. Chem.*, **94,** *661, 1932; Berg, C. P., J. Biol. Chem.*, **104,** *373, 1934.*

8. *du Vigneaud, V., Sealock, R. R., and Van Etten, C., J. Biol. Chem.*, **98,** *565, 1932.*

9. *Wilkening, M. C., and Schweigert, B. S., J. Biol. Chem.* **171,** *209, 1947.*

10. *Albanese, A. A., and Frankston, J. E., J. Biol. Chem.*, **155,** *101, 1944.*

11. *Gordon, W. G., Kaufman, R. E., and Jackson, R. W., J. Biol. Chem.*, **113,** *125, 1936.*

12. *Lepkovsky, S., and Nielsen, E., J. Biol. Chem.*, **144,** *135, 1942.*

13. *Lepkovsky, S., Roboz, E., and Haagen-Smit, A. J., J. Biol. Chem.*, **149,** *195, 1943.*

14. *Fouts, P. J., and Lepkovsky, S., Proc. Soc. Exp. Biol. Med.*, **50,** *221, 1942.*

15. *Wintrobe, M. M., Follis, R. H., Jr., Miller, M. H., Stein, H. J., Alcayaga, R., Humphreys, S., Sukstra, A., and Cartwright, G. E., Bull. Johns Hopkins Hosp.*, **72,** *1, 1943.*

16. *Miller, E. C., and Baumann, C. A., J. Biol. Chem.*, **157,** *551, 1945.*

17. *Musajo, L., Bull. Soc. Ital., Biol. Sper.*, **10,** *290, 1935; Biol. Abstracts*, **29,** *6292, 1935.*

18. *Ried, D. F., Lepkovsky, S., Bonner, D., and Tatum, E. L., J. Biol. Chem.*, **155,** *299, 1945.*

19. *Berg, C. P., Rose, W. C., and Marvel, C. S., J. Biol. Chem.*, **85,** *219, 1929-30.*

20. *Rossiter, R. J., J. Biol. Chem.*, **135,** *431, 1940.*

21. *Berg, C. P., J. Biol. Chem.*, **104,** *373, 1934.*

22. *Bauguess, L. C., and Berg, C. P., J. Biol. Chem.*, **104,** *675, 1934.*

23. *Cahill, W. M., and Kotalik, G. C., J. Nutrition*, **26,** *471, 1943.*

24. *Gordon, W. G., J. Biol. Chem.*, **129,** *309, 1939.*

25. *Gordon, W. G., and Jackson, R. W., J. Biol. Chem.*, **110,** *151, 1935.*

26. *Schweigert, B. S., Sauberlich, H. E., Elvehjem, C. A., and Baumann, C. A., J. Biol. Chem.*, **165,** *187, 1946.*

27. *Axelrod, H. E., Morgan, A. F., and Lepkovsky, S., J. Biol. Chem.*, **160,** *155, 1945.*

28. *Conger, T. W., and Elvehjem, C. A., J. Biol. Chem.*, **138,** *555, 1941.*

29. *Sarma, P. S., Snell, E. E., and Elvehjem, C. A., J. Nutrition, in press.*

30. *Wintrobe, M. M., Stein, H. J., Follis, R. H., Jr., and Humphreys, S., J. Nutrition*, **30,** *395, 1945.*

31. *Krehl, W. A., Teply, L. J., and Elvehjem, C. A., Science*, **101,** *283, 1945.*

32. *Krehl, W. A., Teply, L. J., Sarma, P. S., and Elvehjem, C. A., Science*, **101,** *489, 1945.*

33. *Rosen, F., Huff, J. W., and Perlzweig, W. H., J. Biol. Chem.*, **163,** *343, 1946.*

34. *Woolley, D. W., J. Biol. Chem.*, **157,** *455, 1945.*

35. *Schweigert, B. S., Pearson, P. B., and Wilkening, M. C., Arch. Biochem.*, **12,** *139, 1947.*

36. *Luecke, R. W., McMillen, W. N., Thorp, F., Jr., and Tull, C., J. Nutr.*, **33,** *251, 1947.*

37. *Rose, W. C., Proc. Am. Phil. Soc.*, **91,** *112-116, 1947.*

38. *Adamkiewicz, A., Arch. ges. Physiol. Pflügers*, **9,** *156, 1874; Ber.*, **8,** *161, 1875.*

39. *Hopkins, F. G., and Cole, S. W., Proc. Roy. Soc. London*, **68,** *21, 1901.*

40. *Cary, C. A., J. Biol. Chem.*, **67,** XXXIX, *1926.*

41. *Winkler, S., Z. Physiol. Chem.*, **228,** *50, 1934.*

42. *Shaw, J. L. D., and MacFarlane, W. D., Canad. J. Research*, **16B,** *361, 1938.*

43. *Voisenet, M. E., Bull. Soc. Chem.*, **33,** *1198, 1905.*

44. *Rhode, E., Z. Physiol. Chem.*, **44,** *161, 1905.*

45. *Thomas, P., Ann. de l'Inst. Pasteur*, **34,** *701, 1920.*

46. *May, C. E., and Rose, E. R., J. Biol. Chem.*, **54,** *213, 1922.*

47. *Fürth, O., and Dische, Z., Biochem. Z.*, **146,** *275, 1924.*

48. *Kraus, I., J. Biol. Chem.*, **63,** *157, 1925.*

49. *Kraus-Ragins, I., J. Biol. Chem.*, **80,** *543, 1928.*

50. *Komm, E., Z. Physiol. Chem.*, **156,** *202, 1926.*

51. *Tomiyama, T., and Shigematsu, S., Proc. Soc. Exp. Biol. Med.*, **32,** *446, 1934-35.*

52. *Sullivan, M. X., Milone, H. S., and Everitt, E. L., J. Biol. Chem.*, **125,** *471, 1938.*

53. *Nicols, R. R., and Eckert, H. W., New York State Dept. Health*, **26,** *1941.*

54. *Greene, R. D., and Black, A., Proc. Soc. Exp. Biol. Med.*, **54,** *322, 1943; J. Biol. Chem.*, **155,** *1, 1944.*

55. *Wooley, J. G., and Sebrell, W. H., J. Biol. Chem.*, **157,** *141, 1945.*

56. *Snell, E. E., Arch. Biochem.*, **2,** *389, 1943.*

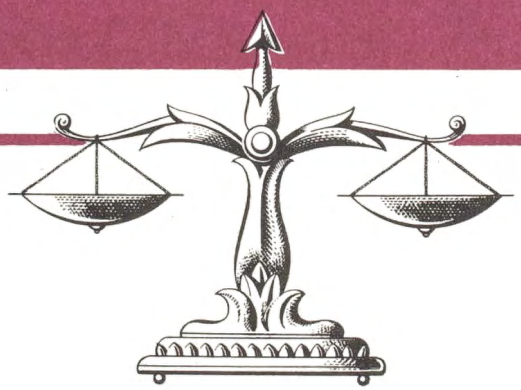
ADVANCEMENTS IN AMINO ACID RESEARCH

Although the study of amino acids has been actively pursued for many years, it is only within the last ten to fifteen years that their direct relationship to nutrition and therapy has been, even in part, understood. One of the reasons for the somewhat slow and painstaking progress in this field has been the lack of sufficient quantities of amino acids for advanced nutritional and clinical studies. However, within recent years more economical methods of synthesis have been evolved which now make several more of the amino acids commercially available.

The Dow Chemical Company, with its vast facilities and knowledge of synthetic organic chemistry, has undertaken the role of leadership in research in this very special field.

TRYPTOPHAN

an important essential Amino Acid



IMPORTANT DISCOVERIES

SEVENTY-SIX YEARS before the actual isolation of tryptophan, Tiedemann and Gmelin (in 1825) reported the development of a *violet color*, now known to be attributable to tryptophan, when chlorine was added to the pancreatic juice of a dog. Claude Bernard, who discovered glycogen, became interested in the observation, probably because the color produced was similar to that obtained on the addition of iodine to glycogen. In 1856, Bernard reported that minced pancreatic tissue failed to give this reddish color before putrefaction had occurred and that minced liver, spleen, and certain other glands behaved similarly. Of particular interest was his observation that boiled pancreatic tissue lacked this property of producing a reddish color with chlorine and that the color was apparently a property of proteins resembling casein. Bernard failed to obtain a similar color test on pancreatic digests with bromine and iodine. In this he was correct only with respect to iodine. The use of bromine water as a test reagent was introduced by Kühne in 1875. ***He was the first to show that indole was not produced when the digestion with trypsin was conducted so as to avoid putrefaction, but that it was formed only when putrefaction occurred; hence he was the first to associate indole with tryptophan. In 1890, numerous investigators, among them Neumeister, Städelmann, Nencki and Beitler, attempted to isolate the then unknown chromogenic substance, without success. Neumeister proposed the name tryptophan for it and this name was later adopted by Hopkins and Cole.***

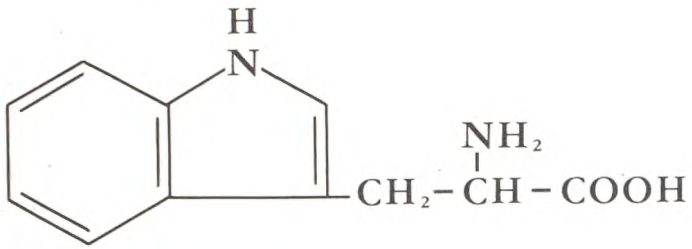
Another interesting color reaction of proteins, now associated with tryptophan, was noted by Adamkiewicz in 1874. When concentrated sulfuric acid was added to a solution of albumin in glacial acetic acid a violet color was produced. According to Hopkins and Cole, the glacial acetic acid used by Adamkiewicz probably gave the color because it was contaminated with glyoxylic acid.

In 1901, Hopkins and Cole succeeded in isolating tryptophan from an enzymatic digest of casein. They used the glyoxylic acid color test to guide them. The isolation procedure finally evolved consisted of adding enough sulfuric acid to the digest to make a 5 per cent solution, then a gram of mercuric sulfate in 5 per cent sulfuric acid for every gram of casein digested. After 24 hours, the voluminous yellow precipitate which formed was filtered off and freed of tryosine by washing it with dilute sulfuric acid. The mercuric sulfate complex was suspended in water and decomposed with hydrogen sulfide and barium hydroxide. The mixture was then filtered. To the clear filtrate, sulfuric acid was added to precipitate the barium, the solution was again filtered and sulfuric acid was added to produce a concentration of 5 per cent by volume. Mercuric sulfate dissolved in 5 per cent sulfuric acid was next added until a slight precipitate formed. This precipitate contained chiefly cystine. After standing for half an hour, the turbid mixture was filtered and an excess of mercuric sulfate in 5 per cent sulfuric acid was added to precipitate the tryptophan. The mercury and the sulfate were removed from the complex as before, and the filtrate was concentrated under vacuum and mixed with alcohol. The tryptophan which separated was recrystallized from 75 to 80 per cent alcohol.

Soon after its isolation, ***Ellinger reported that tryptophan was probably the precursor of the indole produced in the intestine and previously noted in the putrefied digests of proteins.*** In 1907, Ellinger and Flammand synthesized tryptophan by condensing indole-aldehyde with hippuric acid and hydrolyzing the resulting azlactone; they showed that the structure of tryptophan was that of α-amino-3-indole propionic acid.

FORMULA • PROPERTIES • OCCURRENCE

structural formula....



empirical formula....

$C_{11}H_{12}N_2O_2$

C=64.69% • H=5.92% • N=13.72% • O=15.67%

Properties of Synthetic Tryptophan

DL-Tryptophan is almost white in color. It crystallizes in thin, shining rhombic and six-sided plates. The D component gives it a characteristic sweet taste; the L form tastes flat. In water, DL-tryptophan dissolves to the extent of approximately 0.4% at 25°C. and approximately 2% at 100°C. Its solubility in methanol approximates 0.2% at 25°C. In the presence of other amino acids, it is much more soluble. It is stable in alkaline solution at reflux temperatures and above, but unless it is very pure, it tends to decompose in strong acids, even at room temperature.

The melting point of DL-tryptophan varies with mode of determination, hence melting point alone is not an accurate criterion of purity. If the sample is placed in an open capillary tube in a bath at 250°C. and the temperature is raised 3° per minute, melting with decomposition should occur within the range of 278-285°C. Darkening of the sample usually begins at a temperature 10 to 20° lower.

Occurrence of Tryptophan in Nature

Natural L-tryptophan is not found in the free form, except perhaps in small amounts in urine. Its concentration in animal proteins is rarely large, varying usually from 0.5 to 2.5 per cent. It is absent from gelatin, elastin and a number of vegetable proteins, and present in others in amounts ranging up to 1.5 per cent.

L-Tryptophan is destroyed during the acid hydrolysis of proteins and it is racemized by alkaline hydrolysis. It is therefore usually isolated from proteolytic digests by the method outlined earlier, or by some modification of it.

NUTRITIONAL ASPECTS

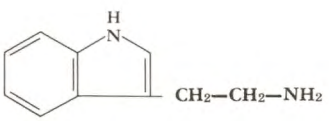
In 1906 Willcock and Hopkins fed zein, the alcohol extractable and tryptophan-deficient protein of corn, to young mice as the sole dietary protein. They found that the animals lost weight rapidly and died, but that the addition of tryptophan to the diet greatly prolonged their survival time. This observation was confirmed by others, including Osborne and Mendel, whose more extensive tests on rats demonstrated conclusively that tryptophan was indispensable for growth. Two types of diets soon came into common use for determining the dispensability or indispensability of an amino acid in an experimental animal. In one, a pure protein devoid of one or more of the common amino acids was fed as the only source of nitrogen; in the other, the source of nitrogen was a protein hydrolysate from which one or more of the amino acids had been removed as completely as possible.

*Indispensability**

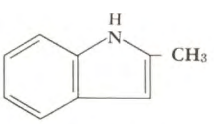
Removal of tryptophan from the diet causes all experimental animals thus far tested, including mice, rats, dogs, chickens and man, to go into negative nitrogen balance[1-3]. Tryptophan is the only natural amino acid having an indole ring as part of its structure. Inability to synthesize the ring may account for its indispensability. Loss of appetite and loss of weight, symptoms characteristic of any essential amino acid deficiency, follow the development of negative nitrogen balance. In the rat, prolonged maintenance on a tryptophan-deficient diet causes a reduction in blood albumin and globulin and the development of cataracts[4, 5]; after an initial period of rapid weight loss, the animal continues to lose weight slowly and finally dies. These consequences can be prevented by the addition of tryptophan to the diet. According to Albanese and Buschke[4], the cataracts induced by the lack of tryptophan are very similar to those induced by riboflavin deficiency. Berg and Rose[6] have observed that tryptophan is nutritionally more effective when it is incorporated intimately in the food mixture than when it is fed separately.

Intestinal Putrefaction

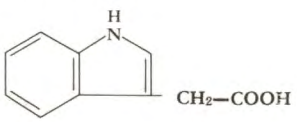
Tryptophan undergoes putrefaction in the intestines to form several so-called toxic compounds, among them indole, skatole, indoleacetic acid and 3(2-aminoethyl) indole. The following are their structural formulas:



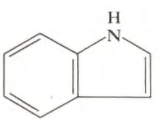
3 (2-aminoethyl) indole



Skatole

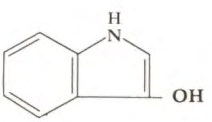


Indoleacetic Acid

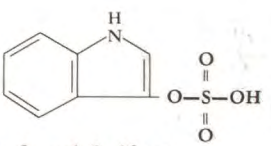


Indole

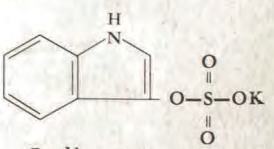
Absorption of indole from the bowel gives rise to the formation of indoxyl which is conjugated with sulfuric acid in the liver and excreted as indican in the urine.



Indoxyl



Indoxyl Sulfate



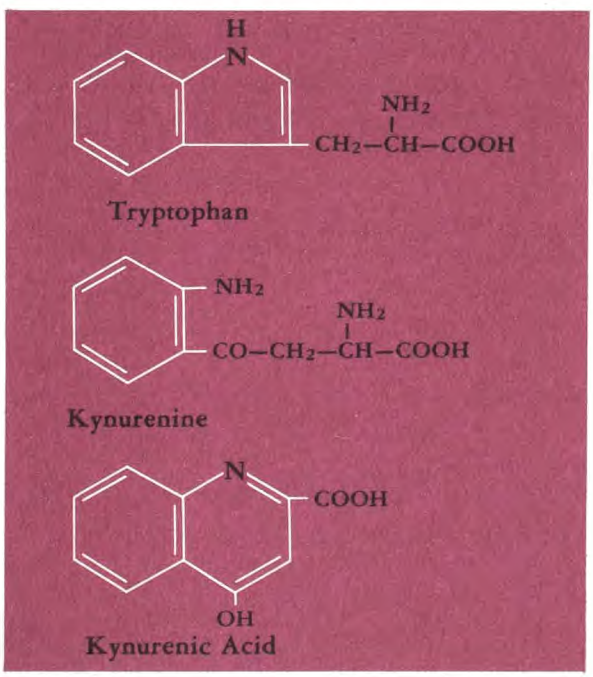
Indican

**A more general and detailed discussion of the dispensability and indispensability of the amino acids will be found in the appended section.*

Metabolic Aspects

Little is known of the intermediary metabolism of tryptophan. ***The early work of Berg and Potgieter[7] and of du Vigneaud, Sealock and Van Etten[8] indicates that the nutritive state of the rat is maintained as well by D-tryptophan as by its natural L isomer. More recent studies on the chick by Wilkening and Schweigert[9] indicate that D-tryptophan is utilized to an extent of 17 to 40 percent.*** Albanese and Frankston[10] have recently reported that the administration of DL-tryptophan to the human subject promotes the excretion of an uncharacterized "aberrant metabolite" which apparently is converted to indigo red by the addition of iodine to the urine; also that this does not occur when L-tryptophan is fed.

It has long been known that rats, guinea pigs, dogs and coyotes synthesize kynurenic acid from L-tryptophan, whereas men and cats do not. Gordon, Kaufman and Jackson[11] have studied still other species. Apparently L-kynurenine is an intermediate product. D-Tryptophan produces D-kynurenine, but no kynurenic acid. It is also the more readily excreted unchanged. The configurational relationship of these substances to tryptophan is shown below:



Effect of Vitamin B_6 Deficiency on Tryptophan Metabolism

Lepkovsky and Nielsen[12] noted that the urine of rats kept on a pyridoxine-deficient diet contained a yellowish-green pigment which they subsequently isolated and identified as xanthurenic acid (4,8-dihydroxyquinoline-2-carboxylic acid). Xanthurenic acid is excreted also by dogs, pigs, and mice, but not by chicks[13-16], on a pyridoxine-free diet containing tryptophan as such, or in the form of protein. The relationship between tryptophan and xanthurenic acid had been noted earlier by Musajo[17] in studies on rats and rabbits. Whenever these animals were deprived of dietary tryptophan, xanthurenic acid disappeared from the urine; when tryptophan was added to the diet, xanthurenic acid reappeared. Ried, Lepkovsky, Bonner and Tatum[18] observed that only L-tryptophan and L-kynurenine are capable of producing xanthurenic acid in the pyridoxine-deficient animal. None is formed from indole-3-pyruvic acid[19, 20], D-tryptophan[8, 21], indole-3-lactic acid[22] or abrine (methyl-tryptophan)[23-24]. When xanthurenic acid is fed to pyridoxine-deficient animals it is excreted unchanged. In addition to increasing the excretion of xanthurenic acid in such animals the feeding of tryptophan or of proteins like casein also aggravates the ill health caused by the deficiency. Schweigert and coworkers[26] observed that the pyridoxine content of animals fed pyridoxine-free diets decreased as the protein content of the diet was increased; also that fatty livers developed. Axelrod, Morgan and Lepkovsky[27] noted that the ingestion of tryptophan by dogs fed pyridoxine-free diets produced nausea, anorexia and sometimes collapse. These symptoms were not produced under similar circumstances when the B_6 deficiency was only moderately severe. Other syndromes, such as dermatitis, were noted in pyridoxine-deficient rats kept on a high protein diet[28]. Sarma, Snell and Elvehjem[29] observed retardation of growth in rats fed indole or DL-tryptophan as supplements to diets low in pyridoxine or pyridoxal. ***Such observations leave little doubt that***

normal metabolism of tryptophan cannot occur in animals unless they are provided with ample amounts of vitamin B_6. Whether this may apply also in man is not known.

Interrelations Between Tryptophan and Niacin

The urinary excretion of nicotinic acid in dogs is influenced by the amount of protein ingested in the diet. In swine, Wintrobe et al.[30] were able to produce niacin deficiency by reducing the protein content of the diet to 10 per cent, but were unable to do so when the diets contained 26 per cent of protein. The niacin deficiency could be prevented by adding niacin to the diet. The white rat is ordinarily able to synthesize this vitamin. In investigating the reason why either corn meal or corn grits[31] should retard growth in rats when added to their protein-low diets, Krehl, Teply, Sarma and Elvehjem[32] found that tryptophan-deficiency was the causative factor. ***Nutritionally, 50 mg. of L-tryptophan proved to be equivalent to 1.0 to 1.5 mg. of niacin. Further research has clearly indicated that high protein diets are beneficial in niacin deficiency because of their tryptophan content. Rosen, Huff and Perlzweig[33] showed conclusively that the urinary excretion of nicotinic acid was increased by increasing the tryptophan intake and decreased by decreasing it. This was true whether the tryptophan was fed as the free amino acid or as a component of protein.*** Schweigert, Pearson and Wilkening[35] have carried out similar studies in the Shetland pony and the cotton rat, obtaining substantially the same results.

The deleterious effect of corn grits in animals normally requiring niacin may be due to a factor other than tryptophan deficiency. Woolley[34] claims to have extracted a "pellagragenic" substance from corn with chloroform. He noted that it was capable of simulating niacin deficiency and that 3-acetylpyridine, an analog of niacin, is also "pellagragenic". ***Luecke and coworkers[36] carried out studies in which weanling pigs on a corn ration showed symptoms of nicotinic acid deficiency, chiefly confined to the large intestine, particularly the colon. Supplementing the rations with 200 mg. of DL-tryptophan per day produced an excellent growth response and reduced considerably the inflammatory conditions.***

The evidence cited seems to indicate either that L-tryptophan is a precursor of niacin or that it induces niacin synthesis in the experimental animal. The mechanism by which the niacin is produced is still obscure.

Fortification of Protein Hydrolysates with Tryptophan

As indicated earlier, no protein, protein hydrolysate, or amino acid mixture which lacks tryptophan is capable of effecting growth or maintaining nitrogen balance in any species of animal thus far tested. Considerable interest has recently been manifested in the clinical use of protein hydrolysates. Those prepared by using acid require the addition of tryptophan to render them satisfactory for clinical application. According to Rose[37], man's minimal requirement of L-tryptophan is about 0.15 grams daily. Ingestion of slightly higher amounts may be more practical. Since Rose's figure is based on the natural L form and the capacity of the human subject to use the D isomer has not yet been definitely determined, it would be well when using the DL-mixture to provide at least 0.3 gm. per day. At least one manufacturer of acid hydrolysates of protein for clinical use has fortified them since 1943 with 1.2 per cent of DL-tryptophan (equivalent approximately to 0.6 gm. of tryptophan per day), with no resulting untoward reaction.

CLASSIFICATION OF THE AMINO ACIDS WITH RESPECT TO THEIR GROWTH EFFECTS

DISPENSABLE	INDISPENSABLE
Alanine	*Histidine* (a)
Aspartic Acid	*Isoleucine*
Cystine	*Leucine*
Cysteine	*Lysine*
Glutamic Acid	*Methionine*
Glycine (b)	*Phenylalanine*
Hydroxyproline	*Threonine*
Proline	*Tryptophan*
Serine	*Valine*
Tyrosine	*Arginine* (c)

(a) Histidine is indispensable to the rat but is not required for nitrogen balance in the human adult.

(b) Glycine is indispensable to the chick.

(c) Arginine can be synthesized by the rat but not rapidly enough to meet the demands of normal growth. It is indispensable in the chick.

TABLE 1

DISPENSABILITY AND INDISPENSABILITY OF AMINO ACIDS

PROTEINS owe their nutritive value to their constituent amino acids. These are liberated by digestion and pass unchanged into the portal circulation. "They are then distributed throughout the system, utilized by the various tissues to form the many characteristic tissue proteins, or they are deaminated by the liver and the carbon-containing residue may then serve as a source of energy. *One of the most astounding phenomena of life is the unerring accuracy with which a specific tissue cell builds up its specific tissue protein out of the mixture of amino acids that are constantly circulating in the blood*".*

Until recently, studies of the nutritive significance of individual amino acids were limited to animals. The prohibitively high cost of pure amino acids in the past may have deterred extending such investigations to man, but the invalid assumptions that the human dietary protein intake usually provides an adequate supply of amino acids, and, hence, that study of the amino acids was only academically interesting, were quite likely also partly responsible.

Hypoproteinemia, attributable to malnutrition, is frequently observed in man and is often associated with digestive disturbances and tropical diseases. To it may in some instances be attributed the rapid onslaught of infectious diseases, slow healing of ulcers and wound ruptures, poor recovery from major surgical operations, etc.

The amino acids whose chemical identity and whose common occurrence as protein components have been definitely established, are divided into two main nutritional classes: (1) *dispensable and* (2) *indispensable amino acids. An* indispensable amino acid *may be defined as one that the body cannot synthesize, at least at a rate commensurate with needs. It must therefore be included in the diet. A* dispensable amino acid *is defined as one that the body can synthesize; its omission from the diet therefore does not retard growth or cause negative nitrogen balance.*

The recent investigation by Rose on histidine serves as an illustration of differences in need, according to species. In the rat, this amino acid is indispensable. In man, Rose found that nitrogen balance could be maintained despite its absence from the diet. Almquist found that glycine, which is dispensable in the rat, the dog and man, is essential in the chick. Interconversion of amino acids also complicates the picture. The indispensable amino acid, phenylalanine, may be used by the animal organism for the synthesis of tyrosine. A larger intake of phenylalanine is required when no tyrosine is supplied in the diet. Methionine and cystine have a similar relationship. *When the methionine content of the diet is sufficient to meet the body's needs, not only for methionine, as such, but also to provide the sulfur needed for cystine synthesis, incorporation of cystine in the diet will not enhance the animal's growth.* When the methionine content of the diet is too low to meet both of these demands, subnormal growth results and supplementation of the diet with cystine stimulates growth by relieving the body of the need of diverting methionine for cystine synthesis.

Tests by Schoenheimer with isotopic amino acids have added to our understanding of the metabolic functions and interconversion of amino acids. Thus, dietary leucine containing deuterium and N^{16} was observed to be used by the animal to replace some of the leucine in the tissue proteins. Part of the leucine nitrogen was transferred, particularly to the carbon chains of the dicarboxylic aspartic and glutamic acids. The N^{16} of isotopic tyrosine was partly transferred to the α-amino group, but not to the imidazole ring, of histidine. From animals fed isotopic phenylalanine, isotopic tyrosine could be isolated. ***Lysine appears to be the only amino acid that does not accept N^{16} from isotopic amino acids incorporated in the diet.***

*Rose, W. C., Physiol. Rev., 18, 109, 1938.

Protein Requirements and Nitrogen Balance

Considerable speculation has prevailed as to the protein requirements of human subjects. Late in the nineteenth century, Voit concluded that the daily consumption of 110 to 120 grams of protein was necessary. Because of Voit's prestige in the field of nutrition, this estimate was not readily discounted, despite lower estimates based on better scientific evidence. Recently, however, the standard of one gram of protein per kilogram of body weight was proposed by Sherman for the adult after careful re-investigation of the subject. This estimate has met with favor. ***The Food and Nutrition Board of the National Research Council recommends 70 grams of dietary protein for a man weighing 70 kg. and 60 grams for a woman weighing 56 kg.*** It is important to note that the requirement of dietary protein per kilogram of body weight is higher than this in the infant, the adolescent, and the pregnant and lactating woman; during the period of recovery from surgery, severe burns, and certain diseases; and in the period required for overcoming the effects of malnutrition.

Nitrogen Balance

Estimation of nitrogen balance is important as a measure of the dietary protein requirement. When the intake of total nitrogen (chiefly protein nitrogen) exceeds the total nitrogen output, the subject has obviously retained nitrogen and is said to be in *positive nitrogen balance*. When the nitrogen ingested in the diet approximates or equals the nitrogen output, neither loss nor storage can have taken place and the subject is therefore said to be in *nitrogen equilibrium*. When the nitrogen output exceeds the nitrogen intake, nitrogen has obviously been lost from the body and the subject is therefore said to be in *negative nitrogen balance*.

A careful study of nitrogen balance requires that the nitrogen content of the food and the nitrogen content of the excreta be determined accurately. Usually 24-hour collections of well-mixed feces and urine are analyzed to determine output. If extreme accuracy is desired, the nitrogen in the perspiration should also be measured, particularly when the perspiration is profuse, as during hot weather, in fever, and during exercise. However, the error introduced by failure to determine the loss of nitrogen through the skin is usually insignificant.

The establishment of nitrogen balance may require adjustment of the diet with reference to caloric intake and the ratio of protein to fat and carbohydrate, as well as with reference to daily protein intake. Obviously an increased intake of protein in the diet of the normal adult will lead to an increased nitrogen output, a decreased intake of protein to a decreased nitrogen ouput.

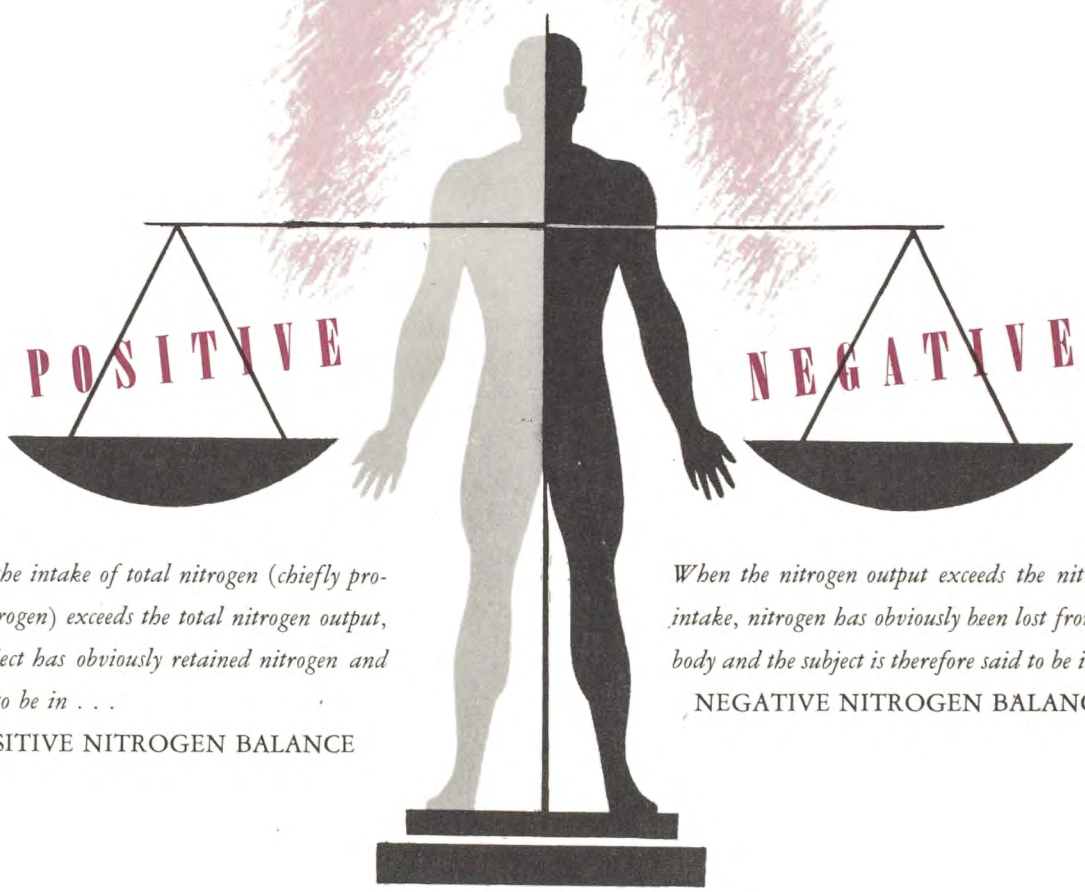
Relation of Indispensable Amino Acids to Nitrogen Balance

The nutritive value of a protein depends upon its digestibility and absorption from the intestinal tract and upon the presence of the indispensable amino acids among those thereby made available. A complete dietary protein contains all of the essential amino acids listed in Table 1. All of the indispensable amino acids must be present to promote optimal growth in the rat. Rose has demonstrated that the omission of any one other than arginine from the ration produces loss of weight. As has already been intimated, Rose has recently extended his studies of amino acid requirements to include man. Healthy young men were fed a diet consisting of purified amino acids, starch, sucrose, centrifugated butter, inorganic salts, and vitamins. When approximately 7 grams of nitrogen were supplied daily in the form of a mixture of the 10 amino acids previously found essential for animals, the subjects reached nitrogen equilibrium in a few days, after which they maintained it as long as the diet was continued (for a period of about 8 days). Hence, Rose concluded that the amino acids which

he had omitted from the diet because they were dispensable to animals, were also dispensable to man.

Subsequent omission singly from the diet of valine, leucine, isoleucine, lysine, methionine, TRYPTOPHAN, phenylalanine or threonine, produced a negative nitrogen balance. Curiously, the omission of histidine did not. Hence in man, histidine is dispensable for the maintenance of nitrogen equilibrium.

TRYPTOPHAN AND NITROGEN EQUILIBRIUM



When the intake of total nitrogen (chiefly protein nitrogen) exceeds the total nitrogen output, the subject has obviously retained nitrogen and is said to be in . . .

POSITIVE NITROGEN BALANCE

When the nitrogen output exceeds the nitrogen intake, nitrogen has obviously been lost from the body and the subject is therefore said to be in . . .

NEGATIVE NITROGEN BALANCE

When the nitrogen ingested in the diet approximates or equals the nitrogen output, neither loss nor storage can have taken place and the subject is therefore said to be in . . .

NITROGEN EQUILIBRIUM

DETERMINATION OF TRYPTOPHAN

SEVERAL METHODS have been devised for the quantitative estimation of tryptophan. These are of two general types (1) chemical and (2) microbiological.

Hydrolysis

Although L-tryptophan is a constituent of many proteins, it is rarely found free in nature. To permit the isolation and estimation of tryptophan proteins must be hydrolyzed and this ordinarily may be done with acids, alkalies, or enzymes. The hydrolysis with acids is not satisfactory since tryptophan is destroyed either in part or *in toto*. When alkalies such as sodium, potassium or barium hydroxide are used for hydrolysis of proteins, L-tryptophan is converted to DL-tryptophan. When barium hydroxide is used great care must be exercised to prevent the absorption of tryptophan on the insoluble salts formed by removing the barium as the sulfate or carbonate.

Proteins may also be hydrolyzed with enzymes trypsin, erepsin, papain and others that are capable of splitting the protein molecule into simple peptides and amino acids. Unless care is exercised to prevent contamination during the process, microorganisms will invade the hydrolysate and destroy the tryptophan molecule or metabolize it to indole or indole derivatives. Usually enzymatic digestion does not go to completion.

Chemical Methods of Analysis

Most chemical procedures for the estimation of tryptophan are based on (1) ***the reaction between tryptophan and glyoxylic acid,*** (2) ***the reaction of tryptophan with bromine,*** (3) ***the condensation of tryptophan with aldehydes or*** (4) ***diazotization reactions.*** Condensation with glyoxylic acid and other aldehydes: As early as 1874 Adamkiewicz[38] observed that the addition of concentrated sulfuric acid to a solution of albumin treated with glacial acetic acid yielded a violet-blue color. In 1901 Hopkins and Cole[39] made use of this test to guide them in preparing tryptophan from casein digests. They considered glyoxylic acid responsible for the color and subsequently employed it in the presence of hydrochloric or sulfuric acid. For many years the method of Hopkins and Cole was used with moderate success. Among its several modifications are those of Cary[40], Winkler[41], and Shaw and MacFarlane[42]. The last method is still frequently used. In 1905, Voisenet[43] reported that other aldehydes reacted with tryptophan in the presence of sulfuric or hydrochloric acid. ***Rhode[44] applied Voisenet's principle and showed that tryptophan – containing proteins reacted with p-dimethylaminobenzaldehyde in sulfuric acid to yield a reddish color.*** He also found that Ehrlick's reagent (p-nitrobenzaldehyde) could be used satisfactorily. Subsequent quantitative modifications of the Voisenet-Rhode method have been developed by Thomas[45], May and Rose[46], Fürth and Dische[47], Kraus[48,49], Komm[50], Tomiyama and Shigematsu[51], and Sullivan and coworkers[52].

Bromine Test

On treating a dilute solution of tryptophan with bromine water a reddish (magenta) color is obtained. This reaction is a sensitive one but has not found a wide quantitative application since the color is destroyed by an excess of bromine. ***Bromine is absorbed by tryptophan and the color is highly specific for*** free ***tryptophan but other amino acids present in protein such as cystine, tyrosine, histidine and phenylalanine and decomposition products of tryptophan such as indole and skatole also react.***

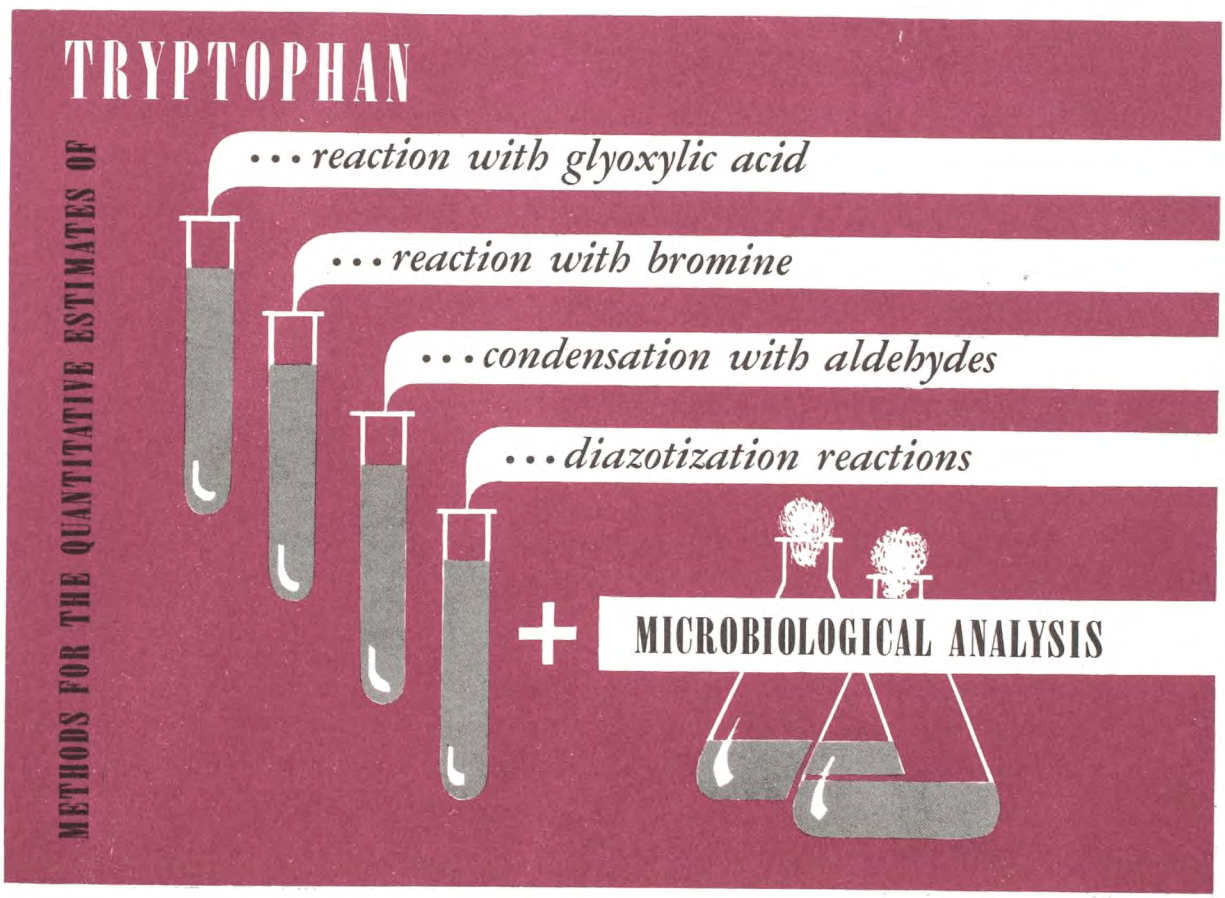
Diazo Reaction

In 1941 Nicols and Eckert[53] found that tryptophan could be treated with nitrous acid and the

resulting product condensed with N-(α-naphthyl)-ethylenediamine to give a reddish color. The microcolorimetric procedure they devised was subsequently modified by Eckert to make it suitable for the microphotocolorimetric determination of tryptophan.

Microbiological Methods of Analysis

In 1943 and 1944 Green and Black[54] outlined a microbiological method for estimating tryptophan which depended upon the response of L. *arabinosus* to this amino acid in a medium complete with respect to all the growth factors, the essential minerals and the other amino acids. ***Since D-tryptophan is inactive in supporting the growth of this organism, the value obtained for DL-tryptophan, present because it has been added to an amino acid mixture or hydrolysate or has been produced by alkaline digestion of protein, should be multiplied by two.*** Values obtained by applying this procedure to enzymatic hydrolysates are somewhat low but consistent with those obtained for other amino acids. Incomplete protealysis is probably responsible. Wooley and Sebrell[55] were unable to recover added tryptophan quantitatively when alkaline hydrolysis was used. According to Snell[56], indole and anthranilic acid can replace tryptophan for growth of L. arabinosus, but these compounds can be removed by extraction with ether at a pH of about 4 before applying the microbiological procedure. According to Snell, Green and Black's method gives consistent values in repeated assays and permits the quantitative recovery of added tryptophan.



NOTES

This review is published with the desire to further the study of the known and unexplored problems involving amino acids. Such studies hold great future promise for the improvement of human and animal nutrition and therapy

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN

LITHO IN U. S. A.

FORM NO. SP5.10M-348

Methionine

an important essential Amino Acid

ADVANCEMENTS IN AMINO ACID RESEARCH

this review on

Methionine

is the second in a series of publications which in due time will cover all of the essential amino acids. It is our firm belief that through the information contained herein other research workers and laboratories will be encouraged to further studies leading to a more complete comprehension of this important field.

THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN

OPPORTUNITY FOR RESEARCH

The recognition of methionine as an essential amino acid in nutrition and the discovery of its diversified metabolic reactions have stimulated increasing interest in the dietary and therapeutic utility of this compound.

Particular attention has been focused upon nutritional studies which have shown that the protein of many commonly used poultry and livestock feed mixtures can often be more economically utilized if synthetic methionine is added in small amounts to correct a naturally existing deficiency.

Other important aspects include the clinical investigations that have indicated the possible value of methionine in the treatment of certain liver disorders and nutritional deficiencies.

The Dow Chemical Company with its interest in agricultural progress and knowledge of synthetic organic chemistry has assumed a leading position in making available DL-METHIONINE and other amino acids.

BIBLIOGRAPHY

Part I

1. *Mueller, J. H. Proc. Soc. Exptl. Biol. Med.* **18,** *14, 1921.*

2. *Mueller, J. H. Proc. Soc. Exptl. Biol. Med.* **18,** *225, 1921.*

3. *Mueller, J. H. Proc. Soc. Exptl. Biol. Med.* **19,** *161, 1922.*

4. *Mueller, J. H. J. Biol. Chem.* **56,** *157, 1923.*

5. *Mueller, J. H. J. Biol. Chem.* **58,** *373, 1923-1924.*

6. *Odake, S., Biochem. Z.,* **161,** *446, 1925.*

7. *Barger, J., and Coyne, F. P. Biochem. J.* **22,** *1417, 1928.*

8. *Pirie, N. W., Biochem. J.,* **26,** *1270, 1932.*

9. *du Vigneaud, V., and Meyer, C. E., J. Biol. Chem.* **94,** *641, 1931-32.*

10. *Barger, J. and Coyne F. P., Biochem. J.,* **22,** *1417, 1928.*

11. *Windus, W. and Marvel, C. S., J. Am. Chem. S.,* **52,** *2575, 1930.*

12. *Emerson, O., Kirk, P. L. and Schmidt, C. L. A., Biol. Chem.* **92,** *449, 1931.*

13. *Barger, G., and Weichselbaum, T. E., Biochem. J.,* **25,** *997, 1931.*

14. *Hill, E. M. and Robson, W., Biochem. J.,* **30,** *248, 1936.*

15. *Gresham, W. F. and Schweitzer, S. N., Patent Application S. N. 522, 966 (pending) filed Feb. 18, 1944 cited by* (7).

16. *Lecky Lecky, H. S., U. S. A. patent 2, 432, 429, Dec. 9, 1947.*

17. *Lecky Lecky, H. S., U. S. A. patent 2, 432, 478, Dec. 9, 1947.*

18. *Kilmer, G. W. and du Vigneaud, V., J. Biol. Chem.,* **154,** *247, 1944.*

19. *Melville, D. B., Rachelle, J. R. and Keller, E. B., J. Biol. Chem.* **169,** *419, 1947.*

20. *Livak, J. E., Britton, E. C., Vander Weele, J. C. and Murray, M. F., J. Am. Chem. Soc.,* **67,** *2218, 1945.*

21. *Barger, G. and Coyne, F. P. Biochem. J.* **22,** *1417, 1928.*

22. *Baernstein, H. D. J. Biol. Chem.* **115,** *25, and 33, 1936.*

23. *Bailey, K., Biochem. J.* **31,** *1396. 1937.*

24. *Kassell, B., and Brand, E., J. Biol. Chem.* **125,** *145, 1938.*

25. *Kuhn, R., Birkofer, L., and Quackenbush, F. W., Ber., Deut. Chem. Gesel.* **72,** *407, 1939.*

26. *Lavine, T. F., J. Biol. Chem.,* **109,** *141, 1939.*

27. *Lavine, T. F., Federation Proc.* **1,** *120, 1942.*

28. *Beach, E. F. and Teague, D. M., J. Biol. Chem.* **148,** *431, 1943.*

29. *McCarthy, T. E., and Sullivan, M. X., J. Biol. Chem.* **141,** *871, 1941.*

30. *White, W., and Koch, F. C., J. Biol. Chem.,* **158,** *535, 1945.*

31. *Tutiya, Y., Chem. Abst.* **36,** *3152, 1942.*

32. *McMahan, J. R. and Snell, E. E., J. Biol. Chem.,* **152,** *83, 1944.*

33. *Schweigert, B. S., J. Biol. Chem.,* **168,** *283, 1947.*

34. *Stokes, J. L., Dwyer, I. M., and Caswell, M. C., J. Biol. Chem.,* **160,** *35, 1945.*

35. *Greenhut, I. T., Schweigert, B. S., and Elvehjm, C. A., Ibid* **162,** *69, 1946.*

36. *Dunn, M. S., Shankman, S., Frankl, W., and Rockland L. B., Ibid* **156,** *703, 1944.*

37. *Guirard, B. M., Snell, E. E., and Williams, R. J. Proc. Soc. Exp. Biol. Med.* **61,** *158, 1946.*

38. *Barton-Wright, E. C., Emery, W. B., and Robinson, F. A., Nature,* **157,** *628, 1946.*

39. *Baumgarten, W., Mather, A. N., and Stone, L., Cereal Chem.* **22,** *514, 1945.*

40. *Dunn, M. S., Shankman, S., and Block, H., J. Biol. Chem.,* **168,** *1, 1947.*

41. *Lyman, C. M., Moseley, O., Butler, B., Wood, S., and Hale, F., J. Biol. Chem.,* **166,** *161, 1946.*

42. *Lyman, C. M., Moseley, O. and Hale, F., Arch. Biochem.,* **10,** *47, 1946.*

43. *Riesen, W. H., Schweigert, B. S., and Elvehjm, C. A., J. Biol. Chem.,* **156,** *347, 1946.*

44. *Sauberlich, H. E., and Baumann, C. A., J. Biol. Chem.,* **166,** *417, 1946*

45. *Barton-Wright, E. C., Analyst,* **71,** *267, 1946.*

46. *Dunn, M. S., Camien, M. N., and Block, H., J. Biol. Chem.,* **163,** *577, 1946.*

47. *Hodson, A. Z., and Kreuger, G. M., Arch. Biochem.,* **10,** *55, 1946.*

48. *Horn, M. J., and Blum, A. E., J. Biol. Chem.,* **166,** *321, 1946.*

49. *Dunn, M. S., and Shankman, S., J. Biol. Chem.,* **161,** *657, 1945.*

50. *Snell, E. E., Arch. Biochem.,* **2,** *389, 1943.*

51. *Snell, E. E., J. Biol. Chem.,* **154,** *313, 1944.*

PART I

DISCOVERY

IN 1922, Mueller (1-5) announced the discovery of a sulfur-containing amino acid in casein hydrolysates to which he finally gave the empirical formula $C_5H_{11}NO_2S$. Curiously enough this was brought about through microbiological research relative to the growth of the streptococcus. When, eventually, this amino acid was isolated in a pure form, it showed no particular influence on the growth of the microorganism.

Mueller's procedure for isolation of this amino acid may be summarized as follows: An acid hydrolysate of casein was neutralized and treated with mercuric sulfate. The precipitate was washed and extracted four times by two per cent hot barium hydroxide. The extracts were freed from mercury and then from barium. After concentrating the filtrates, mercuric chloride was added and the precipitate formed was washed and treated with barium sulfide. Reagents were removed and the filtrate was next concentrated *in vacuo*. A crystalline product was obtained which was subjected to further purification and recrystallization. The final yield varied from 0.2—0.4 per cent of casein.

In 1925 Odake (6) investigated yeast extracts and succeeded in isolating a crystalline substance possessing the properties of Mueller's amino acid. Barger and Coyne (7) were first to synthesize this compound, and after consultation with Mueller, suggested the name METHIONINE in allusion to its structural formula.

Structural Formula

γ-Methylmercapto-α-aminobutyric acid

Empirical Formula

$C_5H_{11}NO_2S$

C=40.2% • H=7.4% • N=9.4% • O=21.5% • S=21.5%

SYNTHESIS • DETERMINATION

The isolation of methionine was also investigated by Pirie (8) and by du Vigneaud and Meyer (9). Biochemical methods for the isolation of this amino acid in a pure state gave very low yields which for more than two decades accounted for its high cost.

DL-Methionine has been synthesized by numerous investigators with varying yields.

Barger and Coyne's (10) attempt to synthesize this amino acid by the hydantoin method was not successful. They finally succeeded, however, by the application of a modified Stecker reaction using beta-methylthiolpropionaldehyde. The over-all yield was only 3.4 per cent of the theoretical amount. Windus and Marvel (11) employed a malonic ester synthesis and their method was modified by Emerson, Kirk and Schmidt (12) with slight improvement in yield. Barger and Weichselbaum (13) applied the phthalimido-malonic ester synthesis and shortly after that Hill and Robson (14) reported another synthesis. Graham and Schweitzer (15) investigated Barger and Coyne's original method and effected some improvement in yield. Lecky (16, 17) studied the acid hydrolysis of methionine nitrile and claimed substantial gain in yield, mostly by refinement in technique.

The synthesis of DL-methionine containing excess quantities of the stable isotopes S^{34} and C^{13} in the beta and gamma positions was accomplished by Kilmer and du Vigneaud (18). Melville, Rachelle and Keller (19) reported the synthesis of L-methionine containing C^{14} in the methyl group. Livak, Britton, Vander Weele and Murray (20) obtained DL-methionine by the alkaline hydrolysis of 5-(beta-methylmercaptoethyl)-hydantoin. Other methods of synthesis and variations thereof are now employed commercially using as starting materials acrolein, hydrogen cyanide and ammonia.

DL-Methionine ($C_5H_{11}O_2NS$) crystallizes from water in lustrous white hexagonal plates. It is soluble in 100 gm. water to the extent of 1.8 gm. at O°C., 3.4 gm. at 25°C. and 17.6 gm. at 100°C. It is slightly soluble in methanol (app. 0.1 gm. per 100 cc. at 25°C.), to a slight degree in ethanol, and almost insoluble in other organic solvents. The melting point varies greatly with the procedure used, but is in the range of 268-270°C. when inserted in an open capillary tube in a bath at 240° and the bath temperature raised at a uniform rate of 3°C. per minute.

Determination of Methionine

Methionine may be determined by chemical or microbiological procedures. The latter methods account for only 50 per cent of DL-methionine.

Chemical Methods

By virtue of the labile methyl group, Barger and Coyne (21) pointed out that methionine may be determined by demethylation with hydriodic acid and the resulting methyl iodide may be quantitatively estimated. Baernstein (22) applied this principle for the determination of methionine found in proteins. His procedure became known as the "methyl iodide method"; however on further investigations, numerous workers found that it gave high values for this amino acid. Bailey (23), Kassel and Brand (24) Kuhn, Birkofer and Quackenbush (25) and Lavine (26) suggested several refinements and modifications for Baernstein's method. Beach and Teague (28) reported a gravimetric method.

MacCarthy and Sullivan (29) found that methionine forms a colored compound with sodium nitroprusside. They developed a satisfactory, rapid, colorimetric method which is now widely used. White and Koch (30) offered minor modifications. Tutiya (31) proposed a colorimetric method based on fusion of methionine with sodium hydroxide, aeration of the solution into isatin and measurement of the green colored compound.

Microbiological Methods

Assay of amino acids by microbiological methods involves the use of certain groups of bacteria known as test organisms. These may be divided into two groups: (a) *Homofermentative*, which comprises bacteria that are capable of almost quantitatively converting glucose to lactic acid and (b) *Heterofermentative*, which can convert glucose into lactic acid and other degredation

products such as ethyl alcohol, acetic acid and carbon dioxide.

Although many organisms have been employed for microbiological assay of amino acids, only a limited number have been carefully studied and recommended by workers in this field. One reason is that different strains of the same species may not give the same response or may differ from one another as to their specific nutritional requirements.

Several microbiological methods for the estimation of methionine have been developed. References to these methods and to the *test organisms* employed are:

Test Organisms	*References to Microbiological Methods for Estimation of Methionine*
Streptococcus faecalis	34, 35, 39, 40, 41, 42, 43, 44
Leuconostoc mesentroides	36, 38, 39, 40, 41, 42, 43, 46, 47
L. arabinosus	37, 40, 43, 44, 46, 48
L. casei	40
L. fermenti	40, 46, 49
L. delbruckii	40

Measurement of Results:

The results may be obtained either by turbidimetric or titrimetric measurements.

For turbidimetric measurements of bacterial growth, photoelectric colorimeters are generally used. Readings are recorded, plotted and checked against a standard reference curve or against readings using known amounts of the standard amino acid. For titrimetric measurements, the acidity of a sample is determined by titration with a standard alkali. For details on criteria for accuracy see Snell (50, 51).

BIBLIOGRAPHY

Part II

1. *Rose, W. C., Physiol Rev.* **18,** *109, 1938.*

2. *Jackson, R. W., and Block, R. J., J. Biol. Chem.* **98,** *465, 1932.*

3. *Weichselbaum, T. E., Weichselbaum, M. B., and Stewart, C. P., Nature,* **129,** *795, 1932.*

4. *Beach, E. F., and White, A., J. Biol. Chem.,* **127,** *87, 1939.*

5. *Tarver, H., and Schmidt, C. L. A., J. Biol. Chem.* **130,** *67, 1939.*

6. *Stekol, J. A., J Biol. Chem.,* **86,** *93, 1930, also* **109,** *147, 1935.*

7. *Madden, S. C., Carter, J. R., Kattus, A. A., Jr., Miller, L. L., and Whipple, J. H., J. Exp. Med.,* **77,** *227, 1943.*

8. *Du Vigneaud, V., Kilmer, G. W., Rachelle, J. R., and Cohn, M., J. Biol. Chem.,* **155,** *645, 1944.*

9. *Borsook, H., and Dubnoff, J. W., J. Biol. Chem.,* **132,** *559, 1940 ibid,* **134,** *635, 1940.*

10. *Bodansky, M., Duff, V. B., and McKinney, M. G., J. Biol. Chem.,* **140,** *365, 1941.*

11. *Bloch, K., and Schoenheimer, R., J. Biol. Chem.,* **131,** *111, 1940.*

12. *Du Vigneaud, V., Chandler, J. P., Cohn, M., and Brown, G. B., J. Biol. Chem.,* **134,** *787, 1940.*

13. *Du Vigneaud, V., Cohn, M., Chandler, J. P., Schenck, J. R., and Simmonds, S., J. Biol. Chem.,* **140,** *625, 1941.*

14. *Lewis, H. B., J. Biol. Chem.,* **110,** *23, 1936.*

15. *Medes, G., Biochem. J., 33,* **1559,** *1939.*

16. *Smuts, D. B., Mitchell, H. H., and Hamilton, T. S., J. Biol. Chem.,* **95,** *283, 1932.*

17. *Payne, S. A., and Perlzweig, W. A., J. Clin. Invest.,* **12,** *899, 1933.*

18. *Sullivan, M. X., and Hess, W. C., J. Bone Joint Surg.,* **16,** *185, 1934.*

19. *Peters, B. A., Lancet,* **1,** *264, 1945.*

20. *MacKenzie, C. G., Chandler, J. P., Keller, E. B., Rachelle, J. R., Cross, N., Melville, D. B., and du Vigneaud, V., J. Biol. Chem.,* **164,** *757, 1947.*

21. *Stekol, J. A., Annual Rev. Biochem.* **10,** *265, 1941.*

22. *Quick, A., Chapter 8, "Outline of Amino Acids and Proteins" M. Sahyun, Reinhold Publishing Corp., N. Y., 1943.*

23. *Stekol, J. A., J. Biol. Chem.,* **138,** *225, 1941.*

24. *White, A., and Jackson, R. W., J. Biol. Chem.,* **111,** *507, 1935.*

25. *West, H. D., and Jefferson, N. C., J. Nutrition,* **23,** *425, 1942.*

26. *Handler, P., and Dann, W. J., J. Biol. Chem.,* **146,** *357, 1942.*

27. *Stekol, J. A., Abst. Biol. Div., A. C. S. (B-9), 1942.*

28. *Goldscmidt, S., Vars, H. M. and Ravdin, I. S., J. Clin. Invest.* **18,** *277, 1939.*

29. *Gyorgy, P., Poling, E. C., and Goldblatt, H., Proc. Soc. Exp. Biol. Med.* **47,** *41, 1941.*

30. *Messinger, W. J. and Hawkins, W. B., Am. J. Med. Sci.,* **199,** *216, 1940.*

31. *Schifrin. A., Virchows Arch.,* **287,** *175, 1932.*

32. *Smith, M. I., and Stohlman, E. F., J. Pharmacol.,* **70,** *270, 1940.*

33. *Snyder, J. C., and Zarafonetis, C. J. D., Proc. Soc. Exp. Biol. Med.* **60,** *115, 1945.*

34. *Goodell J. P. B., Hanson, P. C., and Hawkins, W. B., J. Exp. Med.,* **79,** *625, 1944.*

35. *Miller, L. L. and Whipple, G. H., J. Exp. Med.,* **76,** *421, 1942.*

36. *Heppel, L. A., Neal, P. A., Daft, F. S., Endicott, K. M., Orr, M. L. and Porterfield, V. T., J. Ind. Hygiene and Toxicol.,* **27,** *15, 1945.*

37. *Heppel, L. A., Neal, P. A., Perrin, T. L., Endicott, K. M., and Porterfield, V. T., J. Pharm. Exp. Therap.,* **84,** *53, 1945.*

38. *Milhorat, A. T., Bartels, W. E., and Toscani, V., Proc. Soc. Exp. Biol. Med.* **45,** *394, 1940.*

39. *Baxter, J. H., J. Clin. Invest.,* **25,** *908, 1946.*

40. *Baxter, J. H., J. Pharmacol. Exptl. Therap.,* **91,** *345, 1947.*

41. *Baxter, J. H., J. Pharmacol. Exptl, Therap.,* **91,** *350, 1947.*

42. *Ershoff, B. H., Physiol. Rev.* **28,** *107, 1948.*

43. *Rose, W. C., Physiol. Reviews,* **18,** *109, 1938.*

44. *Glynn, L. E., Himsworth, H. P., and Neuberger, A., Brit. J. Exptl. Path.* **26,** *326, 1945.*

45. *Neuberger, A. and Webster, T. A. cf. Cuthbertson, D. P.*

46. *Cuthbertson, D. P., Annual Rev. Biochem.,* **16,** *177, 1947.*

47. *Wanscher, O., Nord. Med.,* **30,** *1373, 1946.*

48. *Sydenstricker, V. P., Hall, W. K., Hock, C. W., and Pind., E. R., Science,* **103,** *194, 1946.*

49. *Feng, K. C., Dissertation, University of Pittsburgh, 1944.*

50. *Roberts, E., and Spiegel, C. J., J. Biol. Chem.,* **171,** *9, 1947.*

51. *Gordon, G. S. Bennett, L. L., Li, C. H., and Evans, H. M., Proc. Soc. Exptl. Biol. Med.* **65,** *347, 1947.*

PART II

METABOLISM OF METHIONINE

FOR MANY YEARS prior to the discovery of methionine, cystine was the only component of proteins known to contain sulfur. Cystine was first investigated by Osborne and Mendel who observed, that when 18 per cent of casein was incorporated in an otherwise adequate diet, young rats receiving such a food mixture grew at normal rates. When the proportion of casein was progressively decreased in the diet, however, the rate of increase in body weight was inhibited (1). The percentage of cystine in casein was known to be very small and consequently it was assumed that this amino acid was the limiting factor. On the basis of these findings cystine was considered an essential amino acid. Several investigators confirmed these observations, and it was not until Jackson and Block (2) reported that the addition of methionine to a diet low in casein promoted growth in the rat, that the indispensable nature of cystine was questioned. The findings of Jackson and Block were confirmed by Weichselbaum *et al.* (3). Even then, the dispensability of cystine and the indispensability of methionine were not as yet fully established.

1. Indispensability

Since experimental evidence based on diets containing proteins is not conclusive to demonstrate the indispensable nature of an amino acid, mixtures of pure amino acids should be utilized for such purposes. To insure the exclusion of cystine and methionine from a ration, each amino acid employed in the preparation of a mixture of amino acids must be of the utmost purity and free from contamination by other amino acids. Rose and his associates (1) were first to employ such a procedure and painstakingly prepared and recrystallized each amino acid in the diet they used. The cystine employed as a supplement was recrystallized six times until correct chemical data had been obtained. The methionine investigated was a recrystallized synthetic product. Rose's elaborate experiments showed that the addition of cystine to a diet deficient in methionine and cystine was practically without any effect and the animals lost weight; on the other hand the incorporation of methionine in such a diet permitted rapid growth. These data provided the first convincing proof of the dispensability of cystine and the indispensability of methionine. Beach and White (4) also reported that methionine stimulates the growth of animals previously stunted by a diet in which arachin served as the protein; whereas cystine under the same conditions yielded no growth-promoting effect.

Thus it has been shown that the need of the body for protein-sulfur is met by the all-important amino acid *METHIONINE.* In this connection it must be pointed out that although methionine is indispensable in the diets of certain animals, its quantitative requirements vary not only with the species but also with the cystine content of food protein constituents. Experiments have shown that cystine can be synthesized from methionine but the reverse reaction does not occur. The addition of cystine to a diet low in methionine, however, enhances growth and can, to a certain extent, spare methionine.

The requirement for sulfur-containing amino acids by different animal species and by man is of critical importance in nutrition. The high content of sulfur in keratin proteins such as wool, hair, nails, and hoofs, in hormones such as insulin and in many organs of the animal system must be supplied by a diet which contains adequate methionine or a suitable proportion of methionine and cystine. There is considerable evidence in the literature that many well known foods for human and animal consumption are so limited in their methionine and cystine content that the index

Classification of the amino acids with respect to their growth effects

DISPENSABLE	INDISPENSABLE
Alanine	Histidine (a)
Aspartic Acid	Isoleucine
Cystine	Leucine
Cysteine	Lysine
Glutamic Acid	METHIONINE
Glycine (b)	Phenylalanine
Hydroxyproline	Threonine
Proline	Tryptophan
Serine	Valine
Tyrosine	Arginine (c)

(a) Histidine is indispensable to the rat but is not required for nitrogen balance in the human adult.

(b) Glycine is indispensable to the chick.

(c) Arginine can be synthesized by the rat but not rapidly enough to meet the demands of normal growth. It is indispensable in the chick.

of their biological value is low. By incorporating methionine in such foods an enhancement of their utilization ensues, permitting a decrease in the protein intake.

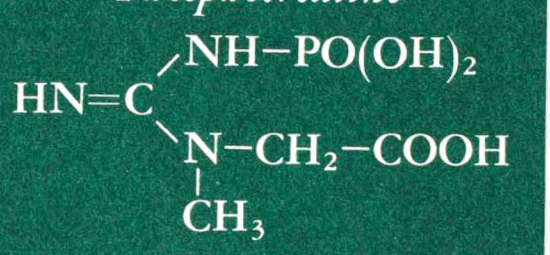
2. *Mechanism of Conversion of Methionine to Cystine*

The mechanism of conversion, *in vivo*, of methionine to cystine has been investigated in considerable detail. In the absence of an adequate supply of cystine in the diet, methionine is converted, probably in the liver, to cystine, but the reverse does not occur. The details of the chemical reactions involved are not fully established. Tarver and Schmidt (5) showed that the sulfur of methionine containing S^{35} is transferable to cystine in the animal body. They also reported that methionine can fulfill all the functions of cystine. Stekol (6) observed that there was no immediate elimination of sulfur nor of nitrogen when either cystine or methionine was given to rats receiving a low protein diet. This investigator concluded that since there was no diminution in the excretion of nitrogen derived from the protein of the diet plus that from endogenous sources, the retained cystine and methionine were not used to form protein but were stored in some other form. Madden and his coworkers (7) observed negative nitrogen balance in the experimental dog when cystine was the only source of amino acid sulfur in the diet. Of considerable importance is the recent finding of duVigneaud *et al.* (8) who performed feeding experiments with rats using methionine containing isotopes of sulfur and carbon. They reported: "On isotopic analysis of the cystine approximately 80 per cent of its sulfur but no significant amount of its carbon had been derived from the tagged methionine." This conclusive experimental evidence establishes that the carbon chain of methionine is not utilized for *in vivo* conversion of methionine to cystine. Since the sulfur of methionine has been found to be transferable to cystine, it has been suggested that the remainder of the molecule could be derived from serine.

3. *Relation of Methionine to Creatine*

The origin and metabolism of creatine and creatinine in the animal body and their relation to the amino acids have long been subjects of considerable research and debate. One reason for the unusual interest in creatine has been the startling revelation made less than two decades ago by Lundsgaard on the mechanism of muscular contraction. Prior to this time, it was assumed by physiologists that the energy released from the breakdown of glycogen to lactic acid was responsible for muscular activity and that muscle fatigue was caused by the accumulation of lactic acid *in situ;* also that muscular contraction was not resumed until the lactic acid formed was converted to glycogen, one fifth of it being oxidized to carbon dioxide and water and the remaining four-fifths being used for glycogen synthesis. Lundsgaard demonstrated that the poisoning of muscle with monoiodoacetic acid would permit muscular contraction even though lactic acid was not produced. This meant that the process of muscle contraction and the production of lactic acid were not inseparably connected. Subsequently, it was discovered that the process of muscular activity was the result of the tremendous energy released by the breakdown of a substance known as phosphocreatine the chemical structure of which is:

Phosphocreatine

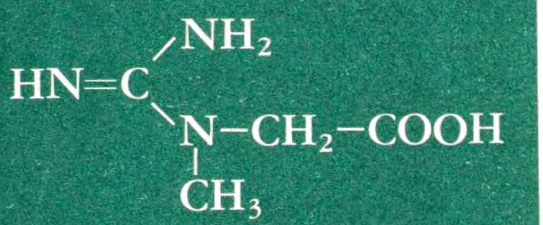


Phosphocreatine was isolated from the extracts of skeletal muscle by Fiske and Subbarrow in 1927 and they, together with the Egglestons, proved that during muscular contraction, this compound is hydrolyzed to phosphates and creatine, and is resynthesized during the period of muscular recovery. When a muscle is poisoned with monoiodoacetic acid it contracts as

long as there is phosphocreatine available, but unlike normal muscle, resynthesis of phosphocreatine does not take place, hence contraction ceases.

Obviously Lundsgaard's discovery and the findings of Fiske and Subbarrow and of the Egglestons, aroused considerable interest in the origin of creatine in the system. Creatine has the following chemical structure:

Creatine



In this presentation we are only concerned with the relationship that exists between creatine and methionine.

Borsook and Dubnoff (9) reported that liver slices synthesized creatine from guanidoacetic acid *in vitro* and that this reaction was accelerated by the addition of methionine. Their findings were confirmed by Bodansky, Duff and McKinney (10). The amidine group of creatine was shown to originate from arginine by Block and Schoenheimer (11) by means of their isotope experiments. However, it remained for duVigneaud and his associates (12, 13) to prove by means of isotopic elements in DL-methionine, that this amino acid contributes its methyl group for the conversion of guanidoacetic acid to creatine.

4. Ultimate fate of Methionine in the system

It has been definitely established that methionine is the precursor of cystine in the animal body. The question arises as to whether or not cystine is an obligate intermediate in the metabolic disposal of methionine. Lewis (14) has shown that methionine is oxidized more slowly than cystine in the normal course of metabolism. The simultaneous oral or parenteral administration of these two substances revealed that methionine remained longer in circulation and formed sulfate more slowly than cystine did. This indicates that the first step of methionine breakdown is its conver-

sion to cystine. Subsequent reduction converts cystine to cysteine. Medes (15) extracted liver enzymes which oxidized the sulfur of both cystine and cysteine to sulfinic acid and the latter was eventually converted to sulfate.

Hair, fingernails, toenails, hoofs, and wool are unusually rich in cystine. Smuts, Mitchell and Hamilton (16) demonstrated that a diet deficient in this sulfur-containing amino acid inhibited the growth of rats and their hair. Payne and Perlzweig (17) observed that the fingernails of pellagrous patients (due to nicotinic acid deficiency) were low in cystine.Sullivan and Hess (18) reported similar findings in chronic deforming arthritis. Peters (19) reported improvements in two cases of exfoliative dermatitis following the administration of cystine. An analysis of the "scaly skin" of these patients showed that one-third of the dietary cystine was lost by the exfoliation.

Hitherto in this presentation no mention has been made of the fate of the labile methyl group of methionine. Recently, MacKenzie and his associates (20) fed rats methionine containing the isotope C^{14} in the methyl radical and reported the presence of radioactivity in the carbon dioxide collected one hour after feeding the tracer compound. This is conclusive evidence that the labile methyl group of methionine can be oxidized to carbon dioxide.

5. *Detoxification Mechanism*

It has been observed (21) that when a toxic substance like bromobenzene is fed continuously to rats, a deficiency in the sulfur-containing amino acids occurs. This deficiency is accompanied by retardation of growth and loss of weight and can be corrected by the intake of adequate amounts of methionine, cystine and homocystine (22). Cessation of growth and loss of weight following bromobenzene poisoning are explained on the basis of utilization of these amino acids for conjugation mechanisms and the *in vivo* synthesis of mercapturic acids; thus preventing methionine from the pursuit of its normal metabolic functions. A similar deficiency has also been noted in animals fed biphenyl or chrysene and this toxicity was shown to be alleviated by methionine, cysteine and glutathione (23, 24, 25).

The administration of 1 per cent nicotinic acid or its amide to rats causes toxic manifestations with concomitant loss of weight. Nicotinic acid apparently creates a labile methyl group deficiency shown to be counteractable by methionine but not by choline, cystine or homocystine. Choline can be rendered effective against nicotinic acid poisoning if given in conjunction with cystine or preferably homocystine (26, 27).

High protein diets have been known to exert a protective mechanism in animals exposed to the toxic effects of selenium, trinitrotoluene, arsephenamine and Mapharsen. The effective agent in proteins against these poisons was recently found to be methionine (28-37). In the depleted dog, Miller et al (38) found that methionine and, to a lesser extent, cystine protected the animal against the ill effects of chloroform.

Baxter (39, 40) investigated the toxicity in rats of 0.1—0.2 per cent pyridine hydrochloride added to a diet moderately low in protein (10 per cent casein) and noted cessation of growth and eventual death within two weeks. The supplementation of the diet with 0.5 per cent methionine permitted growth and increased to a limited extent the survival time. Baxter and Mason (41) studied the relationships between the mechanisms of the liver and kidney injury produced by pyridine and other similar toxic substances and of diets deficient in choline and methionine. They found that, in contrast with the beneficial influence of methionine, the addition of choline to a diet moderately low in protein and containing pyridine afforded no protection to the animal. They therefore concluded that methionine, but not choline, contributes labile methyl groups in the presence of pyridine.

In his recent discussion "Conditioning Factors in Nutritional Disease" Ershoff (42) states: "On a low protein diet, insufficient amounts of such amino acids may be present to meet the requirements for maintenance plus detoxification with the result that an amino acid deficiency may develop. The effects of such a deficiency will be particularly marked in the liver cell, since the liver is the organ where detoxification primarily occurs. The hepatotoxic effects of chloroform, carbon tetrachloride and other toxic substances may be explained therefore, at least in part, on the basis of methionine deficiency in the liver cell. Particularly

pertinent in this regard is the similarity of symptoms resulting from a dietary deficiency of methionine with that obtained on the administration of noxious substances requiring methionine for detoxification. It appears further that the body will employ nutrients preferentially for detoxification, even at the expense of sacrificing its own tissues to obtain the required material."

Bearing in mind that methionine in the animal system can be a precursor of cystine, the av. ilable information on the subject of detoxification indicates that the diet should supply sufficient methionine for: (1) labile methyl groups, (2) conjugation mechanism, (3) synthesis of mercapturic acids, (4) prevention of fatty liver infiltration, (5) normal metabolic functions, (6) maintenance of positive nitrogen balance, and (7) growth.

6. Methionine Deficiency

The complete absence of methionine from the diets of experimental animals causes loss of weight, loss of hair and eventual death. Dietary relationships between methionine and cystine have already been discussed. It is now definitely established that cystine alone does not stimulate growth in the complete absence of methionine. In the presence of suboptimal amounts of methionine, however, cystine does cause growth stimulation (43). Appropriately, Rose stated: "These facts serve to emphasize the importance of knowing the exact composition of the ration before drawing positive deductions from the growth behavior of the animals."

There is a paucity of literature dealing with pathological changes occurring in animals kept not only on diets deficient in methionine but also on suboptimal amounts of this and other indispensable amino acids. Unquestionably, it is not enough to know that a certain amino acid is important in our diet and whether or not it is indispensable to the experimental animal. In addition, we must understand the consequences of deficiency, particularly when the diet contains suboptimal quantities of any of the essential amino acids and of any pathological changes that may occur in the essential organs of the body.

Glynn, Himworth and Neuberger (44) kept rats on a diet deficient in methionine and cystine and observed the development of massive hepatic necrosis and excretion of homogentisic acid-like compounds. These abnormalities were noted to be preventable when either cystine or methionine was added to the deficient diet. Neuberger and Webster (45) as cited by Cuthbertson (46) reported that a deficiency of the sulfur-containing amino acids decreases the ability of the body to metabolize aromatic amino acids to such an extent that even with small intakes of tyrosine and phenylalanine considerable amount of a homogentisic-like acid is excreted. Cystine alone partially protects the animal against this alcaptonuria. They suggested that the important factor which is responsible for the development of necrosis, is the deficiency of cystine and that the protective effect of methionine is due to its transform tion to cystine in the body.

Wanscher (47) found that rats kept on a 5 per cent casein diet develop:

(1) severe degeneration of liver cells concomitant with hemorrhage and intestinal inflammation,

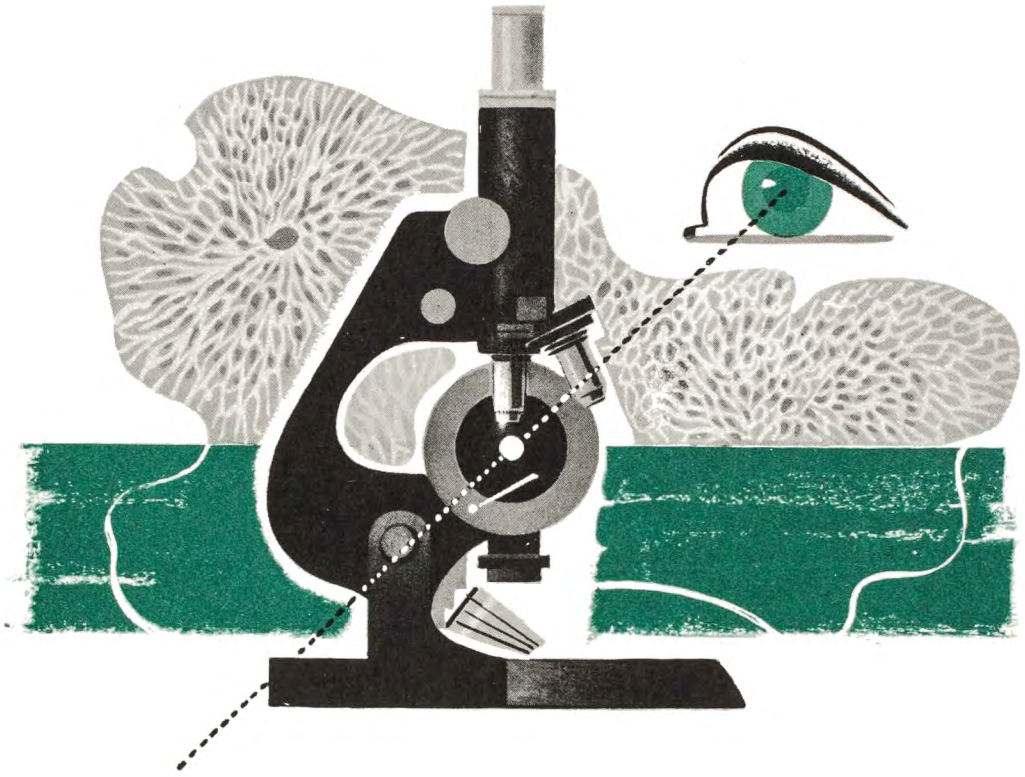
(2) degeneration occurring in the convoluted tubules of the kidneys and

(3) not infrequently severe chronic convulsions. Increasing the amount of casein in the diet from 5 per cent to 10 per cent gives no protection to these animals, but the addition of as little as 0.2 per cent of methionine restores normal growth and causes regeneration of hepatic tissue. The addition of cystine alone yields only partial protection, but does not prevent loss of weight, whereas cysteine affords no beneficial effect whatsoever. Sydenstricker et al (48) observed corneal vascularization in rats kept on methionine deficient diets.

It has recently been reported that chloretone and phenobarbital accelerate the urinary excretion of vitamin C in the rat and cause increased concentration of this vitamin in the small intestine, kidneys, liver and to a lesser extent in the spleen (49). Roberts and Spiegel (50) pursued the latter investigation with rats kept on a 5 per cent casein diet and reported that the ability of these animals to excrete ascorbic acid in response to either chloretone or phenobarbital is greatly limited, as compared with those kept on a diet of 18 per cent casein. The addition of methionine, cystine or both, to the 5 per cent casein diet, in amounts sufficient to supply a sulfur content equivalent to an 18 per cent casein level, increases the excretion of vitamin C by rats treated with either chloretone or phenobarbital.

Roberts and Spiegel are, therefore, of the opinion that methionine and cystine do not act directly as precursors of vitamin C in the rat, but that the accelerating influence on vitamin C excretion is related to the generally beneficial effect observed when the amino acids are added to a low protein diet.

Gordon *et al* (51) studied the effect of the pituitary growth hormone and reported nitrogen retention and cessation of growth to occur in rats kept on a low protein diet of 6 per cent casein. The addition of methionine to this diet permitted prompt resumption of growth as well as nitrogen retention.



BIBLIOGRAPHY

Part III

1. *Allan, F. N., Bowie, D. J., MacLeod, J. J. R., and Robinson, W. L.; Brit. J. Exp. Pathol.;* **5,** *75, 1924.*

2. *Hershey, J. M. and Soskin, S.; Am. J. Physiol.* **98,** *74, 1931.*

3. *Peters, J., and Van Slyke, D. D., "Quantitative Clinical Chemistry"* **1,** *425, 1946, The Williams Wilkins and Co., Baltimore, Maryland.*

4. *Best, C. H., Hershey, J. M. and Huntsman, M. E.; J. Physiol.;* **75,** *56, 1932.*

5. *Du Vigneaud, V. "Biological Symposia"* **5,** *235, 1941.*

6. *Du Vigneaud, V., Chandler, J. P., Moyer, A. W. and Keppel, D. M.; J. Biol. Chem.* **128,** *CVIII, 1939.*

7. *Ibid.* **131,** *57, 1939.*

8. *Platt, A. P.; Biochem. J.,* **33,** *305, 1939.*

9. *Chandler, J. P., and Du Vigneaud, V., J. Biol. Chem.,* **135,** *223, 1940.*

10. *Stetten, DeW., Jr., J. Biol. Chem.* **140,** *143, 1941.*

11. *Ibid.* **142,** *629, 1942.*

12. *Best, C. H., and Ridout, J. H., J. Physiol.,* **97,** *189, 1940.*

13. *Channon, H. J., Manifold, M. C., and Platt, A. P., Biochem. J.,* **32,** *969, 1938.*

14. *Tucker, H. F., Treadwell, C. R., and Eckstein, H. C., J. Biol. Chem.,* **135,** *85, 1940.*

15. *Stetten, DeW., Jr., and Grail, G. F., J. Biol. Chem.,* **144,** *175, 1943.*

16. *Griffith, W. H., J. Nutrition,* **19,** *437, 1940.*

17. *Ibid.* **21,** *291, 1941.*

18. *Griffith, W. H., and Mulford, D. J., J. Nutrition,* **21,** *633, 1941.*

19. *Horning, M. G., and Eckstein, H. C., J. Biol. Chem.,* **155,** *49, 1944.*

20. *Treadwell, C. R., Tidwell, H. C., and Gast, J. H., J. Biol. Chem.,* **156,** *237, 1944.*

21. *Stetten, DeW., Jr., and Salcedo, J., Jr., J. Biol. Chem.* **156,** *27, 1944.*

22. *Fagin, I. D., and Zinn, F. T., J. Lab. Clin. Med.,* **27,** *1400, 1942.*

23. *Fagin, I. D., Sahyun, M., and Pagel, R. W., J. Lab. Clin. Med.* **28,** *987, 1943.*

24. *Beattie, J. and Marshall, J., Nature,* **154,** *547, 1944.*

25. *Barclay, J. A., Kenney, R. G., and Cooke, W. T., Brit. Med. J.,* **2,** *298, 1945.*

26. *Higgins, G. O'Brien, J. R. P., Peters, R. A., Stewart, A. and Nites, L. J., Brit. Med. J.,* **2,** *401, 1945.*

27. *Barclay, J. A., and Cooke, W. T., Lancet,* **2,** *458, 1945.*

28. *Wilson, C., Pollock, M. R., and Harris, A. D. Lancet,* **1,** *881, 1946.*

29. *Ferriman, D. G., Williams, C. E. O. and Cadman, D. S., Lancet,* **3,** *958, 1946.*

30. *Eddy, J. H., Am. J. M. Sc.,* **210,** *374, 1945.*

31. *Eddy, J. H. Indust. Med.,* **14,** *283, 1945.*

32. *Morrison, L. M., J. A. M. A.,* **134,** *673, 1947.*

33. *Beams, A. J. and Endicott, E. T., Gastroenterology* **9,** *718, 1947.*

34. *Editorial, J. A. M. A.,* **136,** *934, 1948.*

PART III

INTERRELATIONSHIPS OF METHIONINE, CYSTINE AND CHOLINE IN FATTY LIVERS

THE ACCUMULATION of fat in the cells of the liver may either arise from exaggerated physiological circumstances or from pathological conditions. As a rule physiological and pathological fatty livers are distinguished, not only by the quantity of lipids they contain, but also by the character and partition of these lipids.

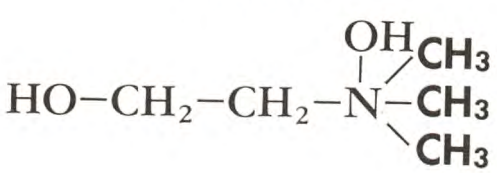
The excessive accumulation of fat in the liver has been recognized since 1889 when Minkowski performed his classical experiment of extirpation of the pancreas in dogs. Since then investigators were more concerned with the metabolic functions of the pancreas and its relation to experimental diabetes than with its relation to fatty liver infiltration. However, following the discovery of insulin it was soon realized that this hormone does not suffice to maintain life of the depancreatized animal (1). An impairment of liver function takes place with rapid accumulation of lipids and ketone bodies. Since the only difference between the depancreatized and the intact animal is the removal of the pancreas, it was found necessary to include raw pancreas in the diet of such animals to permit normal hepatic functions while insulin is being parenterally administered to control blood sugar. Hershey and Soskin (2) also discovered that egg-yolk-lecithin could replace raw pancreas in the diet of the depancreatized animal and restore normal liver function. Thus the term *lipotropic* has been applied to such substances that are capable of eliminating excessive accumulation of fat in the liver caused by dietary deficiencies.

Individuals with pathological fatty livers do not consume an undue amount of fat in the diet nor is there an excess of lipids in the blood stream. In effect there is a diminution of blood lipids, a reduction in cholesterol esters, phosphatides and a disturbed pattern of hepatic lipids. These pathological disorders arising from dietary deficiencies will ultimately lead to a degeneration of liver cells and cirrhosis and not infrequently are accompanied by hemorrhages and degenerative lesions in the kidneys.

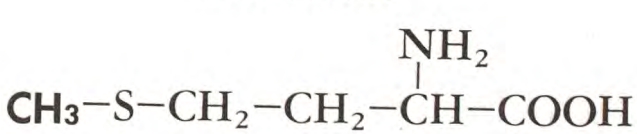
According to Peters and Van Slyke (3), "In almost every instance dietary fatty livers have been traced to the absence from the diet of some components essential for the synthesis of phospholipids or the presence of some compound which interferes with their synthesis."

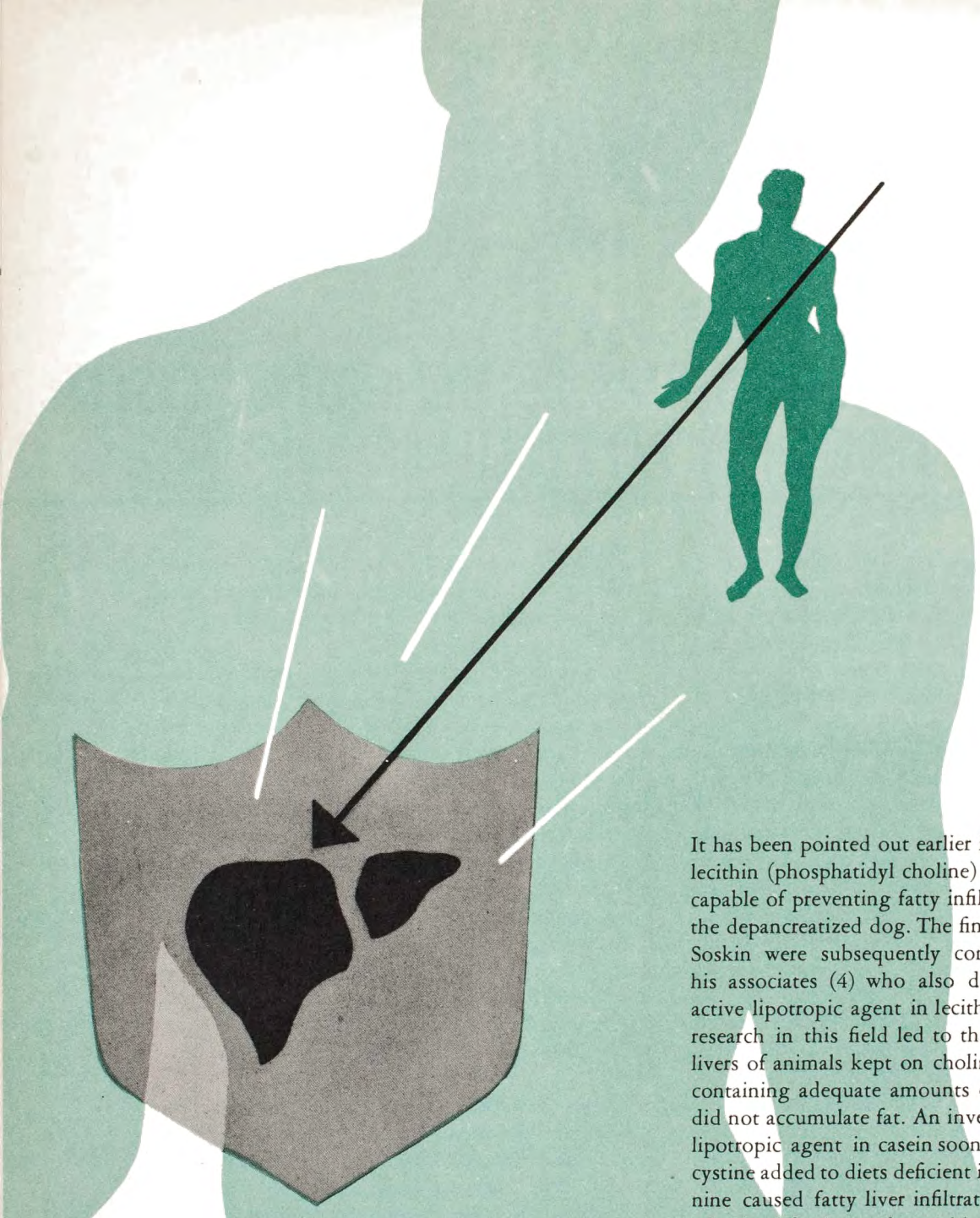
Two of the important dietary factors that have been investigated and found to possess lipotropic activity are choline and methionine. Although these two compounds differ radically from one another in their chemical composition, as can be seen from their structural formulae, nevertheless, they do possess one structural feature in common, viz. *labile methyl group*.

Choline



Methionine

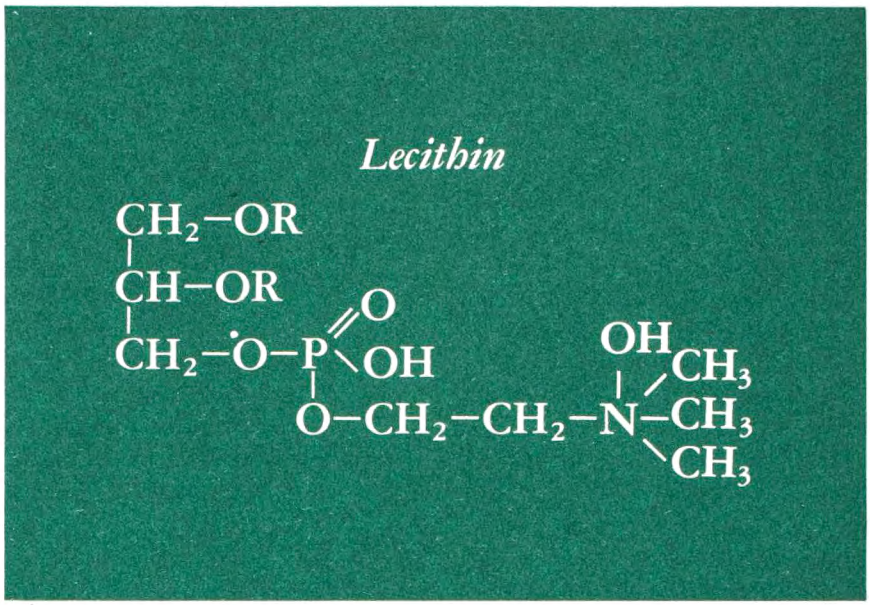




It has been pointed out earlier in this discussion that lecithin (phosphatidyl choline) found in egg yolk is capable of preventing fatty infiltration in the liver of the depancreatized dog. The findings of Hershey and Soskin were subsequently confirmed by Best and his associates (4) who also demonstrated that the active lipotropic agent in lecithin is choline. Further research in this field led to the observation that the livers of animals kept on choline-deficient diets, but containing adequate amounts of casein and sucrose, did not accumulate fat. An investigation of the active lipotropic agent in casein soon revealed that, whereas cystine added to diets deficient in choline and methionine caused fatty liver infiltration in the experimental animal, both D and L methionine did not.

Transmethylation

In reviewing the subject of interrelationships between choline and other methylated compounds, du Vigneaud (5) stated. "The concept that methylation was a general metabolic process was, in fact, explicitly stated some fifty years ago by Hoffmeister (1894) who in his studies on the methylation of tellurium sug-



gested the formation of choline and creatine might be due to the same methylating mechanism. He even postulated that for the purpose of methylation, a methyl group might be split off from some precursor and attached to the new moiety."

On demethylating methionine in the animal system, it has been shown that homocystine is the first intermediate product. Du Vigneaud, Chandler Moyer and Keppel (6-7) demonstrated that rats receiving homocystine in a diet deficient in both methionine and choline were unable to grow. However, it was found that homocystine could support growth of animals on a methionine-free diet only in the presence of choline or betaine. These observations were confirmed by others (8, 9). It therefore appears that choline had probably acted as a donor of a methyl group for the synthesis of methionine from homocystine. The ability of methionine to transfer its labile methyl group in the *in vivo* synthesis of creatine has already been discussed.

The lipotropic action of methionine apparently results from its ability to contribute methyl groups for the synthesis of choline from ethanolamine (10). Evidence for the latter compound as the source of nitrogen of choline is found in the experiments of Stetten (11) on rats in which the feeding of ethanolamine containing N^{15} led to the *in vivo* synthesis of choline containing labelled nitrogen.

Although both choline and methionine are considered excellent transmethylating agents in the animal system, their paths only cross at certain intersections in this complicated system of fat metabolism and evidently only in this respect do they perform similar functions. Thereafter, each assumes more specific roles in metabolism. Choline, for example, is not entirely dependent on its labile methyl group for its lipotropic activity whereas methionine performs this function only by donating its methyl group for the *in vivo* synthesis of choline. The latter is an essential precursor of lecithin and the exact mechanism of its lipotropic activity is yet to be elucidated.

The lipotropic activity of a large number of compounds has been investigated and only those substances which can form choline or donate their methyl groups for its synthesis were found physiologically active in preventing fatty liver infiltration. In the study of casein as a substitute for choline as a lipotropic agent, several workers (12, 13, 14) were unable to ac-

count for the total lipotropic activity of this protein by its methionine content. In view of this, a number of amino acids have been tested and found devoid of this activity. Some observers (14) implied that the opposing action of cystine and methionine together could explain the whole lipotropic effect of casein. This is indeed doubtful, as cystine has a fattening effect and therefore counteracts the lipotropic action of methionine.

Several substances are known to produce fatty livers in the experimental animal despite the presence of protective doses of choline. Among these is guanidoacetic acid (15). It is postulated that the methylation of guanidoacetic acid to form creatine reduces the availability of labile methyl groups responsible for prevention of fatty livers. Like most agents that give rise to fatty livers, guanidoacetic acid induces characteristic lesions in the kidneys.

Ever since its discovery, cystine has been an elusive compound and some one hundred years were required to establish its exact chemical structure. Early investigation showed the important role of cystine in metabolism. As methionine replaced cystine in its classification among the indispensable amino acids, investigators in this field were again puzzled by the peculiarity of cystine to promote hepatic fat infiltration. In young rats, for example, 0.3 per cent of this compound did as much damage as 1.0 per cent, and no greater quantity of choline was necessary to counteract the large dose than was needed for the small dose (16). This effect of cystine was considered by some workers to be connected with methylation. This explanation is also applicable to homocystine which also induces hepatic fat infiltration, and which has been shown by isotopic studies to be a methyl receptor.

The liver fattening effect of cystine, according to Griffith (16, 17) may be accounted for by the appetite-stimulating effect it produces, thus exacting a greater demand for choline or methionine in the diet. This obviously indicates that this particular effect of cystine is not due to any specific toxic action nor to a direct interference with the process of fat transportation. Griffith and Mulford (18) reported that the incidence of fatty livers in rats is related to food-intake. For the prevention of fatty livers caused by cystine, adequate amounts of either choline or methionine must be incorporated in the diet. Thus in rats kept on choline deficient diets and just enough methionine to prevent hepatic fatty liver infiltration, cystine will enhance appetite and causes deposition of fat in the liver. This effect is greater in young growing rats than in mature animals (19, 20).

Recently, Stetten and Salcedo (21) showed that the administration of heavy water to rats kept on choline deficient diets resulted in the finding of deuterium only in the fatty acids of the liver. The addition of cystine to the diets of animals similarly treated, resulted in livers equally fat but with deuterium in the fatty acids of the liver and in the fatty acids of the carcass. It may be concluded therefore that cystine is a general fattening agent; whereas, the lack of choline seems to block the movement of fat from the liver to other tissues of the body.

Hepatic Diseases

In 1942, Fagin and Zinn (22) and in 1943, Fagin, Sahyun and Pagel (23) clinically investigated the lipotropic activity of amino acid mixtures and reported that liver specimens from patients with cirrhosis of the liver and chronic alcoholism, who had received amino acids, contained a greater percentage of protein and a lesser percentage of fat than specimens from patients who had not received amino acid therapy. They postulated that the lipotropic activity of the preparation they used was primarily due to its methionine content. In 1944, Beattie and Marshall (24) published their observations on a case of a young soldier with infective hepatitis who fully recovered following the injection of methionine. However they stated that in chronic cases superimposed on a pre-existing cirrhosis there might be limited improvement with methionine therapy. Barclay *et al.* (25) intravenously administered 10 gm of methionine daily and obtained dramatic results with infective hepatitis in two patients. They felt that the failure of Higgins *et al.* (26) to achieve similar results was the inadequacy of the dosage the latter used in their clinical studies. Barclay and Cooke (27) also obtained remarkable success when both choline and methionine were intravenously injected into a patient who had severe hepatorenal injury as a result of large doses of barbiturates. Wilson, Pollock and Harris (28) orally administered methionine to 52 patients with infective hepatitis. They found a significant shortening of the period of recovery as

compared with 51 control cases. Ferriman, Williams and Cadman (29) gave methionine to a patient with subacute hepatic necrosis. Two months after the methionine treatment complete recovery was observed. Eddy (30, 31) studied the effect of methionine in patients with toxic hepatitis due to trinitrotoluene and carbon tetrachloride poisoning and reported favorable results. Recently Morrison (32) reported studies on a group of 43 patients with cirrhosis of the liver. These studies extended over periods from 1938 to 1940 and 1943 to 1945. Outstanding improvement was observed where methionine, choline, whole liver extract and vitamin B complex were added to the diets.

Beams and Endicott (33) have recently shown the value of methionine based on histologic changes in the livers of patients with cirrhosis. A recent review (34) of the studies using methionine in the treatment of hepatic cirrhosis indicates that this amino acid was responsible for improved histologic changes in the liver which occurred in these patients.

BIBLIOGRAPHY

Part IV

1. *Miller, L. L., J. Biol. Chem.*, **152,** *603, 1944.*

2. *Stevenson, G., Swanson, P. P., William, W. and Brush, M., Proc. Fed. Am. Soc. Exp. Biol.* **5,** *240, 1946.*

3. *Croft, P. B. and Peters, R. A., Nature* **155,** *175, 1945.*

4. *Sahyun, M., Kade, C. F. and Houston, J. J. Dig. Dis.*, **14,** *230, 1947.*
4*a*. *Sahyun, M., Kade, C. F. and Houston, Unpublished data.*

5. *Allison, J. B., Anderson, J. A. and Seeley, R. D., J. Nutrition*, **33,** *361, 1947.*

6. *Brush, M., Willman, W., and Swanson, P. P., J. Nutrition*, **33,** *389, 1947.*

7. *Johnson, R. M., Deuel, H. J., Jr., Morehouse, M. G. and Mehl, J. W.*, **33,** *371, 1947.*

8. *Cox, W. M., Jr., Mueller, A., Elman, R., Albanese, A. A., Kemmerer, K. S., Barton, R. W., and Holt, L. E., Jr., J. Nutrition*, **33,** *437, 1947.*

9. *Tidwell, H. C., Proc. Soc. Exp. Biol. Med.* **63,** *13, 1946.*

10. *Booher, L., Proteins and Amino Acids in Nutrition, Ed. Sahyun, M., p. 132-196, 1948. Reinhold Pub. Corp. N. Y.*

11. *Russell, W. C., Taylor, M. W. Mehrhof, T. A., and Hirsch, R. R., J. Nutrition*, **32,** *313, 1946.*

12. *Peterson, C. F., and Lapman, C. E., Poultry Sc.*, **23,** *287, 1944.*

13. *Lehrer, W. P. Jr., J. Nutrition*, **33,** *469, 1947.*

14. *Murray, H. C., J. Nutrition*, **35,** *257, 1948.*

15. *Russell, W. C., Taylor, M. W., Mehrof, T. G., and Hirsch, R. R., J. Nutrition*, **32,** *313, 1946.*

16. *Geiger, E., J. Nutrition*, **34,** *97, 1947.*

17. *Rose, W. C., Haines, W. J., and Johnson, J. E., J. Biol. Chem.* **146,** *683, 1942.*

18. *Rose, W. C., Proc. Inst. Med. Chicago*, **15,** *24, 1944.*

19. *Madden, S., Proc. Inst. Med. Chicago* **15,** *25, 1944.*

20. *Block, R., Yale J. Biol. Med.*, **15,** *723, 1943.*

21. *Albanese, A. A., Holt, L. Jr., Brumback, J. E., Jr., Frankston, Jane, E., and Irby, V., Bull. Johns Hopkins Hosp.*, **74,** *308, 1944.*

PART IV

METHIONINE IN NUTRITION

WE HAVE SO FAR followed the course of methionine in metabolism with reference to: (1) its indispensability as a constituent of proteins in the diets of animals, (2) its breakdown in the system and the mechanism of its conversion to homocystine and cystine, (3) its ultimate fate, (4) detoxification mechanisms, (5) consequences of its deficiency, and (6) by virtue of its labile methyl group (a) its relation to the *in vivo* synthesis of creatine and choline and (b) the role it plays in fatty liver infiltration—in brief its importance as a transmethylating agent.

Protein-Sparing Action

Recently considerable interest has been shown in the relationship between nitrogen balance and absorbed protein nitrogen. This interest by certain workers has revolved around the regions of negative and low positive nitrogen balances with the object of providing more accurate means of evaluating the biological efficiency of proteins, protein hydrolysates and mixture of amino acids in the diets of animals and man. In 1944, Miller (1) furnished evidence to the effect that methionine reduces the excretion of nitrogen in normal adult dogs receiving a protein-free diet. Stevenson, Swanson and Brush (2) also noted a nitrogen-sparing action for this amino acid in the rat. Croft and Peters (3) demonstrated in burned rats that by doubling the protein-intake and including one per cent of DL-methionine, nitrogen equilibrium was attained. This indeed was a startling revelation. Sahyun, Kade and Houston (4) carefully investigated the biological efficiency in dogs of acid hydrolysates of casein fortified with DL-tryptophan and showed that the addition of DL-methionine to this mixture considerably enhances its biological activity. They also showed that for the maintenance of nitrogen balance in the dog, whereas 140 mg. N of casein per kg. of body weight is required, only 110 mg. N of the acid hydrolysate fortified with DL-tryptophan and DL-methionine is needed. Subsequent investigations by these authors (4a) revealed that in the dog as well as in the rat, the biological value of casein fortified with DL-methionine is on a par with that of lactalbumin which is considered one of the most efficient and complete proteins known.

Allison and coworkers (5) found that the addition of DL-methionine to a low protein diet containing casein or egg white will reduce the excretion of nitrogen in the adult dog. Furthermore, they reported that nitrogen excretions remain lower than those of control values for several days after the amino acid (methionine) has been withdrawn from the diet. Thus they concluded that the addition of 0.025 gm. of methionine-N (0.4 gm. of methionine) to one gram of casein-N (6.25 gm. casein) produces a maximum

nitrogen balance index for this protein. The addition of larger quantities of methionine to the casein does not alter this index. Brush *et al.* (6) also observed a marked reduction in the quantity of nitrogen excreted in the urine by standardized rats partially depleted of their bodily reserves of proteins following the introduction of whole eggs into a protein-free diet. A careful study of the 10 essential amino acids by these workers led to the identification of methionine as being the most effective amino acid in this respect. Analyses of whole carcasses and muscle of adult rats suggest the specificity of methionine in nitrogen metabolism. They stated: "The continued loss in body weight, the lack of change in the total nitrogen content of the carcass, the constancy of the ratio of methionine nitrogen to total nitrogen of the carcass and the increment in hepatic tissue when methionine is fed to the depleted animals, point to the possibility that this amino acid does not act in the general maintenance of body tissues but in the synthesis of functional proteins and important metabolites."

The sparing action of methionine on the urinary nitrogen in men at normal and low levels of protein intake was studied by Johnson and coworkers (7) who reported that under the conditions employed, methionine did not seem to spare body protein, and Cox and coworkers (8) also made similar observations. The latter two observations in man may either mean that methionine sparing effect does not occur in the normal human subject but may be demonstrable in cases of malnutrition and severe hypoproteinemia or that there are species differences. The latter is well known to investigators in this field e.g. histidine which is an essential amino acid to experimental animals is dispensable to man. In the case of methionine, this particular function of protein sparing effect may or may not take place in man, but on the other hand if methionine has no sparing action on urinary excretions of nitrogen when the subject is kept on low-protein diets, its other functions, previously discussed, may be accentuated and the demand for the labile methyl group for the synthesis of creatine and choline *in vivo*, becomes such that there will be no methionine available to exercise a protein-sparing action. The experiments of Tidwell (9) support this theory for he found no diminution in the formation of creatine as measured by urinary excretion in animals kept on a diet deficient in labile methyl groups. He stated: "The available methionine was preferentially used for growth and creatine formation. The increased excretion of creatine by these animals, apparently associated with weight loss, causes a waste of needed methyl groups instead of their conservation."

From the experimental evidence presented in rats and in dogs the effect of methionine should be extensively studied in such animals as sheep, the lactating cow, hogs, chickens and turkeys. This protein-sparing property of methionine should prove of considerable value not only from a metabolic point of view but also for economic reasons. Many feed proteins are low in this amino acid, and if it should prove that the addition of small quantities of methionine to feeds enhances the conversion of feed to meat, then livestock and poultry could be raised at lower costs.

Methionine Supplementation

Of considerable importance to the dietary of both man and animals is the admixture of various types of foods to obtain satisfactory and pleasing diets that provide adequate growth and maintenance of good health at reasonable costs. The economic factors involved are certainly of paramount considerations to all. In a recent publication on this subject, Booher (10) states:

"Carefully considered and circumspect improve-

ments in the nutritive qualities of staple low-cost foods and the development of further low-cost food items of high nutritive value and wide acceptability could expedite the process of obtaining more adequate diets for those of low incomes. However, the inadequate diets of the poor are not of a uniform pattern and the poor, as well as other economic groups, have various food preferences. So it is not to be expected that one or even a few low-cost food items can be ushered in hastily to solve the entire nutritional problem of the poor. Whether or not improvements in nutritive qualities of existing staple articles of food or development of inexpensive new foods would be in the best interests of the poor requires consideration of the cost to the consumers, the degree of acceptability of the items, and the probable level of consumption of the products proposed. Any food purporting to be a boon to the poor must be within economic reach of those with low incomes; it should enjoy ready acceptance by significant numbers of the poor, and it should supply needed nutrients and be consumed in effective quantities by the poor. . . . Ignorance or indolence (both remedial by proper education) almost doom one to the maximal risk of living in poverty; knowledge, training and a willingness to be productive and useful are the best tools we have to avoid the risk of having to live in poverty."

It is true that the proteins of meat are of high nutritive quality, however in order to produce meat proteins, plant proteins must be utilized as feed for conversion into meat proteins. The efficiency of feed proteins to meat protein conversion, obviously, varies with the species and the nature of protein, as well as with the size of the animal produced. For beef proteins, for example, the conversion factor varies throughout the entire range of relatively short-term intensive feeding between 11 and 14.5 per cent (10). However, for less intensive feeding and storing the animals over long periods, the conversion factor is significantly lower. This, in a nutshell, explains the high cost of animal proteins as compared to that of plant proteins and why among many nations and in many areas of the United States plant proteins have to be used almost exclusively as a food.

It is well kown that field peas have been and still are widely used as a source of protein in famine areas in Europe and Asia and in many parts of this country.

Cull peas provide a protein concentrate of high value for animal feeding. Russell and coworkers (11) employing rats, Peterson and Lampman (12) using chickens, demonstrated that the low methionine content of pea protein is a limiting factor when peas were used as the sole source of protein in growth studies. Lehrer, Woods and Beeson (13) showed in the rat that cooked peas alone were a poor source of protein for growth. However, when peas were supplemented with 0.3 per cent DL-methionine growth was increased until it was not insignificantly different from that obtained with whole egg protein.

Murray (14) investigated the protein of Alaska field pea and showed it to be inferior to casein when both were fed at the 10 per cent level. She also observed that canning and baking of peas produce a lowering of the biological value of this protein.

Russell *et al.* (15) investigated several varieties of lima beans, snap beans, chick peas and soy beans and showed that their methionine content ranged from 0.29 to 0.85 per cent. They also studied the nutritive values of these legumes in the rat at a 10 per cent protein level as the sole source of nitrogen intake. They observed little or no growth; however the addition of as little as 0.1 per cent of methionine to the basal diet caused an immediate growth response.

It is therefore obvious from the results obtained by these investigators that the addition of DL-methionine to such a valuable plant protein so abundant and well-liked by animals and man will raise its biological value and renders it a far more useful and nutritive food. Using methionine as a supplement to proteins in food, it is important to bear in mind that it should be incorporated along with the protein of the diet. Geiger (16) has shown that delayed supplementation of such amino acids as tryptophan, methionine and lysine, by several hours after feeding of the incomplete protein or amino acid mixture, will not promote growth.

Methionine in Man

The indispensability of this amino acid to humans has been well established by numerous investigators (17, 18, 19, 20, 21), however its exact requirement has not as yet been quantitatively determined. Man's requirement of this as well as of other indispensable amino acids should probably vary in different subjects depending on age, sex and condition of the individual. For infants, growing children and for the pregnant and lactating woman, dietary protein intake is much higher than for the adult. This fact is universally accepted. Thus if we were to assume that the average adult male and adult female daily require 60 gm. and 50 gm. respectively of a protein such as casein for the maintenance of good health, then approximately 2 gm. of methionine is needed; for casein has been shown to contain about 2.9 to 3.1 per cent methionine. This estimate of 2 gm. of methionine per day should be considered as a minimum requirement, provided adequate amounts of choline and other vitamins have been incorporated in the diet. In deficiency disorders and metabolic dysfunctions, higher quantities of methionine may be necessary.

BIBLIOGRAPHY

Part V

1. *Almquist, H. J. Proteins and Amino Acids in Nutrition. M. Sahyun, Reinhold Publishing Corp., New York, N. Y. p. 221 (1948).*

2. *Rose, W. C., Physiol. Reviews,* **18,** *109, (1938).*

3. *Almquist, H. J., J. Nutrition,* **34,** *543, 1947.*

4. *Nutritional Reviews,* **6,** *148, 1948.*

5. *Almquist, H. J., Trans. Am. Assoc. Cereal Chem. III 158, 1945.*

6. *Grau, C. R. and Almquist, H. J., J. Nutrition,* **26,** *631, 1943.*

7. *Klose, A. A. and Almquist, H. J., J. Biol. Chem.,* **138,** *467, 1947.*

8. *Almquist, H. J. and Grau, C. R., J. Nutrition,* **27,** *263, 1944.*

9. *Almquist, H. J. and Grau, C. R., ibid.,* **29,** *219. 1945.*

10. *Griffith, W. H., J. Nutrition,* **22,** *239, 1941.*

11. *du Vigneaud, V., Chandler, J. P., Moyer, A. W., and Keppel, D. M., J. Biol. Chem.,* **57,** *131, 1939.*

12. *Stetten, DeWitt, Jr., J. Biol. Chem.,* **138,** *437, 1941.*

13. *Almquist, H. J., Science,* **103,** *722, 1946.*

14. *Jukes, T. H., J. Biol. Chem.,* **134,** *789, 1940.*

15. *Jukes, T. H., J. Nutrition,* **22,** *315, 1941.*

16. *Record, P. R., and Bethke, R. M., Poultry Sci.,* **21,** *271, 1942.*

17. *Jukes, T. H., J. Nutrition,* **20,** *251, 1941.*

18. *Nutritional Reviews,* **6,** *230, 1948.*

19. *McKittrick, D. S., Arch. Biochem.,* **15,** *133, 1947.*

20. *Jukes, T. H. and Welch, A. D., J. Biol. Chem.,* **146,** *19, 1942.*

21. *McGinnis, J. Norris, L. C., and Heuser, G. F., Proc. Soc. Exptl., Biol. and Med.,* **56,** *197, 1944.*

22. *Jukes, T. H., Oleson, J. J., and Dornbush, A. C., J. Nutrition,* **30,** *219, 1945.*

23. *Jukes, T. H., and Oleson, J. J., J. Biol. Chem.,* **157,** *419, 1945.*

24. *Lucas, H. L., Norris, L. C., and Heuser, G. F., Proc. Soc. Exptl. Biol. Med.,* **63,** *32, 1946.*

25. *McGinnis, J., Norris, L. C., Heuser, G. F., Proc. Soc. Exptl. Biol. and Med.,* **51,** *293, 1942.*

26. *Almquist, H. J. and Jukes, T. H., Proc. Soc. Exptl. Biol. and Med.,* **51,** *243, 1942.*

27. *Hayward, J. W. and Hafner, F. H., Poultry Sci.,* **20,** *139, 1941.*

28. *Almquist, H. J., Mecchi, E., Kratzer, F. H., and Grau, C. R., J. Nutrition,* **24,** *385, 1942.*

29. *Berry, E. P., Carrick, C. W., Roberts, R. E. and Hauge, S. M., Poultry Sci.,* **22,** *252, 1943.*

30. *Berry, E. P., Carrick, C. W., Roberts, R. E., and Hauge, S. M., Poultry Sci.,* **22,** *442, 1943.*

31. *Marvel, J. A., Carrick, C. W., Roberts, R. E., and Hauge, S. M., Poultry Sci.,* **23,** *294, 1944.*

32. *Marvel, J. A., Carrick, C. W., Roberts, R. E., and Hauge, S. M., Poultry Sci.,* **24,** *46, 1945.*

33. *Marvel, J. A., Carrick, C. W., Roberts, R. E., and Hauge, S. M., Poultry Sci.,* **24,** *181, 1945.*

34. *Bird, H. R. and Mattingly, J. P., Poultry Sci.,* **24,** *29, 1945.*

35. *Clandinin, D. R., Cravens, W. W., Halpin, J. G. and Hart, E. B., Poultry Sci.,* **25,** *509, 1946.*

36. *Mishler, D. H., Carrick, C. W., Roberts, R. E., and Hauge, S. M., Poultry Sci.,* **25,** *479, 1948.*

37. *Gerry, R. W., Carrick, C. W., and Hauge, S. M., Poultry Sci.,* **27,** *161, 1948.*

38. *Mishler, D. H., Carrick, C. W., Hauge, S. M., Poultry Sci.,* **27,** *263, 1948.*

39. *Gerry, R. W., Carrick, C. W. and Hauge, S. M., 37th Annual Meeting, Poultry Science Association Abstracts, page 13, 1948.*

40. *Ibid, page 14.*

41. *Mishler, D. H., Carrick, C. W. and Hauge, S. M., ibid, page 27.*

42. *Hayward, J. W., Halpin, J. G., Holmes. C. E., Bohstedt, G., and Hart, E. B., Poultry Sci.,* **16,** *3, 1937.*

43. *Bird, H. R. and Burkhardt, G. J., Maryland Agr. Exp. Sta. Bull. No., A27, 35, 1943.*

44. *Parsons, H. T., J. Home Econ.,* **35,** *211, 1943.*

45. *Evans, R. J. and McGinnis, J., J. Nutrition,* **31,** *449, 1946.*

46. *Clandinin, D. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., Poultry Sci.*, **26,** *150, 1947.*

47. *Evans, R. J., McGinnis, J., J. Nutrition*, **35,** *477, 1948.*

48. *Melnick, D., Oser, B. L., and Weiss, S., Science* **103,** *326, 1946.*

49. *Westfall, R. J., and Hauge, S. M., J. Nutrition*, **35,** *379, 1948.*

50. *McGinnis, J., and Evans, R. M., J. Nutrition*, **34,** *725, 1947.*

51. *Ham, W. E., Sandstedt, R. M., and Mussehl, F. E., J. Biol. Chem.*, **161,** *635, 1945.*

52. *Patton, A. R. and Pyke, W. E., Abstracts, American Chemical Society 110th Meeting, 10A, 1946.*

53. *Stevens, Joan M., and McGinnis, J., J. Biol. Chem.*, **171,** *431, 1947.*

54. *Peng-Tung Hsu, McGinnis, J., and Graham, W. D., 37th Annual Meeting, Poultry Science Association, Abstracts, p. 18, 1948.*

55. *Patton, A. R., Hill, E. G., and Foreman, E. M., ibid.*

56. *Bethke, R. M., Pensack, J. M., and Kennard, D. C., Poultry Sci.*, **26,** *128, 1947.*

57. *Sarma, P. S., Snell, E. E., and Elvehjem, C. A., J. Nutrition*, **33,** *121, 1947.*

58. *McGinnis, J. and Carver, J. S., Poultry Sci.*, **26,** *489, 1947.*

59. *Patton, A. R., Marvel, J. P., Petering, H. G., and Waddell, J., J. Nutrition*, **31,** *485-95, 1946.*

60. *Kennard, D. C., Bethke, R. M., and Chamberlain, V. D., Poultry Sci.*, **27,** *477, 1948.*

61. *Kratzer, F. H., J. Biol. Chem.*, **153,** *237, 1944.*

62. *Grau, C. R., and Almquist, H. J., Arch. Biochem.*, **6,** *287, 1945.*

63. *Block, R. J., Bolling, Diana, The Amino Acid Composition of Proteins and Foods, C. C. Thomas, Springfield, Ill., 1945.*

64. *McGinnis, J., Peng-Tung Hsu, and Carver, J. S., Poultry, Sci.*, **27,** *389, 1948.*

65. *Petersen, C. F., Lampman, C. E., Bolin, D. W., and Stamberg, O. E., Poultry Sci.*, **23,** *287, 1944.*

66. *Bolin, D. W., Petersen, C. F., Lampman, C. E., and Stamberg, O. E., Poultry Sci.*, **25,** *157, 1946.*

67. *Taylor, M. W., and Russell, W. C., Poultry Sci.* **22,** *334, 1943.*

68. *Bethke, R. M., Kennard, D. C., and Chamberlain, V. D., Poultry Sci.*, **25,** *570, 1946.*

69. *Munks, Bertha, Robinson, A., Beach, E. F., and Williams, H. H., Poultry Sci.*, **24,** *459, 1945.*

70. *Ringrose, R. C., and David, H. A., Poultry Sci.*, **25,** *646, 1946.*

71. *Lucas, H. L., Norris, L. C., and Heuser, G. F., Poultry Sci.*, **25,** *373, 1946.*

72. *Bird, H. R., Marsden, S. J., and Kellog, W. L., Poultry Sci.*, **27,** *53, 1948.*

73. *Shaw, J. C., J. Dairy Sci.*, **29,** *131, 1946.*

74. *Loosli, J. K., and Harris, L. E., J. Animal Sci.*, **4,** *435, 1945.*

75. *Schaub, L. O. and Bauer, North Carolina Agr. Expt. Sta., Ann. Report*, **66,** *1943.*

76. *Ferrin, E. F., J. Animal Sci.*, **5,** *42, 1946.*

77. *Johnson, J. E., and Mussell, D. R., Unpublished Data.*

78. *Mishler, D. H., Carrick, C. W., and Hauge, S. M., Poultry Sci.* **28,** *24, 1949.*

PART V

THE ROLE OF METHIONINE IN POULTRY NUTRITION

METHIONINE, one of the essential amino acids found in protein, plays an indispensable role in avian nutrition. In this review an outline will first be presented which describes the known facts concerning the metabolism of methionine and biochemically related compounds. Secondly, a summary will be given of the practical feeding trials performed to determine the supplemental value of DL-methionine in avian nutrition. A combination of practical feeding trials together with information gained with purified diets has served to build up the present state of knowledge of the biochemistry of methionine in birds.

Biochemical Significance of Methionine in Avian Metabolism

In the chicken, as in the rat, mouse, dog and human, methionine is an indispensable amino acid (Almquist, 1). This means, according to the definition of Rose (2), that methionine cannot be synthesized from the materials normally available in the diet at a rate commensurate with the demands of normal growth. Thus, methionine must be supplied preformed in the feed if chickens are to grow. In the outstanding work of Almquist and coworkers (2, 3, 4, 5) it has been established that the percentage change in body weight bears a linear relationship to the percentage concentration of the limiting essential amino acid in the diet. In the presence of adequate cystine together with other essential nutritional factors 0.5 per cent methionine in the diet is required for optimum growth of the chick. Less than this essential level of methionine results in restriction of the growth rate (Almquist 3, Grau and Almquist 6).

The interrelationships between methionine, cystine, choline and betaine in avian metabolism have an important bearing upon practical feeding problems.

The relationship of methionine to cystine in the chick is similar to that in mammalian species (6, 7, 8, 9). Methionine can be called upon as a precursor of cystine in the body, however, this biosynthesis of cystine from methionine can be spared if the diet supplies adequate cystine to meet metabolic requirements. When cystine is not present 0.9 per cent methionine must be supplied to meet the demand for sulfur-containing amino acids (3, 9). Cystine cannot compensate for a methionine deficiency even when high levels of choline are fed (9). Grau and Almquist (6) have shown that the unnatural isomer or D form of methionine can be utilized by the chick. Thus it is reasonable that DL-methionine could be efficiently employed as a supplement in methionine-deficient diets.

In addition to its role as an essential tissue component, and as a precursor of cystine, methionine serves as a methylating agent in avian metabolism. Unlike the rat (10, 11, 12) however, the growing chick cannot utilize methionine as a precursor of choline; in fact, choline synthesis is extremely limited (13). Methionine does not replace dietary choline in chicks (6, 14, 15, 16, 18, 19, 21) or in turkeys (17) for preventing perosis or slip tendon when an *extreme* choline deficiency exists. The successful substitution of methionine for a part of the choline requirement of chicks appears to be limited to those functions of methylation where either choline or methionine could serve as a methyl donor. When the methylating function is relieved by added methionine, the available

choline may serve in its other essential physiological reactions such as phospholipid synthesis, lipotropic action and perosis prevention.

Betaine, like methionine, can spare choline by assuming its role as a methyl donor (8); however, unlike reactions of betaine in the rat (10, 11, 12) this compound cannot adequately serve as a precursor for choline synthesis in the chick (13, 15, 20, 21). Jukes and coworkers (22, 23) have demonstrated in experiments with chicks that choline synthesis can take place from dimethylaminoethanol in the presence of methionine, however in similar tests monomethylaminoethanol, aside from perosis prevention, does not substitute for choline. McGinnis and coworkers (21) have suggested that precursors of choline are present in the simplified diets in which methionine and betaine prevent perosis. These views are supported by the experiments of Lucas, *et al.* (24) and Jukes, *et al.* (22, 23). Ethanolamine has little or no effect on the occurrence of perosis in chicks (25).

Arsenocholine is a unique analogue of choline in that it does not serve as a methyl donor (26); but does enter into phospholipid synthesis and lipotropic activity and does promote growth and prevent choline-deficiency perosis in the chick (3, 8, 20). The effects of arsenocholine plus those of betaine or methionine are additive. Either methionine or betaine in combination with arsenocholine serves as practically a complete substitute for dietary choline (8).

The relationship of homocystine and methionine in chick metabolism is similar to that observed in the rat. In the presence of choline (6, 7), betaine (8), but not arsenocholine (26), homocystine can be utilized by the chick to form methionine.

Further quantitative evidence regarding the interrelationship between choline, betaine and methionine in chick nutrition has been reported by McKittrick (18, 19). Choline and methionine have at least two metabolic roles as (a) essential tissue components and (b) methyl group donors. For optimum growth 0.5% methionine and 0.10% choline chloride must be present in the diet as "essential" components. These essential levels must be supplemented with a replaceable fraction of choline, methionine or both, (0.25% methionine plus 0.45% choline or an equivalent mixture). Betaine may substitute for the replaceable fraction of choline or methionine but not for the essential quantities of either material.

Excess methionine in the diet of chicks causes a depression of growth rate (19) which can be counteracted by the addition of guanidoacetic acid. High dietary homocystine has no growth depressing effects unless additional choline is added. Guanidoacetic acid is presumed to exert its protective action by serving as a methyl acceptor resulting in the formation of homocystine from methionine. Added serine also counteracts the growth depressing effects of a high level of methionine probably by facilitating the synthesis of cystine. It is likely that methionine is the specific carrier of methyl groups for the conversion of guanidoacetic acid to creatine (1).

Supplemental Value of Methionine in Poultry Nutrition

The first evidence of the supplemental value of DL-methionine used in connection with chick diets containing soybean oil meal was published in 1941 by Hayward and Hafner (27) who reported that the protein of raw or cooked soybeans was improved by the addition of 0.3% L (-) cystine or 0.3% DL-methionine. Almquist *et al.* in 1942, working with a diet consisting principally of purified ingredients, concluded that the principal growth limiting factor in raw soybean protein is that of methionine and that heated soybean protein is slightly deficient in methionine for the chick when fed at the 20% level. The addition of choline to raw soybean protein produced no effect; however, small but definite increases in the rate of gain were produced by adding choline to heated soybean protein. DL-Methionine produced a definite growth response with both raw and heated soybean protein. Almquist and Grau (9), later found that levels of choline in chick diets as high as 0.6 per cent gave only a 4 per cent per day increase in chick growth, whereas 0.55 per cent supplemental methionine gave optimal gains of 6 per cent per day. Furthermore, they concluded that cystine cannot offset a methionine deficiency regardless of the level of choline fed. Experiments have also been described by Almquist and Grau (6, 8) which demonstrate that methionine could be replaced by a combination of homocystine and choline and that methionine could only partially replace choline.

Earlier reports from the Purdue University Agricultural Experiment Station by Carrick *et al.* (29, 30, 31, 32, 33) indicated that choline and methionine exerted an interchangeable supplementary action in a chick diet in which the protein was supplied principally by soybean oil meal and corn. Bird and Mattingly (34), using a ration which contained yellow corn, oats, wheat products, soybean oil meal, alfalfa meal, molasses-butyl-fermentation solubles, vitamin A and D oil and mineral supplements, found that choline and DL-methionine did not produce interchangeable supplementary action. Two-tenths per cent of added DL-methionine produced a growth stimulus slightly exceeding the effect of adding 4% fish meal. The growth was not improved by adding choline chloride. The importance of the method of processing of soybean oil meal was recognized by Carrick *et al.* (32) as an important limitation in the nutritional adequacy of a diet. While no significant differences were observed in the choline content of inferior or superior soybean oil meals, it was suggested that differences in availability of either choline or methionine or other growth factors may have been the cause of the different results reported.

Clandinin *et al.* (35) supplemented a diet containing different samples of soybean oil meal with methionine and choline. All of the meals tested were benefited by methionine addition and one was made adequate thereby for optimum growth. It was concluded, contrary to earlier conclusions by Carrick *et al.*, that choline and methionine cannot be considered as interchangeable supplements to practical soybean oil starter rations. Only one out of the four meals tested was improved by the addition of choline chloride.

Mishler, Carrick, Roberts and Hauge (36) reported that a ration containing corn, soybean oil meal, minerals, fish oil, synthetic riboflavin, pantothenic acid and niacin appeared to be deficient in choline. Continued experiments with this type of diet (37) indicated that either choline or methionine acted as a supplement to the ration but methionine was more effective. These workers concluded, "Althouth methionine and choline may not be entirely interchangeable, it is apparent that in the absence of sufficient methionine, choline was partially effective as a supplement."

A recent report by Mishler, Carrick and Hauge (78) states that choline, betaine and methionine were all effective in supplementing simplified rations similar to those described above. Under the conditions of their experiments, betaine at 0.3 and 0.4 per cent levels appeared to have higher supplementary value than either choline or methionine. The results also indicate that a combination of betaine and methionine may substitute for the growth function of choline.

Experiments by Mishler, Carrick and Hauge (38) demonstrate that the addition of 0.3% DL-methionine to a ration containing corn, soybean oil meal, minerals, fish oil, condensed fish solubles (1.5%), pantothenic acid, niacin and riboflavin gave a significant increase in growth and definite improvement in the efficiency of feed utilization. Similar addition of choline chloride produced only a slight growth re-

sponse. With both choline and methionine additions the results were nearly the same as with methionine alone. It appears, therefore, that methionine was a more effective supplement than choline for the soybean oil meal and fish solubles used.

Carrick and his coworkers (39, 40, 41) have reported further evidence that methionine and choline will not completely replace each other. The amount of methionine which is required for optimum growth varies depending on the type of soybean oil meals used. Betaine shows pronounced supplementary value in the simplified rations. The basal diets contained relatively high percentages of protein (22-24%). It is probable that the requirment for "essential" methionine was largely met by the amount of protein fed and that the response to supplementation with choline, methionine and betaine can be attributed to the role of these compounds as methyl donors. The supplementary effects of methionine would possibly become more obvious in basal diets of somewhat lower protein content.

Variable result by different workers who have investigated the supplementary value of methionine and choline can be best interpreted in the light of the previously stated hypothesis (18, 19) that a certain dietary level of these compounds is essential for chick growth while the methylating function can be assumed by either one, or by supplementation with betaine. Many of the uncertainties of this problem, now gradually being resolved, have contributed to additional lack of agreement by different experimenters. These include the effect of heat treatment upon the chemical nature and availability of the amino acids in protein supplements, the availability of unknown nutritional factors, and the importance of the diet of the laying hen (56) in hatchability and subsequent growth of the chick.

Moderate autoclaving has been shown to increase the nutritive value of raw soybean oil meal for the growth of chicks (28, 42, 43, 44, 46, 47). Prolonged or drastic heat treatment, however, will in turn cause a reduction in nutritive value (43, 44, 45, 46). Melnick *et al.* (48) have suggested that mild autoclaving of soybean oil meal improves the digestability and thereby permits earlier release of methionine from the protein, resulting in more efficient use of other simultaneously-liberated essential amino acids. The information concerning the presence of trypsin inhibitors in soybean has been briefly reviewed by Westfall and Hauge (49). Furthermore, these workers have presented evidence showing that the protein efficiency of soybean meal is increased in direct proportion to the destruction of its trypsin inhibitor potency by heat. It was concluded that the presence of trypsin inhibitor was the chief cause for the poor utilization of the protein of inadequately heated soybean meals. This is in agreement with the explanation advanced by Ham, Sandstedt and Mussehl (57) and by Evans

and McGinnis (45, 47). The latter workers found that mild autoclaving of soybean oil meal increased the percentage of cystine and methionine which was retained by the growing chick.

McGinnis and Evans (50) have performed experiments wherein raw soybean oil meal supplemented with methionine produced a growth response short of the maximum observed with control diets. The addition of methionine to mildly autoclaved soybean meal provided no additional growth stimulus. They have suggested that the methionine requirement for growing chickens may not be more than 0.26% when the diet contains 0.4% cystine. In a later paper (47) Evans and McGinnis indicate that raw soybean oil meal is deficient in amino acids other than methionine because methionine additions to the raw soybean oil meal did not increase growth and protein-utilization to the extent that mild autoclaving did. In view of the observations by McKittrick (19) indicating growth inhibition by diets which contain excessive methionine, this conclusion should be substantiated by further experiments using less than 0.5% of supplemental DL-methionine.

Overheating soybean oil meal can produce deficiencies in the known vitamins and in available lysine, methionine and cystine (45, 46, 47). McGinnis and Evans (50) showed that the deficiency in soybean oil meal autoclaved for 60 minutes at 130°C. was not corrected by adding methionine, cystine or lysine but was corrected by adding a combination of all three. Methionine plus lysine can also correct the heat induced deficiency (53). The yield of methionine by microbiological assay in overheated meal was shown by Clandinin *et al.* (46) to be equal to that in raw soybean oil meal. They concluded that methionine assay of an acid hydrolysate may not be a reliable index of biologically available methionine.

The destruction of certain amino acids, including methionine, by heat treatment in the presence of carbohydrates or aldehydes has been demonstrated (52, 53, 54, 55). This type of reaction may, in part, explain the impaired nutritional value of overheated soybean oil meal. It is obvious that careful control in the processing of soybean oil meal has a very important bearing upon its nutritional quality. Difficulties resulting from overheating are better avoided with the solvent process of soybean oil extraction.

With the exception of choline, little is known about the metabolic interrelationships between methionine and the vitamins. Sarma, *et al.* (57) have shown that DL-methionine has a growth retarding effect when fed to rats on a pyridoxine-deficient diet. Further investigation of unidentified factors will probably render the dietary interrelationships of methionine more understandable because it appears that one or more of these unidentified factors influences the efficiency of amino acid utilization.

McGinnis and Carver (58) have reported that the need for supplementing vegetable protein chick diets with unidentified animal protein factors is general and is not limited only to rations which contain corn and soybean oil meal as suggested by Patton *et al.* (59).

It has been shown (60) that a built up litter can serve as a potent source of the unidentified animal protein factors necessary for supplementing a vegetable protein diet for the production of eggs of maximum hatchability. More information about poultry management coupled with the eventual commercial availability of now unidentified factors will permit more specific planning of amino acid-supplemented diets designed to give the maximum efficiency of protein utilization.

Another problem which has been recognized (48) and which needs further clarification is the relation between absorption rates, digestive mechanism, and blood concentration of the amino acids (61) and their bearing upon the efficiency of the anabolic process.

The methionine content of certain feedstuff pro-

teins has been reported by several investigators including Grau and Almquist (62), Block and Bolling (63) and Almquist (1). By making use of these reported values it is possible to estimate whether or not a composite feed is deficient in methionine. In view of variable availability of methionine in these feedstuffs, however, it is necessary to check the adequacy of supplemented and unsupplemented diets by growth tests.

McGinnis *et al.* (64) have reported that chick growth on a diet containing sunflower seed oil meal was not improved by the addition of methionine. Soybean oil meal used in similar tests was benefited by methionine supplementation (0.1%). Petersen, Lampman, Bolin and Stamberg (65, 66) have demonstrated that methionine is the principal growth limiting deficiency in pea protein. The addition of 0.25% DL-methionine increased the growth response to all diets containing peas.

The supplementation of commercial broiler feeds bought on the open market with 0.1% DL-methionine added gave results which varied both with the brand of feed and the time the feed was procured (77). Some of the mixtures were not benefited by the addition of methionine. Others showed a definite response.

The maximum effect of methionine supplementation was shown with one of the feeds in both laboratory and field experiments. During a 4 week growing period in laboratory tests birds receiving 0.1% supplemental DL-methionine grew ten per cent faster and compared to the controls, consumed only 87% as much feed per unit gain in weight.

In tests, run over a ten week period, using a broiler flock with one thousand chickens per group, the addition of 0.1% DL-methionine to the feed produced an eight per cent increase in the rate of growth. These birds, however, consumed only 80% as much feed per pound gain in weight as a control group fed unsupplemented diet.

Taylor and Russell (67) found that feeding DL-methionine to molting hens neither shortened the molting period nor increased the rate of egg production after the molt. The basal diet used in this experiment was not reported. Bethke *et al.* (68) have found that the hatchability of eggs from hens maintained on a vegetable protein diet was not improved by the addition of DL-methionine, choline or both. Based upon the comparative analysis of amino acids in eggs and in skeletal muscle of chickens, Munks, *et al.* (69) have suggested that the amino acid requirement for growth differs from the requirements for egg production. Ringrose and Davis (70) reported that the choline content of egg yolks did not vary in proportion to the choline or methionine content of the diet. Their conclusions are in agreement with earlier observations by Lucas, Norris and Heuser (71) that substantial amounts of choline are synthesized by the laying hen under conditions of low choline and low methionine intake.

The additon of 0.2% DL-methionine to the diet of turkeys produced a significant stimulus to growth in two of the three experiments tried. Supplements of choline chloride or calcium pantothenate did not improve the nutritional adequacy of the basal-vegetable-protein diets used (Bird, Marsden, and Kellog, 72).

The Effect of Supplemental Methionine in the Nutrition of Farm Animals

Shaw (73) has reported that DL-methionine administered either orally or intravenously to dairy cattle was ineffective in the treatment of ketosis.

Loosli and Harris (74) found that the addition of methionine to the diet of sheep increases the rate of gain and nitrogen retention. Further experiments at Cornell by Lofgreen, Loosli and Maynard (75) showed that the addition of 0.2% DL-methionine to a ration in which 40% of the nitrogen is supplied by urea increased the proportion of dietary nitrogen retained by sheep.

The addition of 0.3% of either cystine or methionine to a raw soybean ration has been reported to increase the rate of growth of pigs (75) Ferrin, (76) however, has reported that the addition of 0.1% DL-methionine to rations containing heated, extraction soybean oil meal, corn, whey, alfalfa meal, irradiated yeast and minerals did not change the rate of growth or feed consumption of swine. Furthermore, the addition of methionine caused scouring within 6 or 7 days. Ferrin considers the scouring to result from an upset of the intestinal flora following the addition of methionine to the ration because it was arrested by the oral administration of sulphathaladine.

NOTES

NOTES

NOTES

This review is published with the desire to further the study of the known and unexplored problems involving amino acids. Such studies hold great future promise for the improvement of human and animal nutrition and therapy

THE DOW CHEMICAL COMPANY

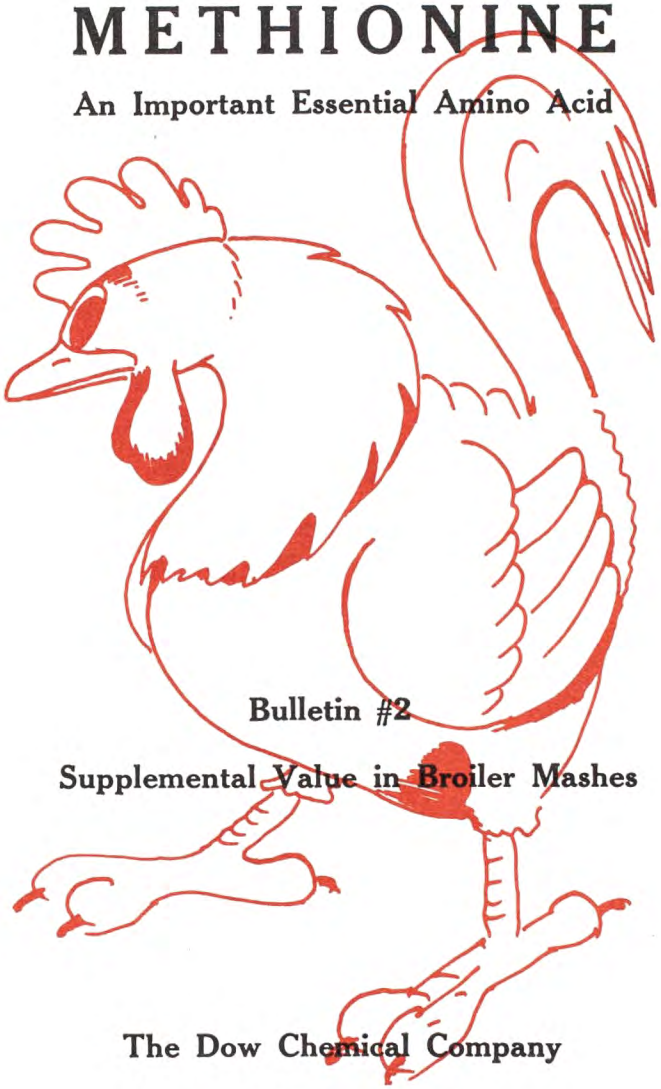
MIDLAND, MICHIGAN

LITHO IN U.S.A.

SP8 N 249

METHIONINE

An Important Essential Amino Acid



Bulletin #2

Supplemental Value in Broiler Mashes

The Dow Chemical Company

Midland, Michigan

Methionine, an essential amino acid, is a required component of poultry feed. This requirement cannot be replaced by APF, Vitamin B_{12}, antibiotics or any other presently known feed supplement. A Methionine deficiency results in reduced growth, lower feed efficiency and consequently less profit to the feed user.

Recent evidence proves the following:

1. Supplemental Methionine helps feed proteins function more effectively.
2. Improved feed performance pays for the Methionine and pays increased profit to the user.
3. Methionine is now effectively used at one-half the percentage (.05% to 0.1%) previously thought necessary (.15% to .3%).
4. Methionine response on addition to feed is usually enhanced by small amounts (1.0% to 2%) of dried whey, fish meal (1% to 2%) or meat scrap (2% to 4%).
5. The addition of Methionine to broiler feed, depending heavily on vegetable proteins, results in significant net profit to the feed user.

Table 1 shows the result of adding dl-Methionine to a practical ration in a battery experiment. From these results it is apparent that growth and feeding efficiency were definitely improved by the addition of Methionine either with condensed fish solubles or aureomycin APF supplements.

TABLE 1

Basal Diet

Component	Per 100 lbs.	Per Ton
Ground yellow corn	58.05 lbs.	1161 lbs.
Pulverized Oats	5.0 lbs.	100 lbs.
A & D Oil (3000A-400D)	0.2 lbs.	4 lbs.
Iodized Salt	0.5 lbs.	10 lbs.
Oyster Shell Flour	1.5 lbs.	30 lbs.
Steamed Bone Meal	3.0 lbs.	60 lbs.
Alfalfa Leaf Meal	3.0 lbs.	60 lbs.
Soybean Oil Meal (Solvent 44% Protein)	26.0 lbs.	520 lbs.
Vitamin B_{12} Supplement	0.05 lbs.	1 lb.
Dried Whey	2.0 lbs.	40 lbs.
BY-500 (Riboflavin Supplement)	0.5 lbs.	10 lbs.
Choline Chloride (25% Dry)	0.2 lbs.	4 lbs.
Manganous Sulfate	7.0 gm.	140 gm.
Niacin	1.0 gm.	20 gm.
Calcium Pantothenate	400 mg.	8 gm.
Potassium Iodide	70 mg.	1.4 gm.

VARIABLE COMPONENTS - %	A	B	C	D
Condensed fish solubles	1.0	1.0	---	---
Aureomycin APF	---	---	0.25	0.25
dl-Methionine	---	0.1	---	0.1
AVERAGE WEIGHT - 8 WEEKS				
Both Sexes	907	1046	925	1069
Males	979	1175	1000	1155
Females	835	917	850	983
FEED TO GAIN RATIO 0-8 WEEKS	2.96	2.65	2.73	2.57

Variable Components added at the expense of corn.
New Hampshire Red - ten males and ten females per group - raised in batteries.
Basal Diet - 19.3% protein.

Choline Chloride was used to fortify the above diet to rule out any question of Methionine response due to labile methyl groups. Vitamin B_{12} was added to assure no deficiency of this factor known to have a sparing effect on Methionine.

Other tests indicate that the magnitude of Methionine response may be influenced by the type of APF, the type of soybean meal, and the type of whey, but in practically all cases similar tests have given comparable results both with 0.1% or 0.05% added Methionine.

The availability of Vitamin B_{12} and antibiotic supplements has now made it possible to throw heavy or complete reliance on vegetable proteins. In spite of this, however, meat and fish proteins will continue to be used in feeds when availability dictates sufficiently low price. Tests were therefore conducted to measure the effect of supplemental Methionine in diets of four basic types: corn-soy, corn-soy-meat, corn-soy-fish, and corn-soy-meat-fish combinations. The compositions are shown in Table 2. Methionine (0.05%) improved the feeding efficiency in every type of ration. Growth improvement also resulted in all groups except the one which received fish meal. Hot weather during the course of this experiment restricted somewhat the growth of all groups.

Field trials were conducted comparing the performance of Methionine supplemented diets containing minimal amounts of meat or fish protein with that of standard high energy broiler feeds. In each case Methionine made it possible to lower substantially the animal and fish protein, and yet attain dietary performance superior to rations containing relatively large amounts of meat and fish proteins.

TABLE 2

Basal Diet

Component	Per 100 Lbs.	Per Ton
Ground yellow corn	57.8 lbs.	1156 lbs.
Pulverized Oats	5.0 lbs.	100 lbs.
A & D Oil (3000A-400D)	0.2 lbs.	4 lbs.
Iodized Salt	0.5 lbs.	10 lbs.
Oyster Shell Flour	1.5 lbs.	30 lbs.
Steamed Bone Meal	2.5 lbs.	50 lbs.
Alfalfa Leaf Meal	3.0 lbs.	60 lbs.
Dried Whey	1.0 lbs.	20 lbs.
Soybean Oil Meal	22.0 lbs.	440 lbs.
Aureomycin APF	0.3 lbs.	6 lbs.
BY-500 (Riboflavin Supplement)	0.5 lbs.	10 lbs.
Choline Chloride (25% Dry)	0.2 lbs.	4 lbs.
Manganous Sulfate	7.0 gm.	140 gm.
Niacin	1.0 gm.	20 gm.
Calcium Pantothenate	400 mg.	8 gm.
Potassium Iodide	70 mg.	1.4 gm.

VARIABLE COMPONENTS* - %	A	B	C	D	E	F	G	H
Soybean meal	4.0	4.0	---	---	---	---	---	---
Meat and bone scrap	---	---	4.0	4.0	---	---	2.0	2.0
Fish meal (70% protein)	---	---	---	---	3.0	3.0	1.5	1.5
Steamed bone meal	1.5	1.5	---	---	1.0	1.0	0.5	0.5
dl-Methionine	---	0.05	---	0.05	---	0.05	---	0.05
AVERAGE WEIGHT - 8 WEEKS								
Both Sexes	899	937	822	918	964	956	922	978
Males	953	998	921	1030	1095	1068	1048	1096
Females	844	876	722	805	832	844	795	860
FEED TO GAIN RATIO	3.40	3.20	3.63	3.20	3.50	3.16	3.26	3.00

Barred Rock - New Hampshire Cross, 114 Birds per group mixed sexes - Servall litter.
*Corn adjusted to total 100 pounds.
Protein - 19.4%.

The object of these tests was to show that Methionine-supplemented feeds pay increased profits to the user.

In the first trial New Hampshire red chicks were raised on a radiant-heated floor (Servall litter). One group was fed high energy commercial broiler mash and the other group was fed a diet (Table 3) containing 1.5 pounds of added Methionine per ton (0.075%). The results show that it is profitable to use Methionine to supplement feeds containing primarily vegetable protein. (See results Table 3).

The trial listed in Table 4 was a commercial fryer operation. The control feed contained high levels of fish and meat protein (9.1%) fish meal: (4.5% meat scrap). The experimental ration contained supplemental Methionine (1 pound per ton), less fish and meat (2.2% each), and more soybean meal. Both rations contained anti-biotic feed supplement. The calculated intake of essential nutrients was approximately equal in these diets. The Methionine supplemented diet showed obvious advantage in feeding efficiency, dressed quality and increased profit. This experiment involved approximately 100 tons of feed which was only a small percentage of the operators' feed manufacture. Feed was mixed at weekly intervals. The continual change in ingredients both as to source, type, and quality was comparable to commercial practice. Methionine was the only ingredient calculated to be potentially limiting, so that variation in the amount and availability of the natural Methionine from the feed ingredients accounts primarily for the difference in the two rations. The supplemental dl-Methionine was all available in the experimental ration and gave insurance against a marginal deficiency.

TABLE 3

Component	Per 100 Lbs.	Per Ton
Ground yellow corn	49.2 lbs.	984 lbs.
Pulverized Oats	10.0 lbs.	200 lbs.
A & D Oil (3000A-400D)	0.2 lbs.	4 lbs.
Iodized Salt	0.5 lbs.	10 lbs.
Oyster Shell Flour	1.3 lbs.	26 lbs.
Defluorinated calcium phosphate	2.5 lbs.	50 lbs.
Alfalfa Leaf Meal	2.0 lbs.	40 lbs.
Corn Gluten Meal	2.5 lbs.	50 lbs.
Soybean Oil Meal (Solvent 44% protein)	25.0 lbs.	500 lbs.
Meat and Bone Scrap (50% protein)	4.0 lbs.	80 lbs.
Condensed Fish Solubles	1.0 lbs.	20 lbs.
Dried Whey	1.0 lbs.	20 lbs.
BY-500 (Riboflavin supplement)	0.5 lbs.	10 lbs.
Choline Chloride (25% Dry)	0.2 lbs.	4 lbs.
Manganous Sulfate	7.0 gm.	140 gm.
Niacin	1.0 gm.	20 gm.
Calcium Pantothenate	400 mg.	8 gm.
Potassium Iodide	70 mg.	1.4 gm.
dl-Methionine	0.075 lbs.	1.5 lbs.

	TEST RATION	HIGH ENERGY COMMERCIAL RATION
PER CENT PROTEIN	21	22
AVERAGE WEIGHT IN POUNDS* (BOTH SEXES)		
4 weeks	0.78	0.84
6 weeks	1.42	1.32
8 weeks	2.26	1.99
10 weeks	3.14	2.88
Females	2.87	2.57
Males	3.41	3.18
POUNDS FEED PER POUND LIVE WEIGHT	3.15	3.23
PROFIT OVER FEED COST	$470	$420
NET PROFIT INCREASE (Supplemental over Commercial)	$ 50	
NET PROFIT INCREASE PER BIRD	5.7 cents	

*876 birds per group, mixed sexes, New Hampshire Red, Servall litter, radiant floor heat.

TABLE 4

EXPERIMENTAL AND CONTROL DIETS

Ingredients	Pounds Per Ton	
	Control	Experimental
Alfalfa leaf meal	100	100
Fish meal	200	50
Meat and bone scrap	100	50
Soybean oil meal-expeller	150	550
Corn	1300	1100
Milo	325	270
Bone-phos	6.5	45
Manganese sulfate	0.5	0.5
Fortafeed 2-22C	3	3
Salt	5	11
Dry D	0.5	0.5
Antibiotic Feed Supplement	3	3
25% Choline Chloride (Dry)	3	4
dl-Methionine	---	1.1
Total Weight - Approx.	2200	2200
% Protein	20	20

CONSOLIDATED RESULTS OF EXPERIMENT

Item	Control	Experimental
Number birds*	10,939	8,118
Live weight of birds marketed	37,360	28,000
Average live weight at market	3.42	3.45
Pounds feed per pound gain	3.49	3.12
Pounds feed consumed per bird	11.94	10.76
% Grade A - New York dressed	68.7	79.3

Net Profit Increase per 1000 birds based on efficiency only -

1-23-50 feed prices	$40.03
6-1-50 feed prices	$23.03

Net Profit Increase per 1000 birds considering all factors (Efficiency, dressed quality, etc.)

1-23-50 feed prices	$77.90
6-1-50 feed prices	$59.40

Net Profit Increase per bird

1-23-50 feed prices	7.79 cents
6-1-50 feed prices	5.94 cents

*Mixed groups - New Hampshire and Cornish New Hampshire Cross, built up litter, radiant heat.

IT IS EVIDENT THAT LOW LEVELS OF SUPPLEMENTAL METHIONINE IN THE PRESENCE OF VITAMIN B_{12} AND ANTIBIOTIC SUPPLEMENT WILL IMPROVE THE QUALITY AND PERFORMANCE OF FEED. SMALL QUANTITIES OF EITHER DRIED WHEY, FISH MEAL, OR MEAT SCRAPS MAY FURTHER IMPROVE THE BENEFITS FROM METHIONINE SUPPLEMENTATION.

Improved feed performance means increased profit to the grower. The following method of translating experimental results into a measure of profit has proven very useful in determining the value of Methionine supplementation.

The profit over feed cost per 1,000 birds is calculated for each diet (similar except for Methionine supplementation) in the manner tabulated below. This method will definitely show which diet is most profitable because the factors of feed efficiency, growth, and feed cost are all taken into consideration.

DIET	AGE DAYS	NO. BIRDS	FEED PER BIRD LBS.	AV. WT. LBS.	*MEAT VALUE PER 1000 BIRDS	**FEED COST PER 1000 BIRDS	PROFIT OVER FEED COST	INCREASED PROFIT RESULTING FROM METHIONINE
Non-supplemented	70	40	9.393	3.037	$911	$376	$535	----
Supplemented (0.05%)	70	40	9.803	3.297	$989	$407	$582	$47

* A price of $.30 per pound is used in figuring meat value in all diets.
**A price of $4.00 per hundred is used in figuring feed costs of non-supplemented diets. Supplemented diets would then be $4.15 per hundred when 0.05% Methionine (one pound per ton) is used.

The column "Increased Profit Resulting From Methionine" shows the value of Methionine supplementation in dollars and cents. It should be emphasized that this is INCREASED profit after taking into account all of the feed expenses including the Methionine supplementation.

Table 5 is a tabulation of a random series of experiments in which the value of Methionine supplementation was determined by the above method. This table includes good, fair, and poor results and includes battery tests, pen tests, and field trials. THERE IS NO QUESTION ABOUT THE TREND OF INCREASED PROFIT TO THE USER WHERE METHIONINE HAS BEEN INCLUDED IN THE DIET.

If you manufacture broiler feed, we strongly recommend that you investigate for yourself, under practical broiler raising conditions, the increased value of feeds containing supplemental Methionine.

TABLE 5

PROFIT OVER FEED COST PER 1,000 BIRDS - TYPICAL OF ALL EXPERIMENTS

(Profit Figures in Dollars)

% Methionine	8 Weeks			10 Weeks		
	Profit over Feed Cost		Increased Profit Resulting From Methionine	Profit over Feed Cost		Increased Profit Resulting From Methionine
	Control	Supplemented		Control	Supplemented	
.05	406	421	15	466	474	8
.05	361	457	96	398	495	97
.05	340	361	21	410	415	5
.05	291	358	67	352	400	48
.05	360	381	21	423	449	26
.05	360	391	31	414	419	5
.05	410	418	8	543	555	12
.05	393	420	27	544	559	15
.05	391	404	13	515	538	23
.05	451	462	11	607	600	- 7
.05	404	443	39	535	582	47
.05	452	464	12	594	600	6
.05	455	474	19	---	---	--
.05	---	---	--	548	589	41
.075	---	---	---	494	527	33
.1	406	454	48	466	513	47
.1	361	404	43	398	456	58
.1	443	433	-10	---	---	--
.1	402	460	58	---	---	--
.1	413	420	7	---	---	--
.1	376	440	64	---	---	--
.1	451	464	13	607	593	-14
.1	452	453	1	594	590	- 4
			Average 28			Average 25
			Average Per Bird 2.8 cents			Average Per Bird 2.5 cents

Printed in U.S.A.

Form No. IN214-W-1150 R