THE METABOLISM OF CYSTINE AND METHIONINE

THE AVAILABILITY OF METHIONINE IN SUPPLEMENTING A DIET DEFICIENT IN CYSTINE*

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Preformed protein, or its hydrolysis products, is one of the inevitable needs of the more complexly organized animal body. The particular metabolic requirement for each individual amino acid yielded by protein is, therefore, a matter of outstanding importance. Although it has been clearly recognized for some 20 years that amino acids might well differ in their degrees of nutritional significance, knowledge in this field is still incomplete. The literature affords only a few examples of protein units which have been repeatedly demonstrated to be essential. That the animal's synthetic powers with respect to even these substances are not absolutely limited is shown by the fact that in specific instances they may be replaced in the diet by the corresponding lactic and pyruvic acids. The apparent dispensability of one amino acid may, moreover, depend on the capacity of another or others to play the rôle of substitute. Such has been thought to be the case with glycine. Metabolic interchange of this type may well explain some of the difficulty encountered in attempts to formulate diets

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† Rose (1931-32) has presented a critical review of the amino acid literature giving the current status of the best facts with regard to the essential or non-essential nature of the known amino acids. Rose and his associates have shown that the known amino acids including methionine are not sufficient to support growth; and that at least one other indispensable factor, apparently an amino acid, is yet to be discovered.
deficient in protein components of more intricate constitution. In fact, there have been reported several studies designed to test such a conceivable physiological connection between amino acids of obviously similar chemical structure, as for example, between tyrosine and phenylalanine.

Cystine (with cysteine) long enjoyed a particular significance by virtue of its being the only naturally occurring amino acid known to contain sulfur and the only apparent major source of reduced sulfur for the body. The discovery of methionine by Mueller in 1923, however, introduced a new aspect to the situation. Methionine, like other amino acids derived from protein, is catabolized in the animal body (Mueller, 1923–24), but there has hitherto not been any basis for predicting a common metabolic behavior for the two sulfur-containing substances. In fact, inspection of the two formulas (cystine, \((-\text{S} \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{NH}_2 \cdot \text{COOH})_2\), and methionine, \(\text{CH}_3 \cdot \text{S} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{NH}_2 \cdot \text{COOH}\)) reveals a lack of striking structural similarity. However, in view of the emphasis which many writers have placed on the presence of sulfur in cystine, the mere fact that methionine is the only known additional sulfur-bearing amino acid thus far isolated from proteins suggests the importance of ascertaining its physiological rôle experimentally. We are not familiar with any previous investigation of the metabolic interrelation of cystine and methionine. These considerations led us to study the effect of administering methionine to animals restricted in growth through the limitation of the cystine intake.

**EXPERIMENTAL**

Male rats were employed in this study. When the animals reached a body weight of 60 to 75 gm. each, they were confined singly in cages equipped with false bottoms and the ration was changed from the stock food mixture to one deficient in cystine.

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2 Sherman and Woods (1925) in commenting upon their biological method for the assay of cystine in casein refer to “cystine (or cystine plus nutritionally equivalent sulfur-containing radicles).” Again, Rimington and Bekker (1932) state, “Evans concludes, as have others, that there must be present in the diet [of sheep] some substance closely related to cystine from which the latter can be synthesized.” Neither Sherman and Woods nor Rimington and Bekker, however, suggest a metabolic relationship between methionine and cystine.
Basal Diet—Different deficient diets fashioned after those of Osborne and Mendel (1915), Sherman and Merrill (1925), Lewis and Lewis (1926), and Rose and Huddlestun (1926) were tried with the purpose of securing a basal regimen which would within reasonable limits just maintain body weight. The lentil food described by Jones and Murphy (1924) was found to permit much too rapid growth. The formula of the basal diet (Diet B) finally selected was as follows: whole milk powder 15, gelatin 2, salt mixture (Osborne and Mendel, 1919) 1, sodium chloride 1.7, cornstarch 54.7, a dry Lloyd’s reagent-vitamin B adsorbate preparation 3 0.6, and lard 25 per cent. These ingredients were thoroughly worked into a doughy mixture which could be dispensed conveniently and quantitatively. This diet and its supplemented forms were, with one exception to be discussed later, fed ad libitum.

Each rat was supplied daily with a supplement of 100 mg. of cod liver oil and 125 mg. of dried yeast. There is no doubt that 100 mg. of an average cod liver oil furnishes an abundance of vitamins A and D for an albino rat. But there is some question as to whether the daily intake of whole milk powder, yeast, and vitamin B adsorbate would meet the animal’s nutritional demands for vitamins B and G and other accessory water-soluble substances throughout an indefinitely prolonged experiment. However, vitiation of our experiments on this or similar account was precluded by the strict control standards imposed on every animal.

The supplementary feeding experiments were not undertaken until the animals on the cystine-poor diet had attained a condition of equilibrium with respect to body weight change. This usually occurred in the course of about 4 weeks. The chief difficulty encountered was that of too rapid growth; and for this reason approximately 50 per cent of all the animals originally placed upon the basal diet was discarded. Variations which different animals thus exhibit even when they are ingesting the same ration are believed to be due to inherent individual differences in appetite or food utilization efficiency. These factors are undoubtedly fairly constant, however, in a given animal restricted in growth in consequence of a constantly operating dietary amino acid deficiency. This point is borne out by the repeated observation that following

3 This concentrate was kindly furnished by Eli Lilly and Company of Indianapolis.
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an animal's initial adjustment to the diet, the food consumption and the body weight change are rather constant over long periods. Accordingly, animals with fairly stationary body weights were selected for the metabolism tests in order that any alteration in the growth rate would be convincingly manifested.

Every experiment was controlled by the behavior of the animal on the basal diet and on the basal diet supplemented with cystine or other growth-stimulating substance. Our experience led us to the conclusion that the general method outlined here, namely that of studying successive periods of feeding on the same animal rather than simply making comparisons of different animals, is the more reliable unless one resorts to the use of large numbers of animals and prolonged feeding periods. However, the growth of our test rats was at all times controlled by the behavior of other animals simultaneously subsisting on the basal diet alone. Thus various general laboratory factors were shown not significantly to affect the outcome of any experiment.

Amino Acid Preparations—The cystine, methionine, serine, alanine, aspartic acid, tryptophane, hydroxyproline, histidine monochloride, glycine, and phenylalanine specimens employed were colorless and characteristically crystalline. Their purity was established by nitrogen determinations. The cystine, aspartic acid, tryptophane, hydroxyproline, and histidine were all the natural optically active forms. The remaining amino acids were racemic preparations. The methionine was synthesized according to the directions of Windus and Marvel (1930). These compounds prior to feeding were thoroughly mixed into the basal diet. 120 mg. were selected as the equivalent of cystine to be used as a supplement for 100 gm. of the basal diet. The other substances, unless otherwise specified, were employed in the amount of 2 or

4 The methionine employed in this research was prepared collaboratively by H. M. Vars and R. W. Jackson, with the purpose of making methionine available for experiments in which they were respectively interested. The authors wish to express their appreciation to Dr. Vars for his cooperation in this arrangement which in a large measure made possible the metabolism experiments. Dr. Marvel, of the University of Illinois, kindly supplemented the original directions for this synthesis with certain modifications (to appear in the "Organic syntheses"). It may be stated that the method of Windus and Marvel proceeds smoothly and may be used to prepare any desired quantity of methionine from readily available starting materials.
4 molecular equivalents: 298 (2 equivalents) and 596 mg. (4 equivalents) of methionine, 420 mg. (4 equivalents) of serine, and 356 mg. (4 equivalents) of alanine, respectively, per 100 gm. of food.

**DISCUSSION**

The administration of methionine to animals subjected to the experimental regimen described above led to unmistakable increments in body weight. This is indicated in Charts I to IV. Attention is directed to the behavior of Rats 65 and 80 (Chart I) following the addition of methionine to the diet. The first of these gained 59 gm. in 7 weeks as contrasted to a previous body weight increase of 2 gm. in 4 weeks. The second, which was originally gradually losing weight, began to grow at quite an appreciable rate. Eight other animals exhibited similar responses to methionine. It may further be pointed out that animals growing in response to a dietary accession of methionine or cystine ceased to grow when deprived of these supplements, as illustrated in the growth curves for Rats 51 and 75 (Chart I). A summary of the effect of methionine ingestion upon the growth of a group of ten rats is presented in Chart IV. The average weight increment during the 2 weeks following methionine administration was 20 times greater than the mean increase preceding the inclusion of this amino acid in the diet.

It appears to be an established fact that methionine, like cystine, supplements the type of diet employed in these studies—a diet ordinarily termed cystine-deficient. This discovery originally reported in *Science* (Jackson and Block, 1931) has now been confirmed by Weichselbaum, Weichselbaum, and Stewart (1932). Their announcement states that the addition of methionine to the Sherman and Merrill (1925) diet fed throughout in constant daily portions caused approximate doubling of the growth rate of their rats. That the limitation of the food intake during the period of methionine administration did not prevent growth stimulation is in agreement with our results of a similar experiment discussed below.

**Dietary Specificity of Methionine and Cystine**—Inasmuch as the protein fraction of the basal diet was relatively small (6 to 7 percent of the total weight), it was thought that perhaps the reaction to methionine might be due simply to an increase in the amount of any kind of usable nitrogen ingested at a given caloric intake. In
Chart I. Growth on methionine and alanine. Rats 80, 65, and 51: growth on the basal cystine-deficient Diet B (B) and on Diet B with 2 equivalents of methionine (2M); in the case of Rat 51, growth finally on Diet B (B). Rat 75: growth on Diet B (B); on Diet B with 4 equivalents of alanine (4A); on Diet B (B); on Diet B with 2 equivalents of methionine (2M) and finally on Diet B (B).

The diet employed during any part of an experiment is indicated on the graph by letter and number notation (see text) with a downward arrow at the point representing the beginning of the period. The average daily food consumption in gm. for the corresponding interval is shown by figures inserted between arrows constructed with broken lines. The slants of the broken lines possess no significance. The initial and final body weights in gm. are printed in parentheses.
CHART II. Growth on methionine, serine, and cystine. Rats 52 and 58: growth on Diet B (B) and on Diet B with 4 equivalents of methionine (4M). Rat 73: growth on Diet B (B); Diet B with 4 equivalents of serine (4S); on Diet B; and finally on Diet B with 1 equivalent of cystine (C). Rat 71: dietary changes similar to those for Rat 73. The designations for diet employed, food consumption, and body weights are the same as in Chart I.
CHART III. Growth on a supplement of eight amino acids without cystine and methionine, on methionine, and on methionine with restricted food intake. Rats 101 and 102: growth on Diet B (B); on Diet B with a supplement of eight pure amino acids (A-A-8, see text) and finally on Diet B with 2 equivalents of methionine (2M). Rats 106 and 107: growth on Diet B (B) and on Diet B with 2 equivalents of methionine (2M), the food intake being restricted to those amounts which the two animals had respectively previously consumed of the basal diet (Diet B) alone. The designations for diet employed, food consumption, and body weights are the same as in Chart I.
other words, any one of the physiologically natural \( \alpha \)-amino acids might cause the same effect. Accordingly, tests were made with serine and alanine which are similar to cystine not only in being derivatives of propionic acid but also in giving rise to glucose in the diabetic organism. The growth curves of Rats 71, 73, and 75 demonstrate that these two substances produce no significant change in the animals' weights, although the subsequent administration of cystine or methionine caused immediate and continued body weight gain.

An additional control experiment of the same type was carried out with a supplement of eight different amino acids selected from those which according to published analyses appear to constitute the lowest percentages of casein. They were each added in 0.5 molecular equivalents related to the standard of 120 mg. of cystine (1 equivalent) employed for supplementing 100 gm. of basal diet. The amino acids and the quantities used were as follows: glycine 38, alanine 45, hydroxyproline 66, phenylalanine 83, aspartic acid 67, serine 53, histidine monochloride 105, and tryptophane 102 mg. per 100 gm. of the basal diet. This combined supplement, like that of the alanine or of the serine, was without any continued appreciable effect on growth (see Rats 101 and 102, Chart III). The conclusion from these experiments is that cystine and meth-

**CHART IV.** Comparison of the average growth in gm. of 10 rats (Rats 80, 65, 51, 75, 52, 58, 101, 102, 106, and 107) for 2 week periods immediately preceding and immediately following methionine administration.
ionine alone among a wide variety of amino acids cause a distinctly specific growth response when fed as a supplement to the basal diet under consideration.

Controlled Food Consumption—Certain aspects of the problem of the relation of food consumption to growth have been reviewed in a previous communication (Jackson, 1929; cf. Berg and Potgieter, 1931–32). Prolonged growth without adequate food consumption of the tryptophane-deficient diet then employed is apparently impossible. Hence, food was given ad libitum in the first of the present experiments in order that possible body weight gains of unquestionable magnitude might be registered. It will be observed from Charts I, II, and III that animals ingesting food ad libitum in every case consumed greater quantities of the diets supplemented with either methionine or cystine. Thus the relief of the deficiency under present discussion, like the amelioration of most, if not all, other nutritional deficiencies, results in an increased consumption of food by the large majority of animals.

To determine what effect the restriction of the intake of the methionine-supplemented diet might have upon growth, an experiment was performed with two animals, Rats 106 and 107 (Chart III). The food consumption was measured for 8 weeks in the ordinary way by means of spring scales calibrated in gm., and for the last 2 of the 8 weeks also by means of laboratory trip scales accurate within 0.2 gm. The latter measurement involving a total over-all possible error of not more than 5 per cent was used as a basis for restricting the food intake of the methionine diet for the next 4 weeks. During this time, the diet was weighed for a 4 day period and then divided into the four equal daily portions.

It is obvious that restriction of the food consumption in this instance does not prohibit methionine from exerting a marked growth-accelerating effect. The response is somewhat greater and more prolonged than in the aforementioned tryptophane experiments perhaps for the reason that the cystine-deficient animals were already growing at a somewhat greater rate than were the ones deficient in tryptophane. These findings constitute additional independent evidence in support of the general principle that growth stimulated in such instances with or without increased food consumption is not, as was vigorously contended to be possible by Mitchell (1927), the result of a direct condimental
influence upon appetite, but rather as Rose (1928) held, the indirect register of increased chemical activity in the cells brought about by enlarging the supply of an indispensable constituent heretofore available in amounts too small for optimum cell activity.

Possible Interpretations—Without further experimental evidence, we hesitate to assume that these results represent an actual conversion of methionine to cystine, though the possibility of such a transformation seems clearly indicated. It should be emphasized that the animals acquired a small but definite intake of both cystine and methionine as well as a certain amount of as yet unidentified sulfur from the proteins of the whole milk powder and presumably also from the yeast. With the latter fact in mind, one may picture the various possibilities as follows: (1) That both cystine and methionine are indispensable; and that with the type of diet used either cystine or methionine supplementation permits growth of the kind depicted in the accompanying charts. Such a circumstance, however, seems unlikely in view of data described in the literature—for example, Osborne and Mendel's finding as to the tryptophane-lysine requirements of animals ingesting zein, indicating that an animal deficient in two necessary substances cannot grow substantially without a supplement of both missing factors. (2) That cystine and methionine make up a freely interconvertible system of which only one member is necessary. (3) That both amino acids are indispensable but each only to a limited degree, and that there is at least one metabolic function which can be cared for by either amino acid or by some common metabolite of the two. This metabolite might conceivably consist of a very small fragment, such as divalent sulfur of such an origin that it could be generated at the proper place and time. (4) That only one of the amino acids, say cystine, is indispensable to a limited degree and that an adequate supplement of the other, in this instance, methionine, may suffice for a common function similar to that indicated under item (3).

The various major and subordinate functions which cystine may serve in the body have been dealt with by various writers (cf., e.g., Garrod, 1923; Lightbody and Lewis, 1929; and Abderhalden and Wertheimer, 1931). Apparently cystine is a precursor or one of the precursors of glutathione, taurine, certain detoxication products, etc., and of insulin, keratin, and other important proteins of the body.
The foregoing hypotheses are all premised on the condition that the metabolic relation of cystine and methionine is circumscribed within the body. Yet it has been clearly recognized by various writers that a substance administered orally is always subject to the action of alimentary bacteria. And indeed, if methionine can be synthesized into cystine in vivo, the lower forms of life with their almost unlimited capacity in general to bring about structural modifications of organic compounds might more readily be expected to achieve the methionine-cystine transformation. Rimington and Bekker (1932) have reviewed the literature bearing on the phenomenon of sheep being able to deposit cystine in their wool to the extent of several times the amount detectable in the pastureage ingested, and have raised the question as to the possible role of bacteria in the gut in converting inorganic sulfur to cystine. These authors, however, lay particular stress on the very favorable conditions for microbiotic activity in the intestinal tract of sheep as contrasted to those in the gut of omnivorous animals such as the rat.

Other speculative conceptions may be elaborated, but those outlined above will serve to show that the relation between the two amino acids may be complicated, and cannot be definitively ascertained from the experimental data submitted at this time. Further study is necessary to permit a precise interpretation of the facts at hand. The successful arrangement of a sulfur-free diet would make it possible to provide the answers to many of the questions to which attention has been directed. Another mode of attack is to study the excretion of bromophenylmercapturic acid following the administration of methionine. A definitely increased output of this product would constitute good evidence of a conversion of methionine to cystine. It will be interesting also to investigate the fate of methionine in the cystinuric individual. It is well known that the cystine output of cystinuric subjects is not increased by the administration of cystine itself but that it is augmented by the ingestion of protein. Is it possible that the cystine originating from protein in the cystinuric individual actually has its origin in the methionine present in the protein? A successful demonstration of such a relation would again be tantamount to proof that the body can convert methionine to cystine.

It is obvious, of course, that, since the addition of methionine to
the diet of animals subsisting on the regimen previously described leads to growth stimulation, the study of the physiological behavior under similar conditions of γ-methio-α-hydroxybutyric acid, other sulfur acids, and the separate optically active forms of methionine becomes important. We have already submitted a tentative report on the first of these problems (Block and Jackson, 1932).

SUMMARY

1. Methionine, like cystine, is capable of unmistakably stimulating growth in albino rats subsisting on a basal diet poor in cystine.
2. Other amino acids, including serine and alanine, are ineffective in this regard.
3. The restriction of the food intake to a constant amount throughout the experiment does not prevent growth acceleration as a result of methionine supplementation.
4. Various interpretations of the experimental findings have been discussed.

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