The Availability of Histidine Derivatives for Growth

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The purpose of the experiments to be described in this paper was to investigate whether the rat can synthesize the glyoxaline ring of histidine, if certain possible precursors are provided in the diet. It was first shown by Rose & Cox (1924) that a diet in which protein was supplied in the form of a casein digest freed of histidine by silver precipitation could not support growth in young rats. This was later confirmed with synthetic diets (Rose, 1938). On the other hand Burroughs, Burroughs & Mitchell (1940) claimed that histidine is not necessary for the maintenance of nitrogen equilibrium in the adult rat, and Rose, Haines, Johnson & Warner (1943) found that a positive nitrogen balance could be maintained in man in the absence of dietary histidine. It appeared to us that these various and to some extent contradictory findings could be explained by assuming that the mammalian organism is able to synthesize histidine from other aliphatic substances, but that the formation of these precursors is slow and is the rate-determining step in the biosynthesis of histidine. If this is the case the addition of such possible precursors to a histidine-deficient diet should lead to a stimulation of growth.

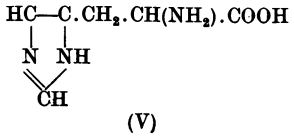
Little is known of the mechanism of the synthesis of iminazole compounds in plants, but by analogy with chemical methods it appeared possible that such a reaction could proceed from ornithine (I) through γ-hydroxy-ornithine to the keto-acid (III). The postulated introduction of a hydroxyl group into a position next to an amino group is a reaction which can presumably be performed by the organism, since both hydroxy-lysine and hydroxy-proline, which have this structure, are found in animal proteins and are not essential amino-acids; they must be presumed to be formed from lysine and proline by such a process of oxidation. The keto-acid (III) could then form histidine by any of the following routes: it could be oxidized to the dicarbonyl compound (IV) which could then condense with ammonia and formaldehyde to give histidine (V); such a reaction would be similar to the formation of 4(5)-methyl-glyoxaline from sugars (Windaus, 1907) which is assumed to involve the intermediate formation of methyl-glyoxal. The keto-acid (III) could also directly combine with formic acid and ammonia; for this there is no exact chemical analogy, but the Bamberger fission of iminazoles represents a reversal of such a reaction. Lastly, the acid (III) might form 2-thiol-histidine (VI) by condensation with thiocyanate, which is normally present in serum and saliva although in small amounts.

$H_2N(CH_2)_3.CH(NH_2)COOH$ (I)

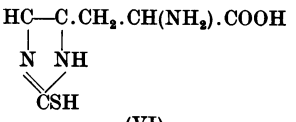
$H_2NCH_2.CHOH.CH_2.CH(NH_2).COOH$ (II)

$H_2N.CH_2.CO.CH_2.CH(NH_2).COOH$ (III)

$OHC.CO.CH_2.CH(NH_2).COOH$ (IV)



(V)



(VI)

It was expected that 2-thiol-histidine would easily be oxidized to histidine in the body. This paper describes experiments to test the ability of these two compounds, 2-thiol-histidine and $\alpha\delta$-diamino-γ-keto valeric acid to replace histidine in the diet. Opportunity was also taken to test the availability of α-*N*-acetyl and of α-*N*-benzoyl histidine for growth.

EXPERIMENTAL

Rats from one litter of black and white animals (National Institute for Medical Research strain) with weights of 46–51 g. were given the experimental diets. Weights were recorded every second day and food consumption was also measured.

Diet. The non-protein part of the basal diet was: cane sugar 40%, corn starch 41%, cod-liver oil in arachis oil (1 : 10) 14%, and salt mixture (*U.S. Pharmacopoeia*, 2, no. 2) 5%. The histidine-free amino-acid mixture had the following composition: glycine 6·0, *dl*-alanine 12·0, *dl*-

valine 28·0, *l*-leucine 20·0, *dl*-isoleucine 20·0, *dl*-serine 6·0, *dl*-threonine 16·0, *l*-proline 12·0, *l*-hydroxy-proline 4·0, *l*-cystine 5·0, *dl*-methionine 10·0, *l*-glutamic acid 44·0, *l*-aspartic acid 8·0, *l*-arginine hydrochloride 13·0, *l*-lysine hydrochloride 14·0, *l*-phenylalanine 8·0, *l*-tyrosine 13·0, *l*-tryptophane 4·0, and $NaHCO_3$ 16·0. Most of these amino-acids were prepared in this laboratory, but some were obtained from Merck and Company, Inc., Rahway, N.J., U.S.A. The purity of the amino-acids was checked by analysis, and where possible, by measurement of optical rotation. The amino-acid mixture was well mixed with any supplement to be tested and the whole ground up with the non-protein part of the diet and made into a stiff paste by the addition of a very small amount of water. Vitamin supplements were added in the same amounts as described in an earlier paper (Gillespie, Neuberger & Webster, 1945)

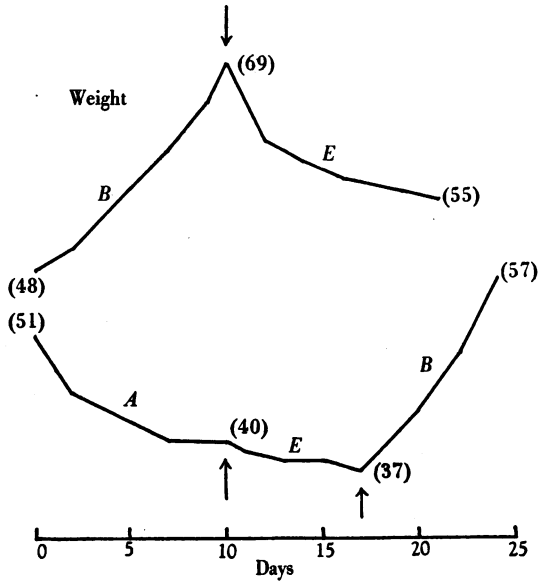


Fig. 1. The availability of 2-thiolhistidine for growth. The arrows indicate a change of diet. The weights of the animals at the beginning and end of each feeding period are shown in brackets. The diets fed to the animals were as follows: *A*, basal diet; *B*, basal diet + 35 mg. *l*-histidine/day; *E*, basal diet + 100 mg. 2-thiolhistidine/day.

except that the amount of choline chloride was increased to 12 mg./day. An amount of a specially prepared protein-free yeast extract (80 mg. (dry weight) equivalent to 0·5 g. of whole yeast) was also given. The intake of amino-acids was kept constant as far as possible, both during the deficiency and control periods, at 1·4 g./day, and variations in appetite were allowed for by adjustment of the intake of the non-protein part of the basal diet. On some days, however, the intake of amino-acids was slightly lower than 1·4 g., especially during the deficiency periods.

Preparation of compounds. *dl*-2-Thiolhistidine and αδ-diamino-γ-keto-valeric acid hydrochloride were prepared by the methods of Harington & Overhoff (1933), whilst for α-*N*-acetyl-*l*-histidine and α-*N*-benzoyl-*l*-histidine the directions of Bergmann & Zervas (1928) and Gerngross (1920), respectively, were followed.

RESULTS

Changes of weight on the basal diet

With histidine absent from the diet there was a loss of weight, amounting on the average to 1 g./day, at least for the comparatively short periods under consideration. The daily food consumption during the periods of deficiency averaged 4·5–5 g. The animals appeared to be quite healthy and lively.

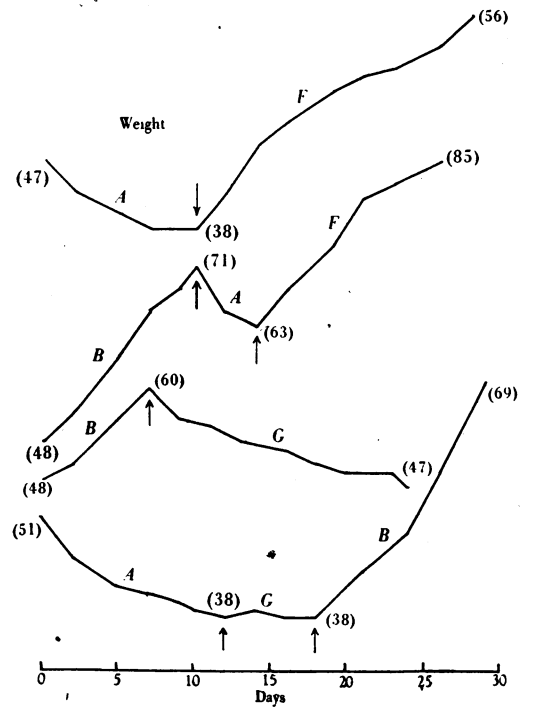


Fig. 2. The availability of *N*-acetyl and of *N*-benzoyl *l*-histidine for growth. The arrows indicate a change in diet. The weights of the animals at the beginning and end of each feeding period is shown in brackets. The diets fed to the animals were as follows: *A*, basal diet; *B*, basal diet + 35 mg. *l*-histidine/day; *F*, basal diet + 56 mg. *N*-acetyl-*l*-histidine/day; *G*, basal diet + 100 mg. *N*-benzoyl-*l*-histidine/day.

Changes of weight on the basal diet supplemented with histidine

An addition of 35 mg. *l*-histidine per day to the diet produces a considerable increase of body weight, the growth rate varying in different animals between 1·9–3·3 g./day, Figs. 1 and 2. It is not easy to compare the growth rates observed in experiments in which some amino-acids are given in the racemic form, with those found in which complete protein is used. If it is assumed, however, that the *d*-forms of the essential amino-acids used in these experiments are not available for growth, it can be calculated

that the amino-acid mixture consumed corresponds to a daily protein intake of 1·0 g. On this basis the average growth rate observed with a complete amino-acid mixture compared not unfavourably with results obtained with casein.

Addition of histidine to the basal diet produced an immediate and pronounced response both as regards change of body weight and increase in food consumption. Weight increased by 5–6 g. during the first 2 days after the addition of the amino-acid, and food consumption rose by about 100%. This quick and marked response facilitated greatly the testing of the ability of the various compounds to replace histidine in the diet.

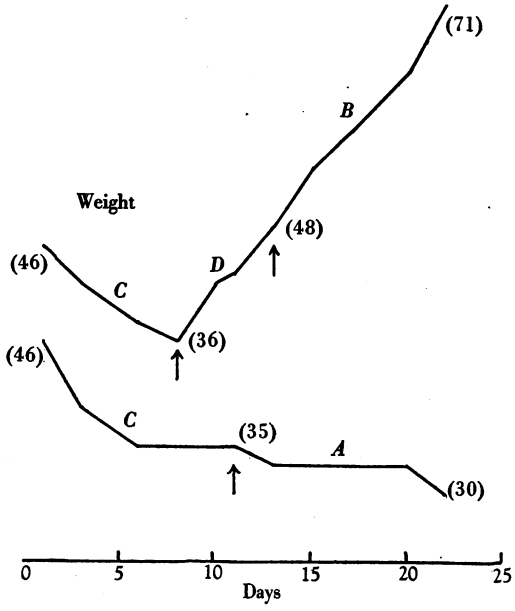


Fig. 3. The availability of αδ-diamino-γ-ketovaleric acid for growth. The arrows indicate a change of diet. The weights of the animals at the beginning and end of each feeding period are shown in brackets. The diets fed to the animals were as follows: *A*, basal diet; *B*, basal diet + 35 mg. *l*-histidine/day; *C*, basal diet + 100 mg. αδ-diamino-γ-ketovaleric acid/day; *D*, basal diet + 100 mg. αδ-diamino-γ-ketovaleric acid/day + 35 mg. *l*-histidine/day.

The availability of αδ-diamino-γ-ketovaleric acid for growth

The inability of this compound to replace histidine is clearly shown in Fig. 3. The average daily loss of weight in the two animals tested amounted to 1·1 and 1·25 g. respectively, and the daily food consumed averaged 4·1 and 4·5 g. respectively. The keto-acid was given as a hydrochloride in a daily dose of 100 mg., which corresponds to about 85 mg. of *dl*-histidine, or if we assume that only the *l*-form is available, to 43 mg. of *l*-histidine. The possibility of the substance being toxic can be excluded, since addition of histidine to a diet containing the keto-acid restored normal growth at once (Fig. 3). It is concluded therefore that αδ-diamino-γ-ketovaleric acid is not converted into histidine to any appreciable extent.

The availability of dl-2-*thiolhistidine for growth*

The results with this amino-acid were equally negative (Fig. 1). The daily dose given was 100 mg. which corresponds to about 83 mg. of *dl*- or to 42 mg. of *l*-histidine. Daily loss of weight in two experiments amounted to 0·4 and 1·5 g. respectively; the differences between these two values can be explained by the different initial weights of the two animals. Daily food consumption averaged 5·0 and 5·5 g. respectively. It is concluded that 2-thiolhistidine does not support growth of young rats kept on a diet free of histidine.

Availability of α-N-*acetyl and* α-N-*benzoyl*-l-*histidine for growth*

Fig. 2 shows clearly that whilst the acetyl compound replaces histidine, the benzoyl compound is inactive. The average daily weight gains of the two animals kept on a diet containing 50 mg. of acetylhistidine were 1·3 and 1·7 mg. respectively and the corresponding figures for average daily food consumption were 7·0 and 11·9 g. It follows that, at least at the level fed, the acetyl compound is as effective or nearly as effective as the amino-acid itself in supporting growth.

In the two experiments in which benzoylhistidine was given there was no increase of weight; in one experiment there was a loss of weight averaging 0·5 g./day, whilst in the other the body weight remained constant. The values for daily food consumption were similar to those found with the basal diet alone; they were 4·8 and 5·2 g. respectively. It is concluded that benzoyl-histidine cannot support growth on a histidine-deficient diet.

DISCUSSION

The results reported in this and two other papers (Glynn, Himsworth & Neuberger, 1945; Fuller, Neuberger & Webster, 1946) show that a diet supplying 1·3–1·4 g. of a mixture of amino-acids, some of which are racemic, can support growth in the rat at a rate of about 2 g./day for long periods. It has been possible to rear rats of initial weights of about 40 g. to full maturity on such diets and the final body weights observed after 100 days were between 230 and 250 g. This compares quite well with growth rates observed on diets containing 8% casein as a sole source of protein. At least at this comparatively low nitrogen intake amino-acid

mixtures are equal to whole protein and the *d*-isomers present in the diet have no apparent deleterious effect. At higher levels of intake, whole protein appears to be superior to amino-acid mixtures (unpublished observations); it is uncertain whether this difference is due to the presence of racemic forms in greater absolute concentrations in such nitrogen-rich diets or to the absence of specific growth factors, such as peptides (Woolley, 1945).

If histidine is left out of the amino-acid mixture there is an immediate and sustained loss of weight, and it is clear that histidine is required for the maintenance of body weight in the young rat. Similar findings have recently been reported by American workers (Albanese & Frankston, 1945; Maun, Cahill & Davis, 1946). These results suggest, but do not prove, that histidine cannot be synthesized at all by the rat. However, the fact that the loss of weight on the histidine-deficient diet found in these and other experiments (Fuller *et al.* 1946) is of the same order of magnitude as that found in deficiencies of essential amino-acids such as methionine, etc., renders very unlikely the assumption that histidine can be made by the rat from other constituents of the diet. The results reported in this paper show clearly that neither αδ-diamino-γ-ketovaleric acid nor 2-thiolhistidine can be converted to histidine by the rat. 2-Thiolhistidine is easily oxidized *in vitro* by ferric chloride to histidine; the observation that such an oxidation can apparently not take place *in vivo* is rather surprising. Ergothioneine does not replace histidine in the diet (Eagles & Cox, 1928), but this is not unexpected since other betaines cannot be converted into their parent amino-acids in the animal body (Jackson, 1929).

After the work described here had been completed a paper by Astwood, Bissell & Hughes (1945) reporting marked goitrogenic activity for 2-mercapto-iminazoles, came to our notice. Unfortunately, the thyroid glands of our animals had not been examined and we are therefore ignorant whether any changes in the thyroid may have occurred. The question, however, arises, whether the observed loss of weight, shown by the animals on the diet containing 2-thiolhistidine, could be ascribed to a goitrogenic or general toxic effect of the compound. Though this interpretation cannot be completely excluded, it appears unlikely. The sudden drop of weight which occurred as soon as histidine was replaced by the thiol derivative, and the immediate growth response on changing back to histidine, are most easily explained by assuming that we were dealing with a simple histidine deficiency. Moreover, the animals appeared to be quite healthy during the period when thiolhistidine was given.

The findings with respect to the two acyl derivatives bring histidine into line with most other amino-acids. In all cases so far tested the *N*-benzoyl derivatives do not support growth whilst the acetyl compounds excepting α-*N*-acetyl-lysine (Neuberger & Sanger, 1943) are fully active. The different behaviour of the α-acetyl derivatives of lysine and histidine may be attributed to the much greater basicity of the ε-amino-group of lysine as compared with that of the iminazole group of histidine. Thus at neutral pH the former exists mainly as a cation and may interfere with deacetylation by the appropriate enzyme, whilst the latter is mainly uncharged. It would be interesting to test this explanation by investigating the availability to birds of α-*N*-acetyl arginine which should resemble α-*N*-acetyl-lysine.

The findings reported by other workers and ourselves permit us to define the structural requirements of substances which are able to replace histidine in the diet, i.e. to be converted into this amino-acid by the organism. It appears that the glyoxaline ring must be present and not substituted in any other position than the 4 (5) position. Thus αδ-diamino-γ-ketovaleric acid, thiolhistidine and *l*-methylhistidine (Sakami & Wilson, 1944) are inactive. The alanine side chain, however, may be modified considerably without affecting activity. Thus the *d*-isomer (Conrad & Berg, 1936), β-iminazole α-hydroxy-propionic acid, the corresponding α-keto acid (Cox & Rose, 1926; Harrow & Sherwin, 1926), α-*N*-methylhistidine (Fishman & White, 1936) and the α-*N*-acetyl compound are all active. These derivatives can be converted into histidine by reactions known to occur with other compounds in the body. β-Iminazole-propionic acid and urocanic acid are, as might be expected, not convertible into histidine, and therefore not available for growth (Cox & Rose, 1926). α-*N*-benzoyl-histidine also belongs to this group.

SUMMARY

1. A diet which provided 1·3–1·4 g. of a mixture of 18 amino-acids, some of which were in the racemic form, produced in young rats a rate of growth which was similar to that found with a diet containing 8% casein. Omission of histidine led to loss of weight.

2. αδ-Diamino-γ-ketovaleric acid and 2-thiolhistidine did not support growth of rats kept on histidine-deficient diets. The inability of 2-thiolhistidine to replace histidine is considered not to be associated with the goitrogenic character of 2-mercapto-iminazoles and it is thought that neither substance can be converted to histidine by the rat.

3. Addition of α-*N*-acetylhistidine to a deficient

diet produced normal growth, whilst *N*-benzoylhistidine was inactive. The structural factors determining the ability of histidine derivatives to replace the amino-acid in the diet are considered.

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The Identification of Amino-acids Derived from Cystine in Chemically Modified Wool

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Consden, Gordon & Martin (1944) showed that all the naturally occurring amino-acids could readily be identified by means of partition chromatography on paper. Using the same methods it is shown in the present work that a number of amino-acids derived from cystine can also be identified as components of hydrolysates of wools which have been modified in various ways. Such hydrolysates have been examined for the following five amino-acids: cysteic acid, lanthionine, djenkolic acid, thiazolidine carboxylic acid and *S*-methyl cysteine. The production of these acids from cystine is represented schematically below: