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Substitution of α-Keto Acids for Five Amino Acids Essential for Growth of the Rat.* (20898)

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The concept of the indispensability of the "essential amino acids" was modified when Rose(1) pointed out that the amino acids, leucine and isoleucine(2), as well as histidine (3,4), tryptophan(5,6), and phenylalanine (7) could be replaced in the diet by the corresponding keto or hydroxy acids. Subsequent studies have extended this list to valine(8-10), methionine(11), and lysine†(12) derivatives. In all of the work cited above, the growth tests were carried out with a single keto acid; the remainder of each diet was adequate with respect to all other essential amino acids. The present study was performed to determine whether 5 essential amino acids could be synthesized simultaneously by young rats at a rate which would permit growth. When leucine, isoleucine, valine, phenylalanine, and methionine were replaced in the diet by the corresponding keto acids, slow growth of the rat was obtained.

Experimental. Dimethylpyruvic acid was prepared by the method of Ramage and Simonson(15) by the condensation of acetone with hippuric acid in the presence of sodium acetate and acetic anhydride. The intermediate, 2-phenyl-4-isopropylidine-5-oxazolone, was hydrolyzed with acid and the keto acid was isolated as the sodium salt. It was characterized by its 2,4-dinitrophenylhydrazone derivative, m.p. 191.5°. *Phenylpyruvic acid* was prepared according to Organic Syntheses (16). It was isolated as the free acid, m.p. 150°. The 2,4-dinitrophenylhydrazone melted at 188°. *α-Ketoisocaproic acid. N*-chloroacetyl-*DL*-leucine was prepared according to the method of Doherty, Tietzmann, and Bergmann(17). The acyl amino acid was heated with acetic anhydride and a trace of pyridine for 3 hours at 60-70°. The mixture was fractionally distilled *in vacuo* and the 2-methyl-4-isobutylidine-5-oxazolone collected between 65-70° at 0.05 mm Hg. It was hydrolyzed to α-ketoisocaproic acid by boiling with *N* hydrochloric acid for 3 hours. The keto acid was extracted with ether, and the ether extract was concentrated to an oil which was dissolved in ethanol. The sodium salt of the keto acid was precipitated by addition of alcoholic sodium hydroxide. The melting point of the 2,4-dinitrophenylhydrazone was 156° (18). *α-Keto-β-methylvaleric acid.* A mixture of *DL*-isoleucine and *DL*-alloisoleucine was converted to the corresponding chloroacetyl derivatives. These were converted to the keto acids by the procedure outlined above for α-ketoisovaleric acid. The *DL*-keto acid was obtained as the sodium salt. *α-Keto-methylthiobutyric acid.* The method of Cahill and Rudolph(11) was modified. *DL*-methionine was converted to an oily chloroacetyl derivative by the action of chloroacetyl chloride and sodium hydroxide at −5°. The chloroacetylmethionine was treated with acetic anhydride and a trace of pyridine. A spontaneous reaction occurred. After 20 minutes the solution was fractionally distilled and the product, 2-methyl-4-(β-methylthioethyl-

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† Since lysine(13) and threonine(14) are not synthesized from the "nitrogen pool", these amino acids fall into a special category and need to be investigated further.

idine)-5-oxazolone, was collected between 40-50° at 0.3 mm of Hg. The oxazolone was converted to the keto acid by acid hydrolysis. The keto acid was isolated by ether extraction and converted to the sodium salt as described above for the keto acid of leucine. The 2,4-dinitrophenylhydrazine melted at 148°(11).

Feeding experiments. White rats, weanling litter mates of both sexes, were used in these studies. Food and water were supplied *ad libitum*. The basal diet consisted of a mixture of 10 essential amino acids plus glutamic acid,‡ 16; sucrose, 15; dextrin, 60; Celluflour, 2; corn oil, 2; salt mixture (Jones-Foster)(20), 4; vitamins A and D mixture, 0.05; inositol, 0.1; choline chloride, 0.2; and liver powder (Wilson's 1:20), 0.4. Water soluble vitamins were added when the diet was placed in the food cup. The supplement furnished per kilo of diet was that utilized by Rose *et al.*(19) excepting the pyridoxine content was doubled: thiamine chloride, 5 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 15 mg; nicotinic acid, 5 mg; calcium pantothenate, 25 mg; *p*-aminobenzoic acid, 300 mg; 2-methyl-1,4-naphthoquinone, 2 mg; biotin, 0.1 mg; folic acid, 0.1 mg. When keto acids were used in the diet they replaced the corresponding amino acids in molar equivalents; a molar equivalent of glycine was also included to maintain a constant nitrogen content. When 4 keto acid sodium salts were utilized initially, the diet mixture was found to be hygroscopic and was often rejected by the animals. Subsequently, diet was placed fresh in the diet cups daily, the thiamine content was doubled, and 0.2% aspartic acid was added as noted in Table I. After these measures were taken the food consumption improved.

Results. Growth rate of young rats on the diet of 10 essential amino acids plus glutamic acid was slow but definite. Table I shows that the animals grew about as well on diets in which various combinations of 2 of the essential amino acids were replaced by keto acids. When combinations of 4 keto acids were substituted the animals ceased to grow, probably because the animals refused to eat, as noted above. When the thiamine supplement was doubled and 0.2% aspartic acid was added to the diet, the animals resumed eating and growth resulted. When the number of

‡ As described by Rose, Oesterling, and Womack (19) excepting the tryptophan was *DL*.

TABLE I. Growth Response to Multiple Keto Acid Supplements.

Initial rat wt (g)	No. of days	Avg wt change/day	Amino acids replaced by keto analog*
59	8	.63	None, basal diet
	8	.83	Leucine, isoleucine
	4	−1.8	Leucine, isoleucine, valine, methionine
	4	1.0	Same as above†
	12	.4	Leucine, isoleucine, valine, methionine and phenylalanine
57	8	.7	None, basal diet
	8	1.4	Leucine and isoleucine
	4	−1.3	Leucine, isoleucine, valine and methionine
	4	.3	Same as above†
	12	.5	Leucine, isoleucine, valine, methionine and phenylalanine
51	4	1.0	None, basal diet
	8	.2	Valine and methionine
	4	−3.7	Leucine, isoleucine, valine and methionine
	4	.8	Same as above†
	12	.9	Leucine, isoleucine, valine, methionine and phenylalanine
61	4	.8	None, basal diet
	8	1.1	Valine and methionine
	4	− .2	Leucine, isoleucine, valine and methionine
	4	1.0	Same as above†
	12	1.0	Leucine, isoleucine, valine, methionine and phenylalanine
63	4	.5	Phenylalanine†
	12	.8	Leucine, isoleucine, valine, methionine and phenylalanine
43	4	1.2	Phenylalanine†
	12	1.1	Leucine, isoleucine, valine, methionine and phenylalanine

* A molar equivalent of glycine was added for each amino acid replaced by its keto analog.

† At this point, the diet was supplemented with .2% aspartic acid and an additional 5 mg/kg of thiamine.

amino acids substituted by keto acids was increased to 5 the growth rate was not appreciably affected.

The keto acids are so unstable that they cannot be considered equivalent to the amino acids on a molecular basis when fed in the ordinary manner. Furthermore, the theoretical equivalence is difficult to judge in the light of present information, since it can only be surmised that α-keto acids are converted exclusively to *L*-amino acids during the metabolic processes. At present there is no reason to assume that amination of the keto acids was produced by intestinal bacteria before absorption of the keto acids took place.

From a consideration of these results with studies using N^{15} labeling it would appear that for the rat at least 8 of the essential amino acids (including arginine) might be replaced in the diet if the corresponding keto acids were furnished with a utilizable source of nitrogen. Such a diet would give a greater indication of the scope of the transamination reaction and the sources of amino acid and nitrogen.

Summary. Young rats grew slowly on a diet in which the nitrogen was furnished exclusively by the 10 amino acids essential to the young rat plus glutamic acid. When leucine, isoleucine, valine, phenylalanine, and methionine were replaced in the diet by the corresponding keto acids plus an equivalent amount of nitrogen as glycine and 0.2% aspartic acid, the animals grew as well as they did on the basal diet.

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