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Mixtures of Pure Amino Acids as Substitute for Dietary Protein.

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Rose[1] has stated that a diet containing 10 essential amino acids is adequate for growth. Animals on this diet gained weight just as rapidly as when protein components were supplied preformed. Evidence has recently been presented by Albanese and Irby[2] showing that a mixture of essential amino acids is inferior from a nutritional standpoint to enzymatic or acid casein hydrolysates or casein. They interpret their results as indicating the toxic effect of unnatural forms of certain amino acids.

The synthesis of vitamins in the intestinal tract of the rat having been amply demonstrated,[3-9] an attempt to obtain evidence suggesting similar synthesis of amino acids was considered advisable.

Albino male rats of the Wistar Institute strain about 35 days old were placed on diets of the following composition: Enzymatic casein digest, vitamin free casein, or amino acid mixture, 16; sucrose, 69; salts, 4, cod liver oil, 2; corn oil, 9; $NaHCO_3$, 2. The vitamin supplements were added at the following levels per kilogram of diet: thiamine hydrochloride, 5 mg; riboflavin, 10 mg; pyridoxine, 5 mg; nicotinic acid, 100 mg; calcium pantothenate, 100 mg; choline chloride. 200 mg; inositol, 200 mg; *p*-amino benzoic acid, 100 mg; ascorbic acid, 100 mg; 2-methyl-1,4-naphthoquinone, 50 mg; ethyl linolate, 10 g; alpha-tocopherol, 100 mg; biotin (conc. No. 5000 SMA), 200 μg. The amino acid mixture had the following composition: *l*-(+)-lysine HCl, 50.5 g; dl-valine, 45.0 g; *l*-leucine, 36.0 g; *dl*-isoleucine, 40.5 g; *dl*-threonine, 23.0 g; *l*-tryptophane, 9.0 g; *l*-histidine, 15.75 g; *dl*-phenylalanine, 33.75 g; *dl*-methionine, 31.5 g; and *l*-(+)-arginine, 13.5 g. To this diet there was added in some instances, succinylsulfathiazole at a 2% level replacing an equivalent amount of sucrose. Six sets of 5 rats each were placed on the various diets. Food consumptions of all the rats were measured in order to eliminate this factor in interpretations of the growth rates. As there was no variation, the values are not listed.

A consideration of the growth values indicates that in the series on Diets A, C, and D the greatest growth was obtained on the casein-containing diet, next on the enzymatic digest of casein, and least on the purified amino acids. The rats receiving purified amino acids doubled their weights in 7 weeks. This indicates that Rose[1] was partially correct in his assumption, and precludes to a certain extent the possibility of toxic action of the unnatural isomers, as in our diet A and the diet EAA of Albanese and Irby[2] the same unnatural amino acids occur. In the diet of du Vigneaud *et al.*,[10] the unnatural isomer used is similar to that of Albanese and Irby.[2] The diet of Bauer and Berg[11] contains unnatural isomers in approximately the same amounts and yet they report moderate growth.

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

2 Albanese, A. A., and Irby, V., *Science*, 1943, **98**, 286.

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5 Daft, F. S., Ashburn, L. L., and Sebrell, W. H., *Science*, 1942, **96**, 321.

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8 Light, R. F., Cracas, L. J., Olcott, C. T., and Frey, C. N., *J. Nutr.*, 1942, **24**, 427.

9 Wakim, K. G., Krider, M. M., and Day, H. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 164.

10 du Vigneaud, Vincent, Dyer, H. M., and Kies, M. W., *J. Biol. Chem.*, 1939, **130**, 325.

11 Bauer, C. D., and Berg, C. P., *J. Nutr.*, 1943, **26**, 51.

TABLE I.
Weekly Weight Gain on Various Diets.

	Diet	Initial wt, g	Weekly wt gain in g, avg for 5 rats 1	2	3	4	5	6	7	% dead end of 7 wks	Avg total gain 7 wks, g
A	16% Amino Acid Mixture	52.0	9.4	5.6	8.4	10.0	3.0	7.3	5.2	0	48.9
AS	16% Amino Acid Mixture + 2% Sulfasuxidine	50.0	1.2	1.6	3.1	—1.0	—3.0	—5.5	—1.0	100	—5.6
C	16% Casein	49.5	17.0	11.4	13.6	21.0	16.0	12.1	15.3	0	106.4
CS	16% Casein + 2% Sulfasuxidine	51.8	17.6	11.2	10.4	10.2	1.0	3.0	11.0	20	64.4
D	16% Enzymatic Casein Digest	42.0	14.0	18.6	12.4	10.0	8.5	7.2	15.5	20	86.2
DS	16% Enzymatic Casein Digest + 2% Sulfasuxidine	47.0	11.6	12.6	7.2	4.0	—10.0	—1.0	8.0	40	32.4

In the present work, the growth could be considered moderate, although not the equal of the rats on casein diet, and there was no evidence of toxicity or nutritive failure such as reported in the rats of Albanese and Irby.[2] One interesting point of difference lies in the somewhat higher content of *dl*-valine in the diet of Albanese and Irby.[2] The unnatural form of valine is not available to the rat. Leucine, isoleucine, lysine, and threonine are other amino acids for which a similar situation holds true but there is no significant difference in the diets in this respect, and one would not expect any toxicity to result from the inclusion in a diet of the unnatural amino acid isomer if that isomer is available.

Comparison of the growth rates of rats on Diet A and Diet AS shows clearly the detrimental effect of the inclusion of succinylsulfathiazole. This drug is not toxic at a level of 2% when included in 18% casein diets supplemented with folic acid concentrates. As is well known, the sulfa drug does precipitate many vitamin deficiencies. The findings here reported are suggestive of inhibition of amino acid synthesis by bacteria in the intestine of the rat. Even on a 16% casein diet plus 2% succinylsulfathiazole, the rats grow very well; this situation also obtains when the casein is replaced by an enzymatic digest of casein incorporated at the same level. The amounts of amino acids available to us were not of sufficient magnitude to permit studies determining which of the other amino acids are essential in experiments of this type. Thus, it is our theory that as in the case of the vitamins so it is with the amino acids: certain members of each group are synthesized in the intestinal tract and thus do not appear to be essential; however, with the inclusion of slowly absorbed sulfonamides this synthesis is reduced and the deficiencies are precipitated.

Enough folic acid concentrate, prepared by the method of Hutchings *et al.*,[12] was available to feed 2 rats on each of the above sulfasuxidine-containing diets for 7 weeks at a level of 5 mg daily. The results indicate exactly the same conclusions as had been obtained with the diets lacking folic acid. Growth on Diet CS supplemented with folic acid nearly equalled that in rats on stock diets. The rats on the AS diet supplemented with folic acid did not gain weight and died in the sixth week. The rats on the diet DS grew but not so well as the rats on diet CS when both were supplemented by folic acid.

Summary. When succinylsulfathiazole is incorporated into a diet containing all known vitamins plus ten essential amino acids, inadequate nutrition is found to result.

[12] Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941, **141**, 521.