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Absence of Lipotropic Activity of Methionine When Pure Amino Acids Were Substituted for Dietary Protein.

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That methionine shows lipotropic activity in preventing the development of fatty livers has been repeatedly demonstrated, but the variation in experimental procedures and in the results obtained has left the magnitude of this effect and the interrelationships of methionine with other lipotropic and anti-lipotropic agents in some question. Lucas and Best[1] have reviewed this subject and have noted that there is disagreement among various workers as to whether the lipotropic action of casein is due solely to its methionine content. It was thought pertinent in this connection to observe the effect of methionine when a mixture of essential amino acids (supplemented with glutamic acid) was substituted for natural protein in the experimental diet.

Experimental. The composition of the amino acid mixture used is shown in Table I. It was designed to contain the essential amino acids (with the exception of methionine) in the proportions in which they occur in casein, glutamic acid making up the nonessential fraction. When the *l*-form was not obtainable, twice the amount of the *dl*-form was used.

TABLE I.
Amino Acid Mixture
(% Composition)

l-Arginine HCl	4.4
dl-Phenylalanine	10.3
dl-Leucine	27.0
dl-Isoleucine	9.7
l-Histidine HCl	2.3
l-Lysine HCl	7.0
dl-Threonine	8.6
dl-Tryptophane	2.4
dl-Valine	10.0
l-Glutamic Acid HCl	18.3

Thirty young adult male rats of a Wistar strain were divided into 2 groups of 15 rats each. All animals were fed on a diet consisting of amino acid mixture, 8.5; cane sugar, 47.5; Crisco, 40; salt mixture No. 2 (U.S.P. XII), 4. Each animal received daily by stomach tube 20 μg thiamine chloride, 100 μg calcium pantothenate, 20 μg pyridoxine and 25 μg riboflavin, dissolved in 1 cc of water. Three drops of percomorph oil furnishing 3750 units of vitamin A and 540 units of vitamin D, and 3 mg of α-tocopherol were given weekly. The methionine-treated group received an additional supplement of 50 mg *dl*-methionine dissolved as the sodium salt in 1 cc of water and administered by stomach tube.[2]

The rats were maintained on experiment for 21 days. Food intake and body weight were recorded daily. At the end of the experimental period the animals were exsanguinated by decapitation under light ether anesthesia and the livers removed, weighed and analyzed for total fat by the method of Tucker and Eckstein,[3] modified slightly. The kidneys were saved for histological examination.

Results and Discussion. The basal diet corresponded in essential amino acid content to one containing about 7% casein and, with the mixture of *l*- and *dl*-acids, to a little less than this in available nitrogen, with no sulphur-containing amino acid present. When supplementary methionine was given, the 50 mg per day represented a level in the diet of 0.8% on the basis of the average food intake of the group, or the same amount of methionine as a diet containing 25% casein.

That the rats should not thrive on the

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1 Lucas, C. C., and Best, C. H., *Vitamins and Hormones*, Vol. I, p. 1, 1943, New York.

2 Machella, T. E., and Griffith, J. Q., Jr., in Griffith-Farris, *The Rat*, 1942, Philadelphia.

3 Tucker, H. F., and Eckstein, H. C., *J. Biol. Chem.*, 1937, **121**, 479.

sulphur-free diet was to be expected. Weight loss was continuous throughout the experiment averaging 30% for the group. The animals did not, however, exhibit any acute symptoms during the 21-day period. Methionine exerted a beneficial effect on the general condition of the animals. The methionine-supplemented group ate 15% more than the control animals. Weight loss paralleled that of the controls for the first week, but during the remaining 2 weeks of the experiment body weight was almost constant. The average weight loss for the period was 21%. This figure does not compare favorably with the average 12% loss of rats on an unsupplemented 8% casein diet which was observed in this laboratory.† This mild effect of methionine on body weight and food intake is in contrast with the results of Glynn, Himsworth and Neuberger.[4] These authors using a high carbohydrate, very low fat diet, with amino acids as the source of nitrogen, found that with the addition of 8 mg of methionine a day to the sulphur-free ration the rats ate two-thirds more, while 80 mg a day more than doubled the food intake. The variation was in the amount of carbohydrate consumed, the amount of amino acids being kept constant at 1.3 g per day. The animals used weighed 80 g at the beginning of the experiment. With no methionine, weight change in 21 days was —2.5%, with 8 mg of methionine, +2.5%, and with 80 mg of methionine, +44%. These workers were studying pathological changes in the liver. That this might be uncomplicated by fatty infiltration, they included in the diet sufficient choline to prevent fatty livers. Our diet was essentially choline-free which may have necessitated a quite different apportionment of the methionine among its various functions.

No necrosis was observed in any of the livers at autopsy, nor did the kidneys show any pathological changes.

Methionine proved quite ineffective as a lipotropic agent in our experiment. The level of liver fat in the control group, 19.7% was of the order usually obtained with a similar diet containing casein. The average for the treated animals was slightly lower, 17.9%, but the difference is insignificant. It has been reported many times that a similar amount of methionine supplementing a ration containing 5% casein has definite lipotropic effect. As examples may be cited the early paper of Tucker and Eckstein[3] in which it was stated that 0.5% of methionine added to the diet reduced the liver fat from 20.4% to 11.1% and the recent work of Clark, Eilert and Dragstedt[5] who found the liver fat changed from 31.5% to 9.3% by the same amount of methionine. In both cases the total level of methionine in the diet was lower than in our experiment: about 0.65% as compared with 0.8%. However, because of the better appetite of the animals on the casein diet, the actual amount of methionine ingested was a little greater than that given to our animals. The rats of Tucker and Eckstein received 71 mg per day and those of Clark, Eilert and Dragstedt, 58 mg, while ours had 50 mg.

The results of the present study appear to support the view that other factors than methionine enter into the lipotropic effect of a high casein diet, or even of a methionine-supplemented low casein diet. Under the conditions of the experiment methionine, in what has been considered as an adequate amount, gave no appreciable protection against development of fatty livers. The amino acid mixture used did not reproduce the composition of casein except for the essential amino acids. Some of the nonessential acids may play a role or, in their absence, the requirement for some of the essential acids may be increased. It has been suggested by du Vigneaud, Chandler, Moyer and Keppel[6] that in low protein diets a deficiency of some other precursor of choline than methionine may be the limiting factor of lipotropic activity. On the other hand,

† Unpublished data.

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

5 Clark, D. E., Eilert, M. L., and Dragstedt, L. R., *Am. J. Physiol.*, 1945, **144**, 620.

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

TABLE II.
Effect on Body Weight, Food Intake, and Liver Fat of Adding Methionine to a Diet Free of Sulfur-Containing Amino Acids.

Group	Treatment	No. of rats	Wt of animals: Initial, g	Wt of animals: Change, %	Food intake, g/day	Liver: Wt, g	Liver: Total lipid, %
I	Basal diet	15	160 (149-172)	−29.8 ± 0.8	5.5 ± 0.2	5.30 ± 0.24	19.7 ± 1.7
II	Basal diet + Methionine	15	164 (146-182)	−21.0 ± 0.8	6.3 ± 0.2	5.50 ± 0.14	17.9 ± 1.4

the amount of methionine required to show a lipotropic effect may be considerably increased in a diet containing minimal amounts of choline. In the present investigation crystalline vitamin B components were used, while in those quoted above[3,5] the animals were fed 0.5 g of yeast daily. This amount of yeast contains more than 1.0 mg of choline[7] which is an appreciable percentage of the daily requirement. It is possible, also, that some other component of yeast works in conjunction with methionine.

Summary. Under the experimental conditions chosen, methionine at a level of 50 mg per day (0.8% of the diet) did not prevent the development of fatty livers in rats fed a diet in which a mixture of essential amino acids replaced protein.

7 Fletcher, J. P., Best, C. H., and Solandt, O. M., *Biochem. J.*, 1935, **29**, 2278.

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Implantation of Riboflavin Pellets in Animals and Man.

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Nutritional studies of the Palestinian population have shown that riboflavin deficiency is very often observed in this country,[1] and that its incidence is far more frequent than that of other vitamin deficiencies.[2] The clinical manifestations of this deficiency, such as glossitis, cheilosis, corneal vasicularization, etc., were particularly common in pregnant women. Low riboflavin excretion in the urine (averaging 95 γ per liter instead of 360 γ per liter in normal pregnant women) was found in 190 (21%) out of 900 pregnant women studied.[3] Furthermore, observations have been reported as to the harmful effect of riboflavin deficiency on the course of pregnancy (prematurity), on the condition of the fetus *in utero* (higher incidence of antenatal death) and upon the postpartum period (agalactia and hypogalactia).[4]

Surprisingly enough, these clinical manifestations appeared not only in the poorer classes, in which riboflavin intake was inadequate, but also in well nourished subjects. The fairly frequent incidence of riboflavin deficiency in well nourished individuals is principally due to inadequate resorption of this vitamin. The frequent occurrence of various intes-

1 Dostrowsky, A., and Sagher, Fr., Harefuath, *J. Pal. Med. Assn.*, 1942, **22**, 1942; *Dermatologica*, 1942, **86**, 325.

2 Berger-Rabinowitz, S., Doctor's Dissertation, 1945, Hebrew University, Jerusalem.

3 Braun, K., Bromberg, Y. M., and Brzezinski, A., *J. Obst. and Gyn., Brit. Empire*, 1945, **52**, 1.

4 Brzezinski, A., Bromberg, Y. M., and Braun, K., *J. Obst. and Brit. Emp.*, 1947, in press.