# Effect of Methionine Deficiency upon Enzyme Activity in the Rat.* (17441)

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The effects of low protein intake upon enzyme activity in the rat have been studied recently by several workers. Seifter *et al.*[1] have shown that during complete dietary protein deprivation the loss of D-amino acid oxidase and arginase activity in rat liver occurs faster than loss of general liver protein. Westerfeld and Richert[2] have reported that xanthine oxidase activity is decreased in rats fed low protein diets. We[3] have observed that liver xanthine oxidase activity is very sensitive to subtle changes in nitrogen intake. Adult rats fed a diet completely adequate for maintenance may show a liver xanthine oxidase content far below normal. Moreover it was shown that addition of 0.25% DL-methionine to the diet increased xanthine oxidase activity to normal.

The present paper is a preliminary report concerning the effects of a true amino acid deficiency upon liver enzyme activity. Because of the observed strongly limiting effects of methionine upon liver xanthine oxidase activity, this amino acid was chosen for the first study. Subsequently we expect to report the effect of other amino acid deficiencies upon liver enzyme activity.

*Experimental.* Eighteen male, weanling albino rats of the Holtzmann strain were placed on a good stock ration for 10 days. They were then given, for one week, a purified ration reported by Ramasarma *et al.*[4] to be the best for growth of young rats. Briefly, this nation consisted of a purified amino acid mix (16%), salts, the known vitamins, corn oil, and sucrose. The animals were fed and watered *ad libitum* during the 7-day period. At the end of that time the animals were divided into 2 groups of 9 animals each. One group was given the complete ration, and the other group was fed the complete ration less methionine. In order to prevent poor food intake due to the methionine-deficiency both groups of animals during the experimental period were force fed in 3 daily portions an amount of the ration equal to the average intake of the animals during the *ad libitum* feeding period. The rations were taken up in distilled water to make a thick suspension and were fed by stomach tube.

After 14 days of the force-feeding period a methionine deficiency was distinctly evident in the animals receiving the methionine deficient diet as evidenced by bleeding of the feet and mouth and general loss of weight. During this period 3 animals of the group receiving the methionine-deficient diet died. The animals receiving the complete diet gained weight throughout the period and showed no detrimental signs because of the regimen.

When the enzyme experiments were begun it was thought best to sacrifice the animals as quickly as possible in order to prevent variations due to different lengths of time on the diets. For this reason 3 animals from the methionine-deficient group were sacrificed on one day; the remaining 3, the next. The animals receiving the complete ration were sacrificed in the following order on consecutive days: 4 animals, 3 animals and 2 animals.

The animals were killed by decapitation and the livers immediately removed. A portion of each liver was quickly weighed out on a delicate torsion balance. The portions of

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1 Seifter, S., Harkness, D. M., Rubin, L., and Muntwyler, E., *J. Biol. Chem.*, 1948, **176**, 1371.

2 Westerfeld, W. W., and Richert, D. A., *Fed. Proc.*, 1949, **8**, 265.

3 Williams, J. N., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, in press.

4 Ramasarma, G. B., Henderson, L. M., and Elvehjem, C. A., *J. Nutrition*, 1949, **38**, 177.

TABLE I.
Effect of Methionine Deficiency Upon Enzyme Activity in Rat Liver.

| Enzyme | Ration | No. of animals | Activity based on wet liver | Activity based on liver N |
|---|---|---|---|---|
| Xanthine oxidase | Basal | 3 | 0* | 0* |
| | | 3 | 0 | 0 |
| | Basal + methionine | 4 | 141 | 4150 |
| | | 3 | 134 | 4100 |
| | | 2 | 137 | 4120 |
| Succinic dehydrogenase | Basal | 3 | 14.8† | 500† |
| | | 3 | 12.5 | 540 |
| | Basal + methionine | 4 | 18.0 | 550 |
| | | 3 | 18.6 | 560 |
| | | 2 | 18.6 | 580 |
| Endogenous respiration | Basal | 3 | 1.06† | 35.0† |
| | | 3 | 1.07 | 45.0 |
| | Basal + methionine | 4 | 1.51 | 44.5 |
| | | 3 | 1.48 | 45.6 |
| | | 2 | 1.56 | 48.5 |

* Units = $\mu$ $10_2$ per hr per g wet liver.
† Units = $\mu$ $10_2$ per hr per mg wet liver.

liver from the animals sacrificed at one time were pooled in 5 volumes of ice-cold 0.039 M sodium potassium phosphate buffer, homogenized for 3 minutes, and strained through gauze. This homogenate was used in the xanthine oxidase determinations according to the method of Axelrod and Elvehjem.[5] A portion of the homogenate was diluted with buffer to give a 10% homogenate, which was employed for measuring succinic dehydrogenase according to the method of Schneider and Potter.[6] Both enzymes were assayed using a Warburg bath maintained at 30°C. Aliquots of each homogenate were taken for nitrogen determinations in triplicate using a microKjeldahl procedure.

*Results.* The enzyme values as presented in Table I are based both upon liver wet weight and liver nitrogen. Since enzymes are protein in nature, loss or gain of enzyme activity as compared to general liver protein can be obtained by basing enzyme activity upon liver nitrogen. This also negates the effects of dilution of liver by glycogen or lipid. Endogenous respiration of the liver homogenates was calculated from the first 10-minute period of oxidation before xanthine was added to the flasks for measuring xanthine oxidase activity. Endogenous respiration is reported since it is believed to be a measure of general liver metabolic rate.

As shown in the table a methionine deficiency induces a significant decrease in all 3 of the activities measured when activity is based upon wet weight of the liver. It was observed that the livers of the methionine deficient animals were somewhat infiltrated with fat, which probably accounts for the more noticeable decrease of wet weight enzyme activity in the amino acid deficient rats. The comparative loss of enzyme activity with general liver protein is not so pronounced in the case of succinic dehydrogenase activity. It appears that activity of this enzyme, which according to Schneider[7] is centered mainly in the mitochondrial elements of the liver cell, is held almost as tenaciously as other liver protein.

The striking disappearance of xanthine oxidase activity during a methionine deficiency is difficult to explain. Seifter *et al.*[1] have reported that during protein starvation the riboflavin content of the liver is lowered. This would not, however, explain such a marked loss in flavin enzyme activity. It ap-

[5] Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1941, **140**, 725.

[6] Umbreit, *et al.*, Manometric technics and related methods for the study of tissue metabolism, 1945.

[7] Schneider, W. C., *J. Biol. Chem.*, 1946, **165**, 585.

pears that the extreme lability of xanthine oxidase activity is due to several unknown factors. It is difficult to believe that activity of this enzyme is lost because of its lack of necessity to the animal since purine metabolism is believed to be very important in animal functions.

As shown in the table, general endogenous respiration of the livers of the animals receiving the complete ration and those receiving the methionine deficient ration are essentially the same. Stare and Elvehjem[8] had reported that endogenous respiration of chick and rat tissue is practically unaffected by various vitamin deficiencies. It has been recently reported[9] that endogenous respiration of chick liver is unaffected by folic acid or vitamin $B_{12}$ deficiencies. It thus appears that while dietary conditions have little effect upon the total endogenous metabolism of the liver individual enzyme activities may be varied significantly by the diet.

*Summary.* It has been observed that a methionine deficiency in the rat reduces liver succinic dehydrogenase activity slightly, completely reduces liver xanthine oxidase activity, and has practically no effect upon endogenous respiration of liver tissue ***in vitro.***

8 Stare, F. J., and Elvehjem, C. A., *Am. J. Physiol.*, 1933, **105**, 655.

9 Williams, J. N., Jr., Nichol, C. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **180**, 689.

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## Lipotropic Effects of Vitamin $B_{12}$ Concentrate. (17442)

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It has recently been reported that crude liver extract exerts a lipotropic effect in rats with dietary induced liver injury.[1] The effect of the liver extract was not due to stimulation of the appetite with a resultant increase in protein intake, nor did the lipotropic effect seem to be related to the choline content of the liver extract. It was also demonstrated that other supplements. with a higher choline content than that of liver extract, exert a lesser degree of lipotropic activity when compared with liver extract.[2] In the present studies the lipotropic effect of a vitamin $B_{12}$ concentrate was determined in rats fed a high-fat diet.

*Methods.* Male rats of the Sprague-Dawley strain, weighing between 115 and 149 grams were used. The high-fat diet (51% lard) and the control diet (6% lard) as recently described,[3] were fed *ad libitum* for the period listed in Tables I and II. When the animals were placed on the synthetic diets injections of vitamin $B_{12}$ concentrate were begun and were administered subcutaneously 3 times a week.* At the end of the experiment sections were taken from the left lobe of the liver and stained with hematoxylin and eosin. Frozen sections were also made and stained with Sudan III. The remainder of the liver was analyzed for total fat.[4]

*Results.* In the first study treatment with a vitamin $B_{12}$ concentrate, in doses of 0.2 $\mu$g of vitamin $B_{12}$ 3 times a week, reduced the average fat content of the liver from 31.5%

1 Hall, C. A., and Drill, V. A., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 3.

2 Drill, V. A., and Hall, C. A., *Am. J. Med. Sci.*, in press.

3 Hall, C. A., and Drill, V. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 202.

* The Oleum Percomorphum used in the diet was kindly supplied by Mead Johnson and Company.

The vitamin $B_{12}$ concentrate was supplied by Dr. T. H. Jukes of Lederle Laboratories Division of the American Cyanamid Company.

4 Outhouse, E. L., and Forbes, J. C., *J. Lab. and Clin. Med.*, 1939, **25**, 1157.