chem. and Biophys., 1953, v42, 61.
2. Mirsky, A. E., and Ris, H., *J. Gen. Physiol.*, 1947, v31, 1.
3. Hiller, A., Plazin, J., and Van Slyke, D. D., *J. Biol. Chem.*, 1948, v176, 1401.
4. Lugg, J. W. H., *Biochem. J.*, 1938, v32, 775.
5. Van Slyke, D. D., Dillon, R. T., MacFadyen, D. A., and Hamilton, P., *J. Biol. Chem.*, 1941, v141, 627.
6. Rees, M. W., *Biochem. J.*, 1946, v40, 632.
7. Hamer, D., *Nature*, 1951, v167, 40.
8. Daly, M. M., Mirsky, A. E., and Ris, H., *J. Gen. Physiol.*, 1951, v34, 439.

Received December 19, 1952. P.S.E.B.M., 1953, v82.

Liver Xanthine Oxidase Activity in Relation to Availability of Methionine from Soybean Protein.* (20153)

IRVIN E. LIENER AND SHOHACHI WADA. (Introduced by M. O. Schultze.)
From the Department of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

It is well known that the nutritive value of the protein from soybean meal can be markedly enhanced by methionine supplementation or by proper heat treatment(1). The demonstration that the amount of sulphur(2) or methionine(3) absorbed from the intestinal tract of the rat is the same whether the animal is fed the raw or heated soybean meal has led to the suggestion that the methionine derived from unheated soybean protein must be absorbed in a form(2), or in a manner(3), which cannot be utilized for growth.

Since the growth depression observed in animals fed raw soybean meal is known to be caused by other factors as well as the availability of methionine(4), it appeared desirable to apply a criterion other than growth as a measure of the utility of methionine from raw and heated soybean protein. The xanthine oxidase activity of the liver was selected for this purpose since the activity of this enzyme has been shown to be a sensitive index of the availability of methionine from dietary protein(5,6).

Experimental. Each kg of basal ration used in this study contained the following components: raw or heated (autoclaved at 15 lb for 20 min.) soybean flour,† 400 g; salts (7), 40 g; Crisco, 60 g; sucrose, 500 g; and vitamin supplements(7) modified to include 40 μg vit. B_{12}. When 0.45% DL-methionine supplemented the basal ration it replaced an equal weight of sucrose. Two types of feeding trials were conducted: *ad libitum* feeding (Exp. A), and paired feeding (Exp. B). In Exp. A, young weanling rats (littermates of the same sex) weighing 35-50 g were distributed into the following groups: (I) raw soy; (II) raw soy + methionine; (III) heated soy; (IV) heated soy + methionine. Each group was composed of 3 females and 5 males; within each group rats of the same sex were housed together in separate cages. Food and water were furnished *ad libitum,* and the weight and food consumption were recorded daily for 6 weeks. The design of Exp. B was identical to that of Exp. A except that each animal was housed in an individual cage, and the food intake of each animal in groups II, III, and IV was restricted to that of its littermate in group I. During the last week of the experiment all animals were transferred to individual metabolism cages for a period of 3 days to permit collection of the feces. The feces were dried in an oven (overnight at 105°C), weighed, ground to a fine powder in a Wiley mill, and 2 g samples were analyzed for methionine using the method of Horn *et al.*(8). Representative samples of each ration were likewise analyzed for methionine. From these data, in conjunction with a measure of the food intake during this period, the amount of methionine absorbed was calculated. At the end of 6 weeks, the animals

* Paper No. 2969 Scientific Journal Series, Minn. Agr. Exp. Station.

† Nutrisoy XXX, Archer-Midland-Daniels Co., Minneapolis, Minn. According to the manufacturer this product has been extracted with hexane and subjected to a minimum amount of heat treatment.

TABLE I. Growth, Absorption of Methionine, and Liver Xanthine Oxidase Activity of Rats Receiving Diets Containing Raw or Heated Soybean Protein with and without Added Methionine.*

Group	Diet: Soybean flour	Diet: Methionine added	Wt gain† (g/6 wk)	Food intake (g/day)	Methionine absorbed§ (mg/100 g rat)	Xanthine oxidase activity‖: mm^3O_2/hr/g dry wt	Xanthine oxidase activity‖: mm^3O_2/hr/g N
			Exp. A: *Ad libitum* feeding				
I	Raw	.0	69 ± 7.5	6.4‡	69 ± 3.7	332 ± 70	5800 ± 1310
II	"	.45%	120 ± 9.6	8.0	159 ± 4.3	785 ± 95	9700 ± 1500
III	Heated	.0	122 ± 8.7	8.5	63 ± 2.4	650 ± 82	7800 ± 1420
IV	"	.45%	152 ±12.4	9.6	147 ± 4.4	792 ± 84	10200 ± 1630
			Exp. B: Paired feeding				
I	Raw	.0	65 ± 5.3	6.3 ± .4	54 ± 4.6	357 ± 48	5200 ± 580
II	"	.45%	84 ± 7.3	6.3 ± .4	153 ± 8.4	769 ± 65	10800 ± 1810
III	Heated	.0	83 ± 6.0	6.3 ± .4	55 ± 2.6	643 ± 92	8200 ± 1310
IV	"	.45%	86 ± 6.6	6.3 ± .4	154 ± 9.7	787 ±103	11000 ± 1760

* All values are means ± stand. error of the mean based on 8 rats per group.

† Based on the "t" test(15) significant differences between groups are as follows: Exp. A and B—I vs II, III, IV ($P < .01$); Exp. A—IV vs II, III ($P < .01$). All other differences are not considered significant.

‡ Stand. error of the mean could not be calculated since individual food consumption data were not available in the *ad libitum* feeding trials.

§ $\frac{\text{Methionine ingested (mg)—methionine in feces (mg)}}{\text{Body wt (g)}} \times 100$. Based on food intake and fecal excretion covering 72 hr. "Body wt" is the avg wt during this same period.

‖ Based on the "t" test(15) significant differences on either a dry wt or N basis are as follows in both Exp. A and B: I vs II, III, IV ($P < .01$); II vs III ($P < .05$); and III vs IV ($P < .05$). All other differences are not considered significant.

were sacrificed by decapitation, and the livers removed and placed immediately on cracked ice. Homogenates were prepared and the xanthine oxidase activity determined in the manner described by Axelrod and Elvehjem (9). In any one run littermates representing each group were selected to provide a more valid comparison between groups. Aliquots of the homogenates were removed for dry weight and nitrogen (micro-Kjeldahl) determinations. In order to compensate for possible variations in liver composition, enzymic activity was calculated on a nitrogen as well as a dry weight basis.

Results and discussion. The data presented in Table I clearly illustrate the beneficial effect on growth produced by heating the soybean flour or by adding methionine to diets containing the unheated flour, this effect being more marked with *ad libitum* feeding. In the latter instance also, methionine added to the ration containing heated soybean flour elicited a further growth response indicating a suboptimal level of methionine even in the heated soybean protein. The activity of xanthine oxidase in the liver, calculated on either a dry weight or nitrogen basis, was likewise increased as a result of heat treatment or methionine supplementation. Heat treatment, however, was somewhat less effective in this respect than the addition of methionine. These data show further that a difference in the quantity of methionine absorbed can be excluded as a possible cause for the enhancement of growth and xanthine oxidase activity effected by heating the soybean flour.‡ The results of the paired feeding experiment also exclude the possibility that a difference in food consumption is a factor contributing to the low level of xanthine oxidase activity in the livers of rats consuming the unheated soybean meal.

One is therefore led to the conclusion that the methionine derived from unheated soybean protein must be inefficiently utilized not only for growth but also for the maintenance of normal levels of xanthine oxidase in the liver. A closer inspection of the data, however, reveals that another factor in addition to the availability of methionine is affecting

‡ This statement is based on the assumption that the short term experiment involving the measurement of methionine absorption is a true reflection of the entire experimental period of 6 wk.

growth. This is particularly apparent if groups II and IV in Exp. A are compared with respect to growth and enzymic activity. Although the xanthine oxidase values of these 2 groups would indicate little difference in the availability of the absorbed methionine, the growth attained by the rats in group IV was significantly greater. The nutritional superiority of heated soybean flour over the raw flour under conditions wherein methionine is no longer limiting has been attributed in part to the growth-inhibiting effect of "soyin," a toxic protein recently isolated from unheated soybean flour(10,11). Since "soyin" was found to limit growth by causing rats to eat less food(11), it is not unexpected that the restriction of food intake in the presence of added methionine obliterates the difference in growth response observed in *ad libitum* feeding trials (compare groups II and IV in Exp. A and B).

These results further emphasize the necessity for specifying the function of an amino acid when referring to its availability from dietary protein. It had previously been reported that the methionine derived from raw soybean protein was equivalent in lipotropic activity to that originating from the heated protein(12). On the other hand, the data presented here show that the methionine requirements for growth and xanthine oxidase activity are only partially met with the unheated soybean flour as a source of protein. Since changes in xanthine oxidase activity, which accompany modification of the dietary protein, have been shown to involve a synthesis of the enzyme *per se*(13), it appears that both growth and xanthine oxidase activity are functions dependent upon a synthesis of protein. Carroll *et al.*(14) reported evidence which indicates that the bulk of the nitrogen from unheated soybean protein is not absorbed until it reaches the cecum or colon, an effect presumably due to the trypsin inhibitors known to be present in the raw meal. It is possible that the methionine absorbed from the large intestine is rendered ineffective as a participant in protein synthesis without suffering any impairment in its function as a donor of labile methyl groups.

Summary. Xanthine oxidase activity in livers of rats consuming soybean protein was increased by feeding heat-treated soybean flour or by adding methionine to diets containing the unheated flour. Since there was no difference in the degree to which the methionine was absorbed, it was concluded that heating increased the availability of methionine for the synthesis of enzyme protein. The poor growth on raw soybean flour was not entirely due to an impairment in the availability of methionine.

1. Hayward, J. W., and Hafner, F. H., *Poultry Sci.*, 1941, v20, 139.

2. Johnson, M. L., Parsons, H. T., and Steenbock, H., *J. Nutrition*, 1939, v18, 423.

3. Melnick, D., Oser, B. L., and Weiss, S., *Science*, 1946, v103, 326.

4. Liener, I. E., Deuel, H. J., Jr., and Fevold, H. L., *J. Nutrition*, 1949, v39, 325.

5. Williams, J. N., Jr., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, v181, 559.

6. Williams, J. N., Jr., Denton, A. E., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 386.

7. Schultze, M. O., *J. Nutrition*, 1950, v41, 103.

8. Horn, M. J., Jones, D. B., and Blum, A. E., *J. Biol. Chem.*, 1946, v166, 313.

9. Axelrod, A. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1941, v140, 725.

10. Liener, I. E., and Pallansch, M. J., *J. Biol. Chem.*, 1952, v197, 29.

11. Liener, I. E., *J. Nutrition*, 1953, v49, 3.

12. Liener, I. E., Spector, H., Fevold, H. L., and Berryman, G. H., *Arch. Biochem.*, 1949, v24, 299.

13. Dhungat, S. B., and Sreenivasan, A., *J. Biol. Chem.*, 1952, v197, 831.

14. Carroll, R. W., Hensley, G. W., and Graham, W. R., *Science*, 1952, v115, 36.

15. Snedecor, G. W., 1946, Statistical Methods, Iowa State College Press, Ames, 4th Edition.

Received February 20, 1953. P.S.E.B.M., 1953, v82.