The Xanthine Oxidase Factor from Liver.* (18644)

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Westerfeld and Richert(1) observed that in weanling rats fed a purified 21% casein diet the initial low level of liver xanthine oxidase remained essentially unchanged for at least 6 weeks. Feeding a 21% crude casein diet produced a significant increase of liver xanthine oxidase after 4 weeks, but not within 2 weeks. Addition of 10% Wilson's whole liver or other natural unpurified foodstuffs to the purified rations increased the xanthine oxidase activity to almost normal levels within 2 weeks. They considered that some unidentified factor present in certain natural materials must be supplied in the diet of the weanling rat for production of normal levels of liver xanthine oxidase. Williams and Elvehjem(2) have shown that liver xanthine oxidase in the adult rat is a sensitive index of amino acid availability in dietary proteins. They demonstrated that methionine in dietary casein is not readily available to the rat but that if either acid hydrolyzed casein or a mixture of amino acids simulating casein is fed as a source of protein, the rat appears to utilize the amino acids much more completely. They also observed that the effects of the low availability of methionine in casein upon liver xanthine oxidase could be overcome by increasing the dietary level of casein to 40 per cent. In addition, Williams *et al.*(3) have shown that the liver xanthine oxidase activity of young rats is especially sensitive to a methionine deficiency.

The studies presented in this paper were carried out to investigate whether an increase in liver xanthine oxidase activity with natural diets over purified diets is due to an unidentified factor or to a better biological value of protein when the natural foodstuffs are incorporated into the diet. Schaefer *et al.*(4) have observed that both young and adult mink fed highly purified rations supplemented with all the known crystalline vitamins require additional unidentified factors for normal nutrition. Preliminary evidence from the fractionation of the factors in fresh raw liver indicated that methanol extraction removed 1 or more of the factors and that the insoluble residue contained still other factors. It appeared possible that these factors might be related to the unidentified xanthine oxidase factor suggested by Westerfeld and Richert (1).

In preliminary experiments in which defatted whole liver (Viobin) was substituted for part of the protein in a purified casein ration, the liver xanthine oxidase activity of young rats did not respond to any greater extent than with the basal casein ration. Therefore, it was decided to test the ether-soluble fraction of whole liver to determine if any factor might have been removed during the commercial defatting process. Since dextrin is known to favor bacterial synthesis of various dietary factors in the intestinal tract, it appeared important to observe the effect of a dextrin ration upon xanthine oxidase activity of young rats. Another dextrin ration containing sulfasuxidine was also fed to eliminate the effects of bacterial synthesis and therefore to observe the effects of the dextrin *per se*.

Experimental. Weanling, male, albino rats of the Sprague-Dawley strain were employed as experimental animals. The basal ration consisted of 18 g casein, 5 g corn oil, 4 g Salts

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

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4. Schaefer, A. E., Tove, S. B., Whitehair, C. K., and Elvehjem, C. A., *J. Nutrition*, 1948, v35, 157.

TABLE I. Effects of Various Liver Fractions and Dextrin Upon Response of Liver Xanthine Oxidase Activity in Weanling Rats.

Group	No. of rats	Carbohydrate	Supplement	Xanthine oxidase activity after 4 wk (μl O_2/hr/g rat liver)
I	11	Sucrose	0	89 ± 7*
II	7	"	Methanol extr. liver	85 ± 0.3
III	7	"	Liver residue after methanol extr.	116 ± 2
IV	7	"	Methanol extr. liver + residue	102 ± 8
V	7	"	Viobin defatted liver	90 ± 3
VI	7	"	Ether extr. liver	89 ± 7
VII	7	"	Liver residue after ether extr.	127 ± 7
VIII	7	"	Ether extr. liver + residue	117 ± 11
IX	7	"	Whole liver	144 ± 5
X	9	Dextrin	0	142 ± 19
XI	9	"	1.5% sulfasuxidine	136 ± 25

* Standard error of the mean.

IV(5), 2 g vitamin mix(2), and carbohydrate (sucrose or dextrin) to make 100 g. In addition the animals were given 2 drops of haliver oil orally per week. The weanling rats were separated at random into 11 groups and fed the rations shown in Table I. Total nitrogen of the liver fractions and residues was determined by a semi-micro Kjeldahl procedure (6). The liver residues were added to the ration at a 10% level, and the methanol and ether soluble fractions were added at a level equivalent to 10% whole liver. In all cases the liver fractions and residues were added to the basal ration at the expense of casein on a nitrogen basis, making a total of 18% protein in the diet (nitrogen x 6.25 = protein). The methanol extract of liver and the residue were prepared as described by Schaefer *et al.* (7). The ether soluble liver fraction was prepared by grinding fresh beef liver, mixing with an equal volume of distilled water in a Waring Blendor, drying in a hot air oven at 70°C and grinding to a powder in a Wiley Mill. The powder was refluxed with ether in a macro Soxhlet apparatus until the ether was no longer colored. Most of the ether was then removed by distillation. After 4 weeks on the respective diets, the animals were sacrificed and liver xanthine oxidase activity determined at 30°C by the method of Axelrod and Elvehjem(8).

Results and discussion. The results of the xanthine oxidase determination are presented in Table I.

It appears that the methanol extract contained no factor which increased the enzyme activity over that obtained on a sucrose basal ration. However, an increase in activity with the residue was noted indicating that if a factor is involved it is probably contained in the liver residue fraction. Upon combination of the methanol extract with the insoluble residue, a significant and at present unexplainable decrease in the enzyme activity was observed.

With Viobin defatted liver no increase in activity over that of the sucrose basal ration was observed, as mentioned above. It appeared possible therefore that a fat-soluble factor had been removed during the defatting process. However, with the ether extract no increase in activity over that given by the sucrose basal ration was observed. The ether extracted residue gave an increase similar to that obtained with the methanol extracted residue. Again a slight decrease in activity was noted here as with the combined methanol fractions when the ether extract and ether-insoluble residue were fed together.

If the response of xanthine oxidase to the feeding of the liver residues were due to an unidentified factor retained in the residue, it

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

appears that essentially the same results should be obtained with the liver residues after extraction as with whole liver. However, as shown in Table I, whole liver causes a significantly greater response than either of the 2 liver residues fed. It is possible that extraction with organic solvents may have decreased the biological quality of the liver protein or the biological activity of the unidentified factor.

The increased activity of the group receiving the whole liver might have resulted from the ingestion of proteins in which the amino acids were more effectively utilized than in the purified casein. As already shown, methionine appears important in xanthine oxidase activity (2,3). Amino acid analyses(9) of liver and casein show that both contain almost identical amounts of methionine, but that the cystine concentration in liver is approximately 1.4 $\pm$ 0.1% while in casein it is present at a level of 0.36 $\pm$ 0.04%. Hence it is possible that the cystine could spare enough methionine to result in a higher level of enzyme activity when the liver was incorporated into the diet.

An increase over the activity of the sucrose basal ration was observed with both diets containing dextrin whether sulfasuxidine was included or not. This leads us to believe that the effect of the dextrin was not through bacterial synthesis of a xanthine oxidase factor but rather by increasing utilization of the ingested ration. Since dextrin is probably less easily digested and absorbed than sucrose because of the size of the molecule, the ration would be held in the intestinal tract longer, thus allowing more complete digestion and utilization of the diet.

Records of the food consumption and weight gains for all groups of animals were kept, but no correlation between these factors with the differences in enzyme activity was observed.

It is possible that extraction of liver with solvents other than those reported might give positive results. It is our interpretation however, that the increase in enzyme activity noted with the liver residue is probably better explained by the superior biological value of the liver proteins as compared to purified casein.

Summary. 1. The xanthine oxidase factor of dietary liver has been investigated by feeding various liver fractions and residues to weanling rats and observing the response of the rat liver xanthine oxidase activity to the various fractions. 2. The results indicate that the unidentified factor is not extractable by methanol or ether but that it is retained in the extracted liver residue. Whole untreated liver gave a more significant response than any fraction or residue tested. 3. Dextrin in the place of sucrose in the diet with no liver or liver fraction added gave a response as great as that given by whole liver. 4. It appears that the increase in xanthine oxidase activity observed in the weanling rats fed liver or liver residue is possibly due to better biological value of the liver protein rather than to a trace factor in the liver.

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