THE RELATION OF AMINO ACID AVAILABILITY IN DIETARY PROTEIN TO LIVER ENZYME ACTIVITY*

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In 1948 Miller (1) demonstrated that the loss of liver xanthine oxidase activity in the rat greatly exceeded the loss of liver protein during acute inanition. Moreover, xanthine oxidase appeared to be the most labile of the four liver enzymes studied. Westerfeld and Richert (2) observed that increasing the level of protein in the diet tended to bring about an increase in liver xanthine oxidase activity, although there was no direct correlation between the level of dietary protein and enzyme activity.

Because of the apparent lability of liver xanthine oxidase, the present work was undertaken to observe whether the measured activity of this enzyme could be used as an index of general protein metabolism. In this work the question of availability of amino acids in dietary protein has been related to liver xanthine oxidase activity under conditions in which gross body changes are not in general sensitive enough to reflect small protein variations in the animal body. It has been found that, at a level of whole dietary protein which maintains and even supports growth of the adult rat, liver xanthine oxidase activity may be appreciably decreased, probably because of incomplete assimilation of the amino acids in the protein.

Early in the experiments it was observed that animals fed a 14.6 per cent casein diet exhibited much lower liver xanthine oxidase activity than rats fed a good stock ration. It was also observed that if the animals were fed acid-hydrolyzed casein at an 18 per cent level (isonitrogenous with 14.6 per cent casein) the xanthine oxidase activity based on liver protein was also much higher than that in animals fed the 14.6 per cent casein diet. If the animals were unable to digest completely the ingested whole protein, the poor availability of one or more essential amino acids could account for the low xanthine oxidase activity. On the other hand if the protein were fed in a predigested form, i.e., as acid-hydrolyzed casein, this difficulty should not be encountered and xanthine oxidase activity should be normal. To rule out the possibility of the formation of unknown factors

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during acid hydrolysis of casein which might in some way increase liver xanthine oxidase activity, a mixture of purified amino acids simulating casein was fed and xanthine oxidase activity of the livers of the animals determined.

If the animals were unable to obtain enough of one or more amino acid from a 14.6 per cent casein diet, it was possible that feeding a higher level of casein might furnish the required amino acid levels by a mass action effect. Therefore, other experiments were carried out in which the animals were given a 40 per cent casein diet.

From calculation of the essential amino acid concentrations in a 14.6 per cent casein diet, methionine was found to be slightly below the level stated by Womack and Rose (3) to be necessary for growth of young, growing rats. Although we used adult animals in our experiments, it was decided to increase the methionine level in the 14.6 per cent casein ration to a value slightly above that required for the young rat. Therefore 0.25 per cent extra methionine was added to the ration and xanthine oxidase activity determined in the livers of the animals fed this ration.

EXPERIMENTAL

Adult, male albino rats of the Holtzman strain weighing 250 to 350 gm. were maintained on a good stock ration for 2 weeks before being placed on the purified rations.

A series of five synthetic rations was prepared, similar in all respects except for the type or quantity of protein included. The rations contained the following common components: Salts IV (4) 4 gm., corn oil 5 gm., vitamin mixture¹ 2 gm., protein at the desired level, and sucrose to make 100 gm. In addition, 2 drops of halibut liver oil were administered each week by dropper. The protein contents of the five rations were as follows: Ration I, 14.6 per cent Smaco casein; Ration II, 14.35 per cent Smaco casein + 0.25 per cent dl-methionine; Ration III, 18 per cent acid-hydrolyzed casein (5) + 0.5 per cent dl-tryptophan; Ration IV, 24.7 per cent purified amino acid mixture² corresponding to casein (6); and Ration V, 40 per cent Smaco casein. The acid-hydrolyzed casein was

¹ 100 gm. of vitamin mixture contained the following vitamins in a sucrose base: thiamine hydrochloride 10 mg., riboflavin 15 mg., niacin 75 mg., pyridoxine 12.5 mg., calcium pantothenate 100 mg., biotin 0.5 mg., pteroylglutamic acid 1 mg., choline chloride 5 gm., and L-inositol 0.5 gm.

² 24.7 gm. of amino acid mixture contained the following amounts of purified amino acids: dl-alanine 0.90, L-glutamic acid 3.7, L-cystine 0.06, DL-leucine 3.92, DL-phenylalanine 0.84, DL-valine 2.26, DL-aspartic acid 1.0; glycine 0.8, L-histidine hydrochloride 0.50, DL-isoleucine 2.10, L-lysine hydrochloride 2.80, L-proline 1.33, L-tyrosine 1.0, DL-methionine 0.57, DL-threonine 1.26, L-arginine hydrochloride 0.45, DL-serine 1.21, DL-tryptophan 0.29 gm.
analyzed microbiologically for arginine, glutamic acid, and the essential amino acids. The amino acids shown to be decreased by the acid hydrolysis from reported casein levels were supplemented with purified amino acids to the required levels. Since tryptophan was completely destroyed during acid hydrolysis, 0.5 per cent DL-tryptophan was added to bring the L-tryptophan level up to that of casein. When the amino acid mixture in Ration IV was fed at a 24.7 per cent level, the concentrations of amino acids utilizable by the rat were equivalent to those found in 14.6 per cent casein, since only the L forms of leucine, valine, isoleucine, lysine, and threonine are active for the rat (7).

The animals were maintained on the respective rations for at least 2 weeks before being used in the enzyme studies. Six animals were maintained on each ration, except that eight animals comprised the group receiving Ration III.

After the feeding period, the animals were stunned by a blow on the head, decapitated, and exsanguinated. The livers were removed in toto, placed immediately into cracked ice, and chilled for several minutes. They were then blotted free of moisture and weighed. A portion of each liver was homogenized in 5 volumes of ice-cold 0.039 M sodium potassium phosphate buffer (pH 7.3), and xanthine oxidase activity was determined according to the method of Axelrod and Elvehjem (8) with a Warburg bath maintained at 30°.

Total nitrogen, non-protein nitrogen, and dry weight of aliquots of each homogenate were determined in duplicate or triplicate. All nitrogen analyses were made by a modification of the micro-Kjeldahl technique. Non-protein N was determined by precipitating the protein in 5 ml. aliquots of the homogenates with 20 ml. of 20 per cent trichloroacetic acid, heating for 2 minutes at 100°, and filtering. The determination of nitrogen in 10 ml. aliquots of the filtrate gave the non-protein N of the liver homogenates. Homogenate protein was calculated from total N less non-protein N per gm. of liver times the protein factor 6.25. To serve as a check on how accurately the liver homogenates were prepared, total N of approximately 0.1 gm. portions of each liver was determined.

The results of xanthine oxidase activity were calculated in terms of liver protein, dry weight, wet weight, and activity per 100 gm. of rat. In this way the relation of xanthine oxidase activity to a variety of liver factors, e.g., total liver protein, liver solids, liver moisture, and body weight, could be obtained.

RESULTS AND DISCUSSION

The results of the enzyme determinations for the various groups of animals are presented in Table I. Although the standard errors of the mean
are given only for enzyme activity based on liver protein, approximately the same relative deviations hold for the other results.

When xanthine oxidase activity was based on liver protein in the animals receiving the different diets, it was observed that, although the animals were gaining weight in every case, the liver xanthine oxidase activity of the group receiving 14.6 per cent casein was much lower than that of any other group. When 0.25 per cent methionine was added to the 14.6 per cent casein ration, liver xanthine oxidase activity returned nearly to normal. Moreover, when the protein was either acid-hydrolyzed casein or the purified amino acid mixture, xanthine oxidase activity based on liver protein was also nearly normal. It was concluded from these results that methionine was probably the limiting amino acid in the 14.6 per cent casein ration. The fact that the level of cystine is so low in whole casein makes it appear doubtful that it was important in this problem. In the acid-hydrolyzed casein ration cystine was probably still lower than in the casein ration because of destruction during the hydrolysis. No extra cystine was added to the acid-hydrolyzed casein ration, however, because of lack of adequate methods for assaying for that amino acid in the hydrolysate. It appeared also that, although there was enough methionine in a 14.6 per cent casein ration to keep xanthine oxidase activity at a normal level, the animals were unable to utilize all the methionine present in the whole protein. This lack of utilization was possibly due to incomplete digestion of the protein, since the same level of methionine in either

**Table I**

*Relation of Liver Xanthine Oxidase Activity in Rat to Dietary Protein*

<table>
<thead>
<tr>
<th>Ration*</th>
<th>Activity per gm. liver protein</th>
<th>Activity per gm. dry liver</th>
<th>Activity per gm. wet liver</th>
<th>Activity per 100 gm. rat</th>
<th>Average gain in weight per week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µl. O₂ per hr.</td>
<td>µl. O₂ per hr.</td>
<td>µl. O₂ per hr.</td>
<td>µl. O₂ per hr.</td>
<td>gm.</td>
</tr>
<tr>
<td>I</td>
<td>590 ± 40</td>
<td>360</td>
<td>99</td>
<td>350</td>
<td>+2</td>
</tr>
<tr>
<td>II</td>
<td>1080 ± 60</td>
<td>680</td>
<td>163</td>
<td>790</td>
<td>+18†</td>
</tr>
<tr>
<td>III</td>
<td>900 ± 100</td>
<td>540</td>
<td>100</td>
<td>490</td>
<td>+3</td>
</tr>
<tr>
<td>IV</td>
<td>1100 ± 90</td>
<td>630</td>
<td>180</td>
<td>630</td>
<td>+3</td>
</tr>
<tr>
<td>V</td>
<td>1200 ± 30</td>
<td>670</td>
<td>182</td>
<td>790</td>
<td>+9</td>
</tr>
<tr>
<td>Stock</td>
<td>1200 ± 150</td>
<td>680</td>
<td>190</td>
<td>720</td>
<td>+8</td>
</tr>
</tbody>
</table>

* See the text for composition.
† These animals were somewhat younger than the other animals when they were placed on the purified ration. They were maintained on the purified ration, however, until their average weight was the same as that of the animals in the other groups. This probably accounts for the large value here, since these figures were calculated from weight changes throughout the period of feeding the purified diets.
acid-hydrolyzed casein or the purified amino acid mixture was enough to keep xanthine oxidase at nearly a normal level.

It is interesting to observe in these experiments that the enzyme activity based on liver protein followed very closely the pattern observed when activity was based on dry weight. However, a somewhat different pattern was observed when enzyme activity was based on wet weight of the liver. The enzyme results based on wet weight of the liver for the two groups of animals receiving 14.6 per cent casein and acid-hydrolyzed casein were nearly the same and also lower than those for the other groups. The reasons for these results are not clear, although they may possibly be explained by a higher salt content of the acid-hydrolyzed casein. This would tend to increase the water intake, and consequently the water content of the liver, with a concomitant decrease in wet weight enzyme ac-

<table>
<thead>
<tr>
<th>Ration*</th>
<th>Nitrogen per gm. liver</th>
<th>Non-protein N per gm. liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.032</td>
<td>0.0032</td>
</tr>
<tr>
<td>II</td>
<td>0.050</td>
<td>0.0032</td>
</tr>
<tr>
<td>III</td>
<td>0.050</td>
<td>0.0033</td>
</tr>
<tr>
<td>IV</td>
<td>0.050</td>
<td>0.0035</td>
</tr>
<tr>
<td>V</td>
<td>0.053</td>
<td>0.0032</td>
</tr>
<tr>
<td>Stock</td>
<td>0.081</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

* See the text for composition.

tivity. The possibility of the presence of factors toxic to the rat in acid-hydrolyzed casein also should not be overlooked.

When the results were expressed as enzyme activity per 100 gm. of rat, the pattern most nearly approximated that observed when the results were based upon liver dry weight. The differences in enzyme activity between the groups of animals were not greatly amplified, as sometimes occurs when enzyme results are expressed in this manner. This was probably due to the fact that the weights of the animals of all groups were approximately the same when used in the enzyme studies.

In Table II the results of the total N and non-protein N determinations for the various groups of animals are presented. The total N results were obtained from the nitrogen determinations upon portions of the whole livers. Very few if any significant differences were observed either in total N or non-protein N among the different groups of animals. Other workers (9) have reported measurable decreases in liver protein per gm. of liver under severe conditions of protein depletion. It thus appears
that xanthine oxidase activity may decrease markedly without a noticeable decrease in non-enzyme liver protein.

The changes in xanthine oxidase activity observed in these experiments were probably due to an actual decrease in enzyme protein rather than to variations in dietary riboflavin (10), since all groups of animals received the same adequate level of riboflavin throughout the feeding period. Similarly, although pteroylglutamic acid has been shown to influence xanthine oxidase activity profoundly both in vitro (11) and in vivo (12), the effects we have observed in the present experiments cannot be attributed directly to that factor.

SUMMARY

1. Evidence has been presented that liver xanthine oxidase activity can be used as a sensitive index of amino acid availability in dietary proteins.

2. With use of liver xanthine oxidase activity as a criterion, it has been demonstrated that methionine in dietary casein is not readily available to the rat. However, if either acid-hydrolyzed casein or a mixture of purified amino acids simulating casein is fed as the source of protein, the rat appears to utilize the ingested amino acids much more completely.

3. The effects of the low availability of methionine in casein fed at the 14.6 per cent level upon liver xanthine oxidase can be overcome by feeding a 40 per cent casein diet.

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