ANEMIA FROM LYSINE DEFICIENCY IN DEAMINIZED CASEIN*

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It was reported in earlier papers (1, 2) that if rats receive deaminized casein as the only source of protein they survive for only a few weeks. When this protein component was reinforced with a mixture of gelatin and gliadin, the animals failed to grow, became anemic, and died. Practically the same result was obtained when either wheat gluten, corn gluten, or a laboratory preparation of lactalbumin was substituted for the gelatin-gliadin mixture. If casein, however, was included in the ration with deaminized casein, the rats grew rapidly and did not become anemic. This ration also cured animals in which anemia had already developed. Subsequently (3) it was shown that a casein hydrolysate is also effective, as is likewise the copper salt fraction of the hydrolysate which is soluble in water but insoluble in methyl alcohol. These later observations indicated that the anemia is partly due to the presence of a toxic agent in deaminized casein, and partly due to a deficiency of one or more amino acids. However, all attempts to identify the active agents by feeding pure amino acids, singly or in combination, failed. The suggestion was made that when deaminized casein is included in the ration some amino acid may be required in unusually large quantities, possibly to detoxicate the anemia-producing agent. Our more recent studies, with larger amounts of amino acids, are described in this report.

EXPERIMENTAL

If normal animals are placed on the deaminized casein rations, they do not become anemic until several weeks have elapsed,

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and so the experimental periods could be greatly shortened by beginning with rats which are already anemic. This was accomplished by rearing the experimental animals on a milk ration by the method of Elvehjem and Kemmerer (4). By this procedure the effectiveness of a substance as an antianemic agent can be determined very quickly. The rations used in these studies are described in Table I.

**Deaminized Casein-Wheat Gluten Ration**—It was deemed necessary in earlier studies to combine deaminized casein with some other protein that was suitable for the purpose, and wheat gluten was among the most useful of these. Ration 3577 contains this constituent. As was mentioned previously, anemia produced by deaminized casein is cured by a casein hydrolysate, and by that copper salt fraction which is soluble in water and insoluble in methyl alcohol. According to Caldwell and Rose (8) this fraction contains the essential amino acids arginine, histidine, and lysine, and in addition alanine, glutamic acid, hydroxyglutamic acid, glycine, serine, and tyrosine. It was decided to add the essential

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Ration No.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3577</td>
</tr>
<tr>
<td>Deaminized casein</td>
<td>10</td>
</tr>
<tr>
<td>Wheat gluten</td>
<td>20</td>
</tr>
<tr>
<td>Lysine†</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td></td>
</tr>
<tr>
<td>Corn-starch</td>
<td>46.5</td>
</tr>
<tr>
<td>Milk fat</td>
<td>12.5</td>
</tr>
<tr>
<td>Salts (5)</td>
<td>4.0</td>
</tr>
<tr>
<td>Agar</td>
<td>2.0</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>2.0</td>
</tr>
<tr>
<td>Water extract of yeast</td>
<td>2.0</td>
</tr>
<tr>
<td>Tikitiki (6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Alcohol extract of dried beef liver, No. 3849 (7)</td>
<td></td>
</tr>
</tbody>
</table>

* In addition 200 γ of thiamine, 400 γ of riboflavin, and 300 γ of vitamin $B_6$ per 100 gm. of food were included.
† Calculated as the free base.
amino acids of this group to the diet, in various combinations, and methionine was included also because an earlier trial had indicated that it might improve the rate of growth.

It will be observed in Fig. 1 that each of the combinations improved the basal diet very materially. The animals made some gains in weight, they recovered from the anemia, and though there are minor variations it is apparent that all of the mixtures are about equally effective. Since the only amino acid which appears in all groups is lysine, it is concluded that it alone was responsible for the improvement. The next step was the addition of lysine alone to the basal diet. This amino acid alone is as effective as any of the combinations tried, 130 mg. daily peranimal being slightly more effective than 65 mg.
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Deaminized Casein As Only Protein—For the next group of experiments the ration was further simplified by omitting all protein except 20 per cent of the deaminized casein itself. Both the weights and the red blood cell counts of the control animals, on Ration 4033, declined rapidly and the animals all succumbed, as shown in Fig. 2. Apparently both declines were due to a deficiency of lysine. When the ration contained 2 per cent of d-lysine, the rats recovered slowly; when it contained 4 per cent they recovered rapidly. There was a short period when d-lysine was not available, and dl-lysine was substituted for it. The rats which received 4 and 8 per cent of the racemic mixture recovered as rapidly as the rats which received 4 per cent of d-lysine.

Fig. 2. The response to the control ration, No. 4033, is shown in the upper right-hand corner. The animals declined in weight and the anemia became more extreme. The rations of the other animals differed from Ration 4033 by containing lysine, which displaced an equal weight of starch. The animals which received lysine recovered from anemia. When the vitamin carriers were fortified with thiamine and riboflavin, they also made considerable gains in weight.
response of the one rat on 4 per cent of the mixture suggests that l-lysine may be effective in detoxicating deaminized casein, but additional data are required to decide that point.

It seemed surprising that rats would recover promptly from anemia and still fail to gain consistently in weight, and this observation suggested that the vitamin carriers may have deteriorated. In an attempt to improve the vitamin supply 2 per cent of milk fat was replaced by wheat germ oil and 400 \( \gamma \) of thiamine and 200 \( \gamma \) of riboflavin per 100 gm. of food were added to the ration. When the new vitamin mixture was supplied to male rats, on a diet that contained 18 per cent of casein as the only source of protein, they made an average gain of 25 gm. per week over a 6 week period. The results obtained when this vitamin mixture was incorporated in a deaminized casein-lysine ration are shown at the right of Fig. 2. Though there was marked improvement in the rate of growth, it was still subnormal.

The lysine was purchased from reliable manufacturers, but we assured ourselves that it was sufficiently pure for our purposes by determining some of the more important constants.

Comparison of Casein and Deaminized Casein plus Lysine—None of the rations that contained deaminized casein supported the optimum rate of growth, and this suggested that some essential amino acid, other than lysine, had been partly destroyed. No attempt was made to estimate the biological value of deaminized casein, but in an attempt to determine whether or not it is grossly deficient in any of the essential amino acids, it was compared, roughly, with casein.

The comparison was between Ration 4722, which contained deaminized casein and lysine, and Ration 4723, which contained casein. All rats were anemic at the beginning of the experimental period. Two of them received Ration 4722 ad libitum, but their food consumption was determined each day. Two others received Ration 4723, weighed out daily, but the amount was restricted to the quantity that had been consumed by the two on Ration 4722. The rates of recovery from anemia, and the gains in weight, are shown graphically in Fig. 3. The rats on the casein diet recovered from anemia more rapidly than did those that received the deaminized casein plus lysine, but the growth rates for the two groups were almost exactly the same. These data indicate that,
except in lysine, deaminized casein is not grossly deficient in any essential amino acid, and the slower growth rate is explained by a

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**Figure 3.** The response to the control ration, No. 4721, is shown in the upper left-hand corner. The animals shown at the lower left, on Ration 4722, and at the upper right, on Ration 4723, consumed the same amount of food and made approximately the same gains in weight. The rats on the casein diet recovered normal red blood cell counts the more rapidly. It is shown at the lower right that an increased food consumption did not greatly accelerate the recovery from anemia. The data indicate that the deaminized casein-lysine mixture and casein have approximately the same biological value.

**Table II**

<table>
<thead>
<tr>
<th>Ration No.</th>
<th>No. of animals</th>
<th>Observations recorded</th>
<th>Data by weekly periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gain in weight</td>
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</tr>
<tr>
<td>4721, ad libitum</td>
<td>2 M., 2 F.</td>
<td></td>
<td>gm.</td>
</tr>
<tr>
<td>4722</td>
<td>2 &quot;</td>
<td>Gain in weight</td>
<td>-13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food consumed</td>
<td>17</td>
</tr>
<tr>
<td>4722, limited</td>
<td>2 &quot;</td>
<td>Gain in weight</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food consumed</td>
<td>41</td>
</tr>
<tr>
<td>4723, ad libitum</td>
<td>2 &quot;</td>
<td>Gain in weight</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food consumed</td>
<td>41</td>
</tr>
</tbody>
</table>

lowered food intake. It would be difficult to decide whether the reduced food consumption is due to the toxicity of deaminized
casein, or whether this protein is merely unpalatable. As shown in Fig. 3 four rats received Ration 4723 ad libitum, and their food intake was much higher than that of those which received the deaminized casein-lysine ration.

As a control, four rats were given Ration 4721, which contains deaminized casein but no lysine. They lost weight rapidly and none survived longer than 17 days. The average weekly gain in weight and food consumption of the four groups is shown in Table II.

DISCUSSION

The many earlier failures to cure anemia produced by deaminized casein with lysine must have been due to the fact that the amino acid was not given in sufficient quantity. Steudel (9) supplied only 20 mg. of lysine per rat per day. Hogan and Ritchie (1) used 25 mg. in most of their trials, although they reported one failure with 52 mg. of lysine per day. Smith and Stohlman (10) fed a mixture of tyrosine, histidine, and lysine at levels intended to be equivalent to 18 per cent of casein. At that time casein was thought to contain only about 4 per cent of lysine, so their ration must have contained less than 0.75 per cent of lysine. The results with gelatin are in accord with this. 10 per cent of gelatin, supplying 0.59 per cent lysine (11) to the ration, had failed. Our more recent results with gelatin are not described in detail, but it was observed that 20 per cent, supplying 1.18 per cent of lysine, permits recovery. According to Rose (12) the ration should contain 1 per cent of lysine in order for the rats to grow at a satisfactory rate. Our results indicate that if both growth and recovery are to occur on a deaminized casein ration over twice this much lysine must be supplied.

As to the cause of this high lysine requirement one can only speculate. Since deaminized casein presumably contains a toxic agent, a detoxication by the amino acid lysine would seem plausible. However, an attempt to isolate lysine from urine collected while the rats were being supplied with this amino acid ended in failure. Lysine could not be isolated even after strong acid hydrolysis.

Muller (13) has demonstrated a marked reticulocyte response in pigeons injected with lysine. From studies of the bone marrow
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He concludes that this response is due to "stimulation and proliferation of red blood cells and an extension of blood-forming tissue." It may well be that the large amount of lysine enables the blood-forming organs to keep pace with destruction by the toxin and thus overcomes the anemia.

Hemoglobin is known to contain lysine (14); hence a lysine deficiency would prevent its synthesis. Also, the heme group itself must be synthesized, presumably from amino acids, and lysine may be a precursor.

Some comments on the earlier data concerning the antianemic activity of various proteins are suggested now that lysine is known to be the antianemic agent. Since autoclaving destroys this substance (2), it must also partly destroy lysine. The failure of many of the lactalbumin preparations (2) must have been due to thermal damage to lysine. This explanation finds added support in the observations of Greaves, Morgan, and Loveen (15) and of Waisman and Elvehjem (16). The lack of activity in corn and wheat gluten (3) is explained by their low lysine content. Gelatin failed (1) because it was supplied at too low a level.

These observations should serve to emphasize again the importance of suitable dietary protein in relation to recovery from anemia. The role of lysine in erythropoiesis may prove to be of importance.

SUMMARY

1. Lysine is the antianemic agent in the deaminized casein-anemia syndrome.
2. The biological method indicates that deaminized casein is not seriously deficient in any essential amino acid other than lysine.
3. If deaminized casein is the only protein in the diet and other conditions are optimal, the lysine requirement is increased two to four times the normal.
4. An interpretation of earlier data is attempted and applications of these observations are suggested.

BIBLIOGRAPHY

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