# The Effect of Coprophagy Prevention on Amino Acid Deficiency[1] (36178)

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(Introduced by F. J. Stare)

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The effect of coprophagy prevention in rats has been studied extensively by Barnes and his coworkers. These workers found that using tail cups on rats resulted in a decrease in growth rate which was reversible when the animals were allowed to eat the feces as they emerged from the anus (1). The same workers also reported that complete prevention of coprophagy creates major changes in the micronutrient requirements of the rats, and deficiencies of certain vitamins, such as thiamine (2), vitamin $B_{12}$ (3), and essential fatty acids (4) develop more quickly.

In earlier studies (5) on the requirements of essential amino acids, it was observed that the weight loss following the removal of single essential amino acids from the diet varied greatly. Deficiencies of threonine, isoleucine and total sulfur amino acids resulted in a very rapid weight loss comparable to that obtained with a complete deficiency of dietary protein. On the other hand, a complete deficiency of lysine resulted in only a very slow loss of weight. Deficiencies of other amino acids produced intermediate responses between these extremes. In studies on the fecal excretion of amino acids, Chang found that rats excreted more fecal lysine (6) than methionine[2] when fed a protein-free diet and that the level of excretion was partially dependent on the nature of the diet fed. Since these fecal amino acids may be synthesized by the intestinal flora, one of the possible explanations for the small loss of weight caused by the lysine-free diet may have been the ingestion of fecal lysine (7). The studies reported in this paper were done to test the effects of coprophagy, if any, upon the response to amino acid and protein deficiencies.

*Methods.* Thirty-two young male Charles River rats were utilized in these studies. Half of the animals were fitted with fecal collection cups according to the method of Kwong and Barnes (8). For the first 4 weeks all of the animals were fed an adequate purified diet shown as Diet 5 (casein) in Table I. Food intake and body weights were recorded weekly. This period allowed the animals to adapt to the use of the fecal collecting device.

After the 4-week adjustment period, each of these groups with and without tail cups was divided into 4 subgroups of 4 animals each. These received the diets shown in Table I as the lysine-free diet, the threonine-free diet, the protein-free diet and the lactalbumin diet. The latter was estimated from prior studies (9) to supply approximately the minimal level of lactalbumin required for maintenance of body weight in adult rats. The lysine- and threonine-free diets contained no protein and all of the dietary nitrogen was supplied by the amino acid mixture shown in Table II. Lysine and threonine were omitted to produce the lysine- and threonine-free diets, respectively. The experimental period lasted 4 weeks during which time body weight and food intake were recorded weekly.

*Results.* The data on body weights and food intake during the 4-week adjustment

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[2] Chang, Y. O., unpublished data.

TABLE I. Composition of Diets.

| Ingredients | DIETS (g/kg) | | | | |
|---|---|---|---|---|---|
| | 1 Lactalbumin | 2 Protein-Free | 3 Lysine-Free | 4 Threonine-Free | 5 Casein |
| Vitamin-free casein | 0 | 0 | 0 | 0 | 200 |
| EAA mix | 0 | 0 | 11.4[a] | 11.9[b] | 0 |
| Non-EAA mix[c] | 0 | 0 | 21.2 | 21.2 | 0 |
| Lactalbumin | 22 | 0 | 0 | 0 | 0 |
| Cornstarch | 378 | 389 | 373.4 | 372.9 | 0 |
| Sucrose | 378 | 389 | 372.0 | 372.0 | 0 |
| Glucose | 0 | 0 | 0 | 0 | 578 |
| Spry | 165 | 165 | 165.0 | 165.0 | 165 |
| Salt mix[d] | 50 | 50 | 50.0 | 50.0 | 50 |
| Choline chloride | 2 | 2 | 2.0 | 2.0 | 2 |
| Vitamin mix[e] | 5 | 5 | 5.0 | 5.0 | 5 |

[a] Complete essential amino acid mixture minus lysine; [b] Complete essential amino acid mixture minus threonine; [c] Non-essential amino acid mixture.

[d] Hegsted, D. M., *et al.*, J. Biol. Chem. **138**, 459 (1941).

[e] Hegsted, D. M., *et al.*, J. Nutr. **92**, 403 (1967).

period when all animals received the same 20% casein diet are shown in Table III. As expected (1) the tail cups produced modest retardation in weight gain of approximately 20%. The food intake did not appear to be significantly affected.

Comparable data during the subsequent 4-week period when the animals received the various experimental diets are shown in Table IV. As in earlier experiments (5), threonine-free and protein-free diets produced a rapid and marked loss of body weight whereas the animals fed the lysine-free diet lost weight only slowly. The level of lactalbumin which was fed was slightly less than the amount required for the maintenance of body weight. At the end of the experiment, the animals which received the threonine- and protein-free diets were emaciated while those receiving the lysine-free diet or the lactalbumin diet appeared essentially normal. Comparable data were obtained whether the animals carried tail cups or not.

*Discussion.* As reported by previous inves-

TABLE II. Composition of Amino Acid Mixtures.

| Complete Essential Amino Acid Mixture (g/kg diet) | | Non-essential Amino Acid Mixture and Ammonium Citrate (g/kg diet) | |
|---|---|---|---|
| L-arginine HCl | 1.07 | L-cystine | 1.08 |
| L-histidine HCl | 0.90 | L-tyrosine | 0.95 |
| L-isoleucine | 1.75 | L-glutamic acid | 8.41 |
| L-leucine | 2.19 | Glycine | 4.29 |
| L-methionine | 0.50 | Ammonium citrate | 6.46 |
| L-phenylalanine | 1.34 | | |
| L-threonine | 1.50 | | |
| L-valine | 1.78 | | |
| L-tryptophan | 0.35 | | |
| L-lysine | 2.00 | | |
| TOTAL | 13.38 | | 21.19 |

TABLE III. Effect of Coprophagy Prevention on Growth and Food Intake in Young Rats Fed a 20% Casein Diet.[a]

| | Initial Weight (g) | Final Weight (g) | Total Gain Per Week (g) | Food Intake Per Week (g) |
|---|---|---|---|---|
| Without tail cups | 83 | 297 | 53.5 ± 2.5[b] | 144 ± 2.2 |
| With tail cups | 83 | 263 | 45.0 ± 1.2 | 137 ± 1.1 |

[a] Four weeks on experiment; [b] Mean ± SE.

tigators (1) the prevention of coprophagy in young growing animals resulted in some reduction in food intake and a depression in growth. In the adult animals the animals with tail cups lost slightly more weight than the comparable groups without tail cups but the differences in food intake or weight gain were not statistically significant for any of the diets fed. It may be noted that none of the diets investigated allowed the normal gain in weight which occurs in adult animals of this size.

The specific reason for these studies was to determine whether the modest loss in weight caused by feeding a lysine-free diet compared to the marked loss caused by the threonine-free diet might be due to the ingestion of lysine from the feces. It is apparent that the differences between the effects of the lysine-free and threonine-free diets persist whether the animals had tail cups or not. Thus, this possibility appears to be eliminated. The amount of lysine which animals obtain from their feces under these conditions must be too small to be of nutritional significance.

The reasons for the ability of animals to adapt to diets free of the various essential amino acids to varying degrees are unknown. Since it is not doubted that all amino acids must be present in the tissues at the sites of protein synthesis, it would appear that the animal must have mechanisms to conserve certain amino acids when they are in short supply or that deficiencies of various essential amino acids have specific effects upon the rates of protein synthesis or breakdown. No data are as yet available to distinguish between these possibilities but we consider the first as the more likely.

We would note that in this study as in previous studies[3] the animals fed the lysine-free diet had very fatty livers when they were killed at the end of the experiments.

[3] Said, A. K., and Hegsted, D. M., unpublished data.

TABLE IV. Effect of Amino Acid Deficiency and Coprophagy Prevention on Growth and Food Intake of Adult Rats.[a]

| | Diet | Initial Weight (g) | Final Weight (g) | Total Loss in 4 Weeks (g) | Food Intake Per Week (g) | Liver Weights (g) |
|---|---|---|---|---|---|---|
| | | | | With Tail Cups | | |
| 1 | Lactalbumin | 323 | 315 | − 8 ± 1.3[b] | 136.0 ± 3.8 | 12.8 |
| 2 | No protein | 289 | 230 | −59 ± 4.7 | 99.9 ± 4.3 | 11.8 |
| 3 | No lysine | 296 | 283 | −13 ± 2.4 | 122.2 ± 4.4 | 16.3 |
| 4 | No threonine | 301 | 241 | −60 ± 2.1 | 109.5 ± 5.8 | 8.7 |
| | | | | Without Tail Cups | | |
| 1 | Lactalbumin | 322 | 318 | − 4 ± 2.3 | 128.0 ± 3.8 | 13.2 |
| 2 | No protein | 307 | 251 | −56 ± 2.2 | 104.2 ± 3.4 | 9.0 |
| 3 | No lysine | 287 | 279 | − 8 ± 0.9 | 136.0 ± 5.0 | 13.0 |
| 4 | No threonine | 297 | 244 | −53 ± 2.7 | 97.0 ± 2.8 | 9.5 |

[a] Four weeks on experiment. [b] Mean ± SE.

Many investigators (10, 11) have reported that threonine- and protein-free diets produce fatty livers but this was less evident in these studies than the effects of lysine deficiency. This aspect is under further study. However, it does suggest that specific amino acid deficiencies may have specific effects upon certain organs and thus overall measures of performance, such as the maintenance of body weight or nitrogen balance, may be quite inadequate as a measure of amino acid requirements.

*Summary.* Animals were fed diets free of threonine, lysine or protein with or without tail cups to prevent coprophagy. The animals fed the threonine- and protein-free diets lost weight rapidly whereas those fed the lysine-free diet lost weight slowly regardless of whether coprophagy was prevented or not. It is concluded that the modest weight loss caused by the lysine-free diet is not due to the availability of lysine from fecal material.

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