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**Proteolytic Enzymes and the Question of Protein Storage.\***

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Many observers have noticed that the nitrogen excretion of an animal on a N-free diet will decline for long periods of time. This fact is difficult to explain on the basis of the depletion of a supply of "reserve" or "deposit" protein. The amounts of nitrogen excreted may account for a much larger storage of protein in the body than anyone has ever been able to demonstrate. That the decline in the rate of excretion of nitrogen may be attributed to a change in the mechanism for the catabolism of protein was suggested by the author several years ago.<sup>1</sup> The assumption is made that an animal with an enzyme system elaborated to catabolize excess dietary protein, which may very likely first be converted to tissue protein, will go on tearing down tissue protein when the supply of dietary protein is suddenly removed. Gradually, however, as the animal continues to live on a diet free of protein, the enzyme system will deteriorate and the catabolism of protein will decrease.

This contention is borne out by the data of Table I. These data show the arginase content of the livers of rats after living on a N-free diet more than 30 days, as compared with normal controls taken from a stock diet.

The method for arginase assay was a modification of that described by Lightbody.<sup>2</sup> A 30-minute digestion period at 37° and pH 9.4 was followed by aeration of the ammonia liberated at pH 5.0

TABLE I.

Rat pair No. and sex	29F	30M	32M	33M	34F
Days on the N-free diet	35	35	33	34	34
Body wt of N-free rat:					
Beginning, g	258	216	114	110	170
Final, g	180	185	70	72	133
Body wt of control	285	415	220	76	163
Arginase activity*					
N-free rat	0.14	0.13	0.12	0.08	0.14
Control rat	0.25	0.24	0.26	0.24	0.25

\*Arginase activity is expressed as mg of urea-N liberated per mg of fresh liver tissue during a digestion period of 30 min at 37°.

\* Research Paper No. 521, Journal Series, University of Arkansas.

<sup>1</sup> Ashworth, U. S., and Brody, S., *Missouri Res. Bull.*, 1933, No. 190.

<sup>2</sup> Lightbody, H. D., *J. Biol. Chem.*, 1938, **124**, 169.

by glycerol urease. Manganous sulfate was used as an activator. Every precaution was taken to treat identically the samples taken from the experimental and control livers. Of course the N-free animals lost considerable body weight while subsisting on the N-free diet. There was some question of whether to use controls weighing the same as the original weight of the experimental animals or the same as their final weight. However, when the arginase activity is calculated per unit weight of fresh liver it seems to matter little which weight control animals are used. The ages of N-free rats Nos. 32M and 33M were 68 days when they were killed. The control for 32M was also 68 days old (same litter) while the control for 33M was 27 days old but had about the same body weight. In each case there is considerably more arginase activity in the livers of the control animals.

Supplementary evidence is found in the fact that the proteolytic enzymes of the pancreas possess less activity after a month of nitrogen starvation. Assay was conducted by means of formol titration. Casein was the substrate. The slope of the titration curve was found by plotting the logarithms of the amounts of alkali used against the time of digestion at 37°. The average slope, measured in arbitrary units, was  $26 \pm 3$  for 11 N-free rats as compared with  $43 \pm 7$  for their 11 controls.

The conclusion is reached then that the arginase activity of the liver, the tryptic activity of the pancreas and presumably the activity of the other proteolytic enzymes of the body may be gradually lowered by the removal of dietary protein. This would account for the continued drop in the endogenous nitrogen excretion during long periods of nitrogen starvation, and would be in agreement with the hypothesis that there is no strictly minimum level of nitrogen excretion.