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Metabolism of Nitrogen and Acetone Bodies in Fasting Hypophysectomized Rats After Low Protein Diet.

REGINALD A. SHIPLEY.

From the Department of Medicine of Western Reserve University, School of Medicine, and the Lakeside Hospital, Cleveland, Ohio.

It has been known since the work of Voit[1] that nitrogen excretion during fasting is directly related to the protein content of the preceding diet. MacKay and his associates[2] have recently shown that in the rat the administration of a low protein diet is followed by a decreased excretion of nitrogen during fasting. The latter workers have attributed this finding to the impoverished supply of storage protein available for release. There is a question, however, whether protein may ever be regarded as an inert component of protoplasm, and it is therefore conceivable that when protein intake is restricted a mechanism for the conservation of cellular protein comes into prominence and that its influence persists during subsequent fasting. The well-known exaggeration of the ketosis of fasting after a low protein diet might depend upon the relative deficiency of anti-ketogenic substances derived from protein incident to a suppression in protein catabolism. Inasmuch as the anterior pituitary is intimately concerned with the anabolism of protein, it might be suspected that this increased ketosis and decreased nitrogen elimination are due to a persistence of pituitary hypersecretion in response to the threat of cellular protein depletion when the dietary intake of protein is low. In order to determine the part played by the pituitary in the metabolic adjustments resulting from a restricted protein intake, the response of hypophysectomized rats to fasting was observed in a group of animals receiving a low protein diet and was compared to the response of another group receiving a normal diet. For controls, 2 similar groups of unoperated rats were also observed.

Methods. All rats were of Sprague-Dawley strain and weighed between 96 and 135 g. The hypophysectomized animals were observed for stationary weight for one to 3 months before use, and the completeness of the operation was verified by serial microscopic sections. All of the unoperated control rats were males while about half of the operated animals on each diet were females. No sex difference in response was noted. Purina dog chow served as the normal diet. The low protein diet which was given for 2 to 4 weeks before fasting was composed of the following:

	%
Casein (Labco "Vitamin free")	8
Hydrogenated Cotton Seed Oil	38
Rice Starch	44
Cod Liver Oil	2
Brewers' Yeast (Anheuser-Busch Strain G)	3
Salt Mixture (McCollum and Simmonds No. 185 with added fluoride, iodide, and manganese)	5

The body weight of both operated and unoperated rats remained practically constant while this diet was being given.

After a 24-hr fast a sample of tail blood was taken from all animals and analysis for the total content of blood acetone bodies was made by a micro-method.[3] During the second 24 hr, the urine was collected in small glass metabolism chambers and the total nitrogen excretion determined. Animals that did not survive more than a day after cessation of the fast were excluded.

Results. It may be observed from Table I that like normal rats the hypophysectomized animals excreted less nitrogen and exhibited greater ketonemia after a low protein diet than after a normal diet. It is therefore

[1] Voit, C., *Z. f. Biol.*, 1866, **2**, 307.

[2] MacKay, E. M., Carne, H. O., Wick, A. N., and Visscher, F. E., *J. Biol. Chem.*, 1941, **141**, 889.

[3] Shipley, R. A., and Long, C. N. H., *Biochem. J.*, 1938, **32**, 2242.

TABLE I.
Blood Acetone Body Levels and Excretion of Nitrogen.

	No. of rats	Blood acetone bodies (mg%)	No. of rats	Nitrogen excr. mg/100 g—24 hr	% loss body weight—48 hr
Normal Rats					
Dog Chow Diet	18	10.8 ± 1.5*	9	124 ± 5.8	23 ± 0.66
Low Protein ''	17	17.5 ± 2.6	13	84 ± 3.4	11 ± 0.43
Hypophysectomized Rats					
Dog Chow Diet	29	4.6† ± 0.40	15	93 ± 3.5	12 ± 0.32
Low Protein ''	28	10.1‡ ± 0.85	11	73 ± 4.6	8 ± 0.40

*Standard error of the mean.
†Avg level of 7 of these rats before fasting 0.4 ± 0.04.
‡Avg level of 9 of the group before fasting 1.0 ± 0.5.

evident that these metabolic responses to protein restriction are not entirely dependent upon the hypophysis but must depend at least in part upon adjustments within tissues elsewhere. It may be noted that the hypophysectomized animals on both diets showed less ketonemia than the corresponding control rats. This cannot be due to increased protein catabolism inasmuch as the nitrogen excretion was lower in the operated animals. The decrease could be due, however, to the absence of the hypophyseal ketogenic principle. The presence of a definite ketosis after hypophysectomy indicates that during fasting the ketogenic principle of the pituitary is not indispensable to the operation of the ketogenic mechanism as a whole. Oastler and Anderson[4] have observed that a high degree of ketosis can be induced by fat feeding in the hypophysectomized rat.

Weight loss differed significantly among the several groups during fasting. The hypophysectomized rats lost less weight than did the animals in the unoperated groups. This is undoubtedly attributable to the low basal metabolism which follows hypophysectomy. The relatively small weight loss of both normal and hypophysectomized animals after a low protein diet is striking. Protein oxidation is decreased in these animals and fat oxidation presumably is high. The greater energy which is supplied by the oxidation of fat in preference to protein could account in part for the comparatively slight decline in body weight. Substitution of an equicaloric weight of fatty tissue for muscle would require the consumption of only one-tenth the mass of body substance. The decreased weight loss after a low protein diet may also be due in part to the decreased metabolism which has been observed in rats receiving a diet of this nature.[5]

Conclusions. Hypophysectomy does not prevent the decrease in nitrogen excretion or increase in ketosis during fasting after a low protein diet. These responses therefore have their origin at least in part in other tissues.

The technical assistance of Miss Ethel Buchwald is gratefully acknowledged.

[4] Oastler, E. G., and Anderson, A. B., *Biochem. J.*, 1939, **33**, 1094.

[5] Horst, K., Mendel, L. B., and Benedict, F. G., *J. Nutrition*, 1934, **8**, 139.