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Protein and Amino Acid Feeding upon Creatine Formation in Muscle, and Creatinine Elimination in Urine.

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It is generally believed that creatine is a tissue constituent with a special function and that it arises in the body as a result of a specific cellular demand for it. There is also much evidence to show that it may also be derived from certain precursors, *e. g.*, arginine,[1] glycine,[2] cystine,[3] and histidine,[4] in the diet.

About 3 years ago, using young rats and mice, it was observed in this laboratory that the feeding of d-arginine monohydrochloride gave slightly larger increases in muscle creatine than creatine itself, when each of these substances formed 5% of the standard casein diet. The possibility that this amino acid was one of the precursors of creatine in the animal body was suggested. With positive evidence for the other amino acids mentioned above, a systematic study was begun to determine the influence of feeding proteins, amino acids, and related substances upon creatine formation in the muscles, and creatinine elimination in the urine, the results of which are presented below.

Young rats were placed on Sherman's Diet B for a period of 10 days after weaning. Amounts of purified amino acids up to 1.5 gm. were fed either as such or mixed with a small amount of the stock diet. In other experiments casein or edestin were fed. At the end of 17 to 48 hours the animals were killed and the muscle creatine determined by the method of Rose, Helmer and Chanutin.[5] The litter mate control animals received no amino acid or protein supplement. The average creatine content of the muscles of 118 control rats was 0.40%. The average results obtained are given in Table I.

The effect of amino acid feeding upon creatinine elimination in the urine was next studied with the hope that further light might be thrown upon the origin and metabolism of creatine. Rats weighing between 200 and 300 gm. were used. They were fed on Sher-

[1] Knoop, F., *Z. physiol. Chem.*, 1910, **67**, 489.

[2] Brand, E., Harris, M. M., Sandberg, M., and Ringer, A. I., Abs. 13th International Physiol. Congress, Boston, August, 1929.

[3] Harding, V. J., and Young, E. G., *J. Biol. Chem.*, 1920, **41**, xxxvi.

[4] Abderhalden, E., and Baudze, S., *Z. physiol. Chem.*, 1930, **189**, 65.

[5] Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

TABLE I.
Influence of Feeding Proteins, Amino Acids or Related Substances upon Creatine Formation in Rat Muscle.

Substance Fed	No. of Rats	Creatine, %	Increase Over Controls, %
Glycine	9	0.46	15.0
dl-Alanine	17	0.45	12.5
dl-Valine	6	0.54	35.0
d-Glutamic acid	8	0.48	20.0
l-Aspartic acid	10	0.47	17.5
l-Cystine	10	0.55	37.5
Histidine	4	0.49	22.5
dl-Phenylanine	5	0.49	22.5
l-Tyrosine	10	0.49	22.5
d-Arginine-HCl	11	0.51	27.5
l-Leucine	4	0.49	22.5
Choline-HCl	6	0.49	22.5
Glycocyamine	3	0.59	47.5
Casein	6	0.52	30.0
Edestin	6	0.52	30.0
Creatine	8	0.49	22.5

man's diet B and a 2-day control output of creatinine determined. On the morning of the third day, 1 or 1.5 gm. of a purified amino acid was fed as above, the stock diet removed, and replaced again when practically complete consumption of the acid was observed, and a second 2-day experimental creatinine output determined. All conditions were uniform throughout and the results obtained are due to the amino acid feeding. See Table II.

Further evidence confirming these results was obtained with several students and members of the laboratory staff, including the writer. The subjects were on a meat-free diet. The following supplements were taken in milk, 100 gm. casein; 100 gm. edestin; 5 gm. arginine-HCl; 8 gm. glycine; 10 gm. alanine. A rest period of at least 4 or 5 days always preceded the 24-hour experimental period. With one or 2 exceptions a definite and large increase in the daily elimination of creatinine was observed.

TABLE II.
Influence of Amino Acid Feeding upon Creatinine Elimination.

Amino Acid Fed	No. of Experiments	Average Increase in the Excretion of Creatinine %
Glycine	21	35.9
l-Aspartic Acid	11	14.2
d-Arginine-HCl	7	26.8
dl-Alanine	8	28.0
l-Cystine	8	28.1
dl-Valine	5	14.8
l-Tyrosine	8	30.7
d-Glutamic Acid	9	25.1
Histidine	6	19.5

Evidence was obtained which shows that the results of these studies were not due to a stimulation of the endogenous metabolism nor to the specific dynamic action of the proteins or amino acids.

The following conclusions were drawn: Both creatine and creatinine were formed, under the conditions of these experiments, as a result of an increased exogenous protein or amino acid metabolism per unit of time. Muscle creatine is an intermediate product and urinary creatinine a waste product of this metabolism. Evidence was obtained which showed that creatine may also have an endogenous origin from amino acids.

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Cure of Rickets by Water Soluble Extract of Yeast and Sodium Phosphate.

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Five albino rats, 28 days old, were removed from the mother to a dark room and placed in individual cages. The rats were of a known inbred stock of laboratory animals which had been under observation for 2 years. The animals were placed on Steenbock's Rachitogenic Diet No. 2965* and given distilled water only. This diet was continued for 30 days, when the animals were X-rayed, and all were found to have advanced rickets.

They were then returned to the dark room and fed the following diet: 960 gm. of Steenbock's Rachitogenic Diet No. 2965, thoroughly mixed with 40 gm. of $Na_2HPO_4 \cdot 12H_2O$. In addition one gram of a water soluble extract of yeast was added to each day's feeding. The water soluble extract of yeast was made by filtering cold double distilled water through brewers' yeast and evaporating the filtrate to a gum. This material was then dried by repeated trituration with absolute alcohol. The residue left after evaporation of the alcohol was combined with the original material and the whole powdered. This water soluble extract gave a negative test for sterols after it had been shaken out with chloroform and treated

* Steenbock's Rachitogenic Diet consists of: 76% whole yellow corn; 20% gluten; 3% CaCl; 1% NaCl.