one and 2% trypsin at pH 8, 60% of the total iodine was rendered acid soluble in 4 hours. With each enzyme, about 70% of the total hydrolysis into acid soluble and acid insoluble iodine fractions occurred during the first 15 minutes of digestion. Incubation of the desiccated thyroid in human gastric and duodenal juice resulted in the liberation of the acid soluble iodine at the same rate as when concentrated solutions of potent enzymes were used.

Since the liberation of amino and carboxyl groups in such digests continues for a number of days,[3, 4] it would appear that one of the first steps in the digestion of desiccated thyroid is the severing of linkages binding the 2 iodine fractions.

The very rapid hydrolysis of desiccated thyroid in peptic and tryptic solutions and in human digestive juices would seem to indicate that therapeutically administered desiccated thyroid is rapidly hydrolysed into acid soluble and acid insoluble iodine fractions.

These data do not seem to support the suggestion of Lerman and Salter[5] that the whole thyroglobulin molecule "is probably absorbed" from the gastrointestinal tract "for the most part unchanged."

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Influence of Glycine on Depleted Creatine Reserves of Skeletal and Cardiac Muscle in Experimental Hyperthyroidism.*

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The use of glycine in the treatment of muscle disease is based partly on the assumption that it is a precursor of creatine, a number of investigators sharing the view that the transformation of glycine into creatine is direct. In support of the contention that creatine is a product of exogenous protein, or amino acid metabolism, Beard and Boggess[1] have recently presented data to show that the creatine content of muscle of rats may be increased 20 to 56% from the low levels caused by restricted protein intake by refeeding these animals on 25% casein, or egg albumin, or 21%

[3] Harington, C. R., and Salter, W. T., *Biochem. J.*, 1930, **24**, 456.

[4] Barnes, B. O., Carlson, A. J., and Riskin, A. M., *Am. J. Physiol.*, 1931, **98**, 86.

[5] Lerman, J., and Salter, W. T., *Endocrinology*, 1934, **18**, 317.

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[1] Beard, H. H., and Boggess, T. S., *Am. J. Physiol.*, 1935, **113**, 647.

glycine, or glutamic acid for a period of 4 weeks. This procedure, which presumably first restricts the intake of creatine precursors and subsequently provides a liberal supply of the same was adopted in pursuit of Rose's suggestion[2] that the origin of an anabolic substance may perhaps be better studied by limiting the intake of a suspected precursor below the required level, rather than by adding excessive quantities to a diet already carrying sufficient amounts to meet the needs of synthesis.

The logic of this approach to the problem is evident although it is to be observed that an increase in the concentration of creatine in muscle and other tissues occurs after feeding creatine.[3] According to Beard and Barnes[4] the same effect may be obtained in normal rats by feeding various amino acids and protein. MacKay[5] found that feeding glycine and *dl*-alanine definitely raised the creatine content of the myocardium, while others,[6] in rabbit-heart perfusion experiments, observed that the addition of glycine to the perfusion fluid produced little, if any, effect, but that alanine caused a definite increase in heart creatine.

In agreement with the work of Brand[7] and others in man and similar studies in experimental animals, one of us has shown[8] that the administration of glycine increases the excretion of creatine in rats. The possibility that this may have no relation to actual production of creatine was emphasized and is further supported by the observation that with the exception of the kidney the creatine content of the tissues is not increased (unpublished data), but on the contrary there is a tendency to loss of creatine from muscle, this occurring in experiments of both short and long duration.

As shown previously[9, 10] hyperthyroidism is associated with a moderate reduction of muscle creatine and a much greater loss from the myocardium. Because of the depleted reserves it was supposed that the tendency to form creatine from its precursors and its retention would perhaps be more readily demonstrable than

2 Rose, W. C., *Ann. Rev. Biochem.*, 1933, **2**, 187.

3 Chanutin, A., and Silvette, H., *J. Biol. Chem.*, 1928, **80**, 589.

4 Beard, H. H., and Barnes, B. O., *J. Biol. Chem.*, 1931-32, **94**, 49.

5 MacKay, E. M., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1562.

6 Decherd, G., Herrmann, G., and Davis, O., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1302.

7 Brand, E., Harris, M. M., Sandberg, M., and Ringer, I., *Am. J. Physiol.*, 1929, **90**, 296.

8 Bodansky, M., *J. Biol. Chem.*, 1936, **112**, 615.

9 Bodansky, M., *J. Biol. Chem.*, 1935, **109**, 615.

10 Bodansky, M., Pilcher, J. F., and Duff, V. B., *J. Exp. Med.*, 1936, **63**, 523.

normally. The present report is based on a study of 68 hyperthyroid rats, the data in the accompanying table representing a number of typical experiments.

Rats 271, 278 and 282 received daily doses of 1 mg. of thyroxine (Hoffman-LaRoche). Muscle was removed for analysis as indicated. Detailed analyses of the urine revealed that following the operation the daily excretion of creatine and creatinine continued at approximately the same level, or slightly above that before the operation. Obviously the continued loss of creatine in these and similar experiments was uncompensated, as the creatine content of the muscle obtained at the conclusion of the experiment was almost invariably lower than the creatine content of the biopsy tissue. The one exception occurred in the case of Rat 282, which died one day after the operation as a result of the exertion incidental to the passage of a stomach tube. The tendency to heart failure in hyperthyroid rats has been commented upon elsewhere.[9, 10, 11] In all 3 animals the creatine concentration of the myocardium had fallen to approximately 50% of normal.

Rats 273 279, 281, 286, 326, and 407 received thyroxine throughout the experimental period. Glycine, one gm. daily, was given by stomach tube following the biopsy as indicated. The addition of amino acids and other substances to the rations was abandoned, because in our experience many rats are sensitive to such changes and consequently eat less food than usual. Analyses of the urine showed an inconstant effect, the excretion of creatine exhibiting wide daily fluctuations, being often depressed sufficiently to suggest a tissue-sparing action. In the aggregate, however, the post-operative excretion of creatine and creatinine exceeded the preoperative excretion. As the concentration of muscle creatine continued to diminish it may be surmized that if any were formed from the administered glycine, it must have been completely destroyed.

Work in this laboratory suggests that the maintenance of the creatinine reserve of muscle, especially of the myocardium, is intimately dependent on the maintenance of an adequate glycogen reserve. The daily administration of one gm. of glucose to the hyperthyroid rats (323, 324, 405) seemed to exert a sparing effect on the tissues. There was a definite lowering of the creatine output, despite the continued administration of thyroxine. However, there was no demonstrable improvement of the creatine content of the

11 Bodansky, M., and Pilcher, J. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 597.

muscle, but considering the longer survival of these animals, the rate of depletion was perhaps less rapid.

In the rats receiving creatine (275, 283), the tendency to further depletion seemed to be checked somewhat, an effect that occurred more consistently when glucose was given in addition (Rat 329). Only about 40% of the administered creatine was recovered in the urine. As the remainder did not accumulate in the tissues, it may be concluded that a considerable part followed other paths in metabolism.[12] It should be noted that the analyses outlined in Table I were of animals that died, (+) or were sacrificed (S) 18 to 24 hours after the last administration of creatine, glycine, etc.

Guanidine acetic acid produced an effect comparable to that of creatine (Rats 285, 403). This becomes more evident if allowance is made for the considerable proportion of the acid that is excreted unchanged. The extra daily output of creatine due to the guanidine acetic acid was 7-9 mg. in Rat 285 and 4-5 mg. in Rat 403.

When the administration of thyroxine was discontinued, the creatine concentration of the myocardium was rapidly restored, while in the skeletal muscle little change was noted (Rat 327). When creatine was administered during the convalescent period, the concentration in the muscle increased, but restitution in the myocardium was not especially accelerated, comparable results being obtained in the controls, or when glucose was given. As regards the tissue changes convalescence was not strikingly different in the rats receiving glycine than in their controls. In the case of Rat 322, which showed a sharp increase in heart creatine, but no change in the skeletal muscle, the excretion of creatine and creatinine remained practically unaltered during the first 6 days of convalescence, then diminished somewhat. This is in contrast to the reduced creatine output which occurred almost immediately in the control rats and especially in those receiving glucose.

Conclusions. The results of these experiments offer no evidence of the conversion of glycine into creatine. That this amino acid is not a precursor of creatine is indicated by some of the data, but admittedly more conclusive evidence is needed to establish this view. Difficulty in the interpretation of the results is due partly to the fact that the fate of creatine is not limited to storage, excretion, and conversion into creatinine. A large part is used up

[12] Compare with Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1923, **56**, 29; and Rose, W. C., Ellis, R. H., and Helming, O., *J. Biol. Chem.*, 1928, **77**, 171.

TABLE I.

Rat No. and Sex	Creatine in muscle, mg. per 100 gm.		Myocardium			Creatine, mg. per 100 gm.		Thyroxine	Biopsy	Remarks
	Biopsy specimen	Postmortem specimen	Wt. of ventricles, gm.	Creatine in ventricles, mg. per 100 gm.	Creatine in ventricles, mg.	Liver	Testes			
271 ♂	413	384	1.127	88	.99	24	313	1/9-1/28(+)	1/26	
278 ♀	475	398	.995	101	1.01	42		1/9-2/2(+)	1/26	
282 ♀	414	430	.826	111	.92	39		1/9-1/30(+)	1/29	
273 ♂	328	278	.929	94	.87	29	353	1/9-1/30(+)	1/26	Glycine, 3 daily doses of 1 gm.
279 ♀	420	383	.923	113	1.05	42		1/9-2/7(+)	1/29	" 6 " " " "
281 ♀	423	356	.918	93	.85	29		1/9-2/2(+)	1/30	" 2 " " " "
286 ♀	432	366	.965	94	.91	37		1/9-2/9(+)	2/7	" 2 " " " "
326 ♀	484	435	.980	105	1.03	18		2/8-3/22(S)	3/13	" 7 " " " "
407 ♂	468	388	1.081	99	1.07	38	398	3/15-3/31(+)	3/28	" 3 " " " "
323 ♀	458	370	1.103	89	.98	31		2/8-3/24(+)	3/10	Glucose, 13 " " " "
324 ♀	475	379	.97	107	1.04	22		2/8-3/23(+)	3/11	" 12 " " " "
405 ♂	473	414	1.02	108	1.10	25	355	3/15-4/5(+)	3/28	" 8 " " " "
275 ♂	399	382	1.11	81	.90	25	341	1/9-2/29(+)	1/26	Creatine hydrate, 1 daily dose 100 mg.
283 ♀	464	440	.83	130	1.08	29		1/9-2/3(+)	1/29	" " 3 " doses " "
329 ♀	467	400	1.06	124	1.31	17		2/8-3/26(S)	3/14	" " 10 " " " " and glucose, 10 daily doses 1 gm.
285 ♀	450	438	1.07	113	1.21	21		1/9-2/12(+)	1/30	Guanidine acetic acid 10 daily doses 100 mg.
403 ♂	472	445	1.04	105	1.09	39	367	3/15-4/2(+)	3/28	Guanidine acetic acid 4 " " 100 "
327 ♀	435	446	.88	158	1.39	17		2/8-2/26(S 3/6)	2/26	
321 ♀	451	486	.96	143	1.37	18		2/8-3/4(S 3/14)	3/5	Glucose, 8 daily doses 1 gm.
322 ♀	434	436	.92	178	1.64	18		2/8-2/27(S 3/6)	2/27	Glycine, 8 " " " "
292 ♀	425	488	.99	144	1.43	19		1/30-3/4(S 3/15)	3/5	Creatine hydrate, 7 daily doses 100 mg.

in other ways. Moreover, in hyperthyroidism, as in certain muscle diseases,[13] the capacity of muscle to take up and retain creatine is deficient, even though the concentration may be considerably below normal.

[13] Bodansky, M., Schwab, E. H., and Brindley, P., *J. Biol. Chem.*, 1929, **85**, 307.