nine could be replaced by citrulline but ornithine was not active. When arginine was deleted, the typically rounded cell of Novikoff hepatoma was no longer apparent; but a predominance of fibroblasts remained attached to the surface of the flasks.

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Creatine Metabolism in Hyperthyroidism.* (24543)

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Present knowledge of the influence of hyperthyroidism on creatine metabolism has been summarized(1). It was pointed out that the disease leads to an elevated creatinuria and reduction in concentration of muscle creatine. Several possible explanations for this metabolic defect were suggested although no direct experimental evidence supported them. The present report presents the results of a study of creatine metabolism in hyperthyroidism utilizing radioactive creatine precursors in a manner similar to that employed in a study of creatine metabolism in Vit. E deficiency (3).

Methods. Two series of experiments were conducted. In the first, Sprague-Dawley rats of both sexes, weighing initially between 80 and 100 g were given a diet of laboratory chow. Four rats were given daily subcutaneous injections of 0.5 mg of sodium thyroxine. Four other rats were given no injections and served as controls. After 3 weeks the rats were each injected intraperitoneally with 100 microcuries of glycine-1-C^{14} (specific activity 0.81 microcuries per μmole) per kilo of body weight. The animals were killed 2 or 3 hours after the injection. Since there appeared to be no significant difference in results obtained from animals killed 2 hours after glycine injection as compared to those killed after 3 hours, the results were combined. Glycocyamine and creatine concentrations and specific activities were determined as previously described(3). In the second series of experiments, weanling Sprague-Dawley rats of both sexes were given a purified diet consisting of casein, 18 g; sucrose, 74.5 g; hydrogenated vegetable fat (Crisco), 3 g; cod liver oil, 2 g; salt mix(2), 2 g; choline chloride, 0.1 g; thiamine chloride, 1.5 mg; riboflavin, 1.5 mg; niacin, 6 mg; inositol, 30 mg; calcium pantothenate, 3 mg; pyridoxine hydrochloride, 1.5 mg; biotin, 15 μg; 2 methyl 1, 4 naphthoquinone, 75 μg; Vit. B_{12}, 10 μg. Each rat was given 10 mg alpha

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TABLE I. Some Metabolic Effects of Hyperthyroidism of Rats.

Animals	Avg wt gain	Avg food intake	Urinary excretion, mg/100 g body wt /day		Organ wt, mg/g body wt		Heart creatine	Skeletal muscle creatine	Kidney glycocyamine
	g/day		Creatinine	Creatine	Heart	Kidney	mg/100 g		
Normal	3.9	9.2	4.61	.52	4.3	8.0	222	389	8.61
Hyperthyroid	2.9	8.7	5.33	.84	8.8	11.3	115	349	13.95
P value*	<.001	<.3	<.025	<.001	<.001	<.4	<.001	<.20	<.10

* Probability that differences observed are due to chance.

tocopherol 2 times weekly. One group was given this diet without supplement and will be referred to as normal while a second group was given in addition 2.0% desiccated thyroid (Nutritional Biochemicals Corp.). After 2 weeks' feeding, the animals receiving the desiccated thyroid exhibited the usual signs of hyperthyroidism. Four animals from each group were then injected with glycine-1-C^{14} at the same dosage as used for series 1. Two animals from each group were killed 30 minutes after glycine injections and 2 after one hour. Glycocyamine and creatine concentrations and specific activities were determined as previously described(3). Skeletal muscle protein specific activity was determined after repeated precipitation with trichloroacetic acid. Eight animals from each group in the second series were injected intraperitoneally with 0.5 microcuries of creatine-1-C^{14} (specific activity 10 microcuries per mg) per 100 g body weight. Within each group 3 animals were killed one hour after creatine injection, 3 after 2 hours and 2 after 4 hours. Blood was collected at this time and the C^{14} counts of the serum were measured. Skeletal muscle and heart creatine were then isolated for counting(3). During the last day of the feeding period animals were placed in metabolism cages and urine was collected for creatine and creatinine determination(4). The data were analyzed for statistical significance by the t-test(5).

Results. Specific activities of kidney glycocyamine and skeletal muscle creatine from hyperthyroid rats fed chow diet and injected with glycine-1-C^{14} were reduced to approximately ¼ the values obtained with normal rats. The reduced specific activity of kidney glycocyamine in the hyperthyroid rats may be related to the time interval between glycine injection and sacrifice of the animals. Other experiments have indicated that kidney glycocyamine specific activity reaches a peak within a few minutes after glycine injection. In spite of the reduced specific activity of kidney glycocyamine, liver creatine from hyperthyroid rats exhibited an elevated specific activity. These results suggest that rate of creatine synthesis is increased in hyperthyroidism. The reduced specific activity of skeletal muscle creatine from hyperthyroid rats indicates that in hyperthyroidism rate of entry of creatine into skeletal muscle is depressed.

The data in Tables I through III were obtained in the second series of experiments. The hyperthyroid animals gained less weight although food intake was not significantly reduced. Creatine excretion was elevated in the hyperthyroid rats as would be expected; creatinine excretion was unaffected. There was a striking increase in heart weight of the hyperthyroid rats with a decrease in concentration of heart creatine. Skeletal muscle creatine concentration was only slightly reduced.

The data in Table II confirm the findings made in the first series of experiments. The results indicate that hyperthyroidism in the rat leads to an elevated rate of creatine synthesis with reduction in incorporation of creatine into skeletal muscle. Incorporation of creatine into cardiac muscle was also depressed in hyperthyroid rats. Hyperthyroidism led to an increased incorporation of glycine into skeletal muscle protein. This result indicates an elevated rate of protein turnover in muscle of hyperthyroid rats which may explain the well-documented increase in uri-

TABLE II. Incorporation of Glycine-1-C^{14} into Protein, Glycocyamine and Creatine by Normal and Hyperthyroid Rats.

Interval after inj., hr	Animals	Kidney glycocyamine	Liver creatine	Skeletal muscle creatine	Heart creatine	Skeletal muscle protein, c.p.m./mg
		c.p.m./μmole				
½	Normal	1697	57	19	66	7.2
½	Hyperthyroid	656	64	13	38	11.2
1	Normal	1397	27	27	88	15.3
1	Hyperthyroid	551	36	15	48	18.3

nary nitrogen in this disease(1).

The data obtained from creatine-C^{14} injections are given in Table III. Serum creatine-C^{14} was considerably elevated in the hyperthyroid rats at all time intervals. There was essentially no effect of hyperthyroidism on specific activity of muscle creatine. If muscle creatine specific activity is divided by serum creatine-C^{14}, which presumably is the immediate precursor of muscle creatine, then rate of incorporation of creatine into muscle would appear to be considerably reduced in the hyperthyroid rats.

From the combined results of these experiments it appears that in hyperthyroidism the rate of synthesis of creatine is elevated while incorporation of creatine into skeletal muscle and cardiac muscle is considerably depressed. This situation may be contrasted with Vit. E deficiency in which the creatinuria is due to an inability of muscle to retain creatine after its incorporation. In the latter condition the entry of creatine into the muscle proceeds at a greatly increased rate(3). Thus the creatinuria of hyperthyroidism and Vit. E deficiency are the result of fundamentally different metabolic lesions.

TABLE III. Metabolism of Creatine-1-C^{14} by Normal and Hyperthyroid Rats.

Interval after inj., hr	Animals	Serum C^{14}, c.p.m./ml	Skeletal muscle creatine	Heart creatine
			c.p.m./μmole	
1	Normal	20	82	91
1	Hyperthyroid	87	76	109
2	Normal	23	92	110
2	Hyperthyroid	45	101	107
4	Normal	13	120	125
4	Hyperthyroid	35	119	188

Summary. Hyperthyroidism was induced in rats by thyroxine injections and by feeding of desiccated thyroid. Normal and hyperthyroid rats were injected with glycine-1-C^{14} and incorporation of the isotope into glycocyamine and creatine was determined. In other experiments normal and hyperthyroid rats were injected with creatine-1-C^{14} and its incorporation into skeletal muscle and heart creatine was determined. Hyperthyroidism resulted in an increased creatinuria, and increased heart weight and a decreased heart creatine concentration. Hyperthyroidism led to increased incorporation of glycine into liver creatine and to decreased incorporation into skeletal muscle and heart creatine. It is concluded that the creatinuria of hyperthyroidism is the result of a block in incorporation of creatine into skeletal muscle and cardiac muscle.

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