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Phosphate Metabolism in Nutritional Muscular Dystrophy and Hyperthyroidism.* (24572)

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Impairment of phosphorylation of creatine has been suggested as the cause of creatinuria of both hyperthyroidism and Vit. E deficiency (1,2). In hyperthyroidism it is possible to demonstrate *in vitro* the uncoupling of oxidative phosphorylation in mitochondria(3), but recent evidence indicates that this may be due to a primary effect on the mitochondrial membrane(4). A method for measuring oxidative phosphorylation *in vivo* is not available, but it is possible to determine the entry of radioactive phosphorus into tissue phosphates. This approach is used in the following experiments to measure phosphorylation of creatine.

Methods. Vit. E deficiency was produced in white male New Zealand rabbits, weighing approximately 500 g, and in weanling Sprague-Dawley rats of both sexes by feeding previously described purified diets deficient only in Vit. E(5,6). Oral supplementation of control animals with Vit. E and general handling of animals were also the same as in previous studies(5,6). Rabbits on the deficient diet exhibited signs of muscular dystrophy after 3 to 4 weeks, then were used in experiments. The group of rats given Vit. E-deficient diet exhibited marked creatinuria and significant decrease in growth rate when compared to normal rats, and there was microscopic evidence of muscular dystrophy after 7 months of feeding, then chosen for the experiments. Two other groups of weanling Sprague-Dawley rats of both sexes were placed on regular laboratory chow diet. One group served as controls while hyperthyroidism was induced in the other group by daily subcutaneous injections of 0.5 mg of sodium thyroxinate for 21 days. The thyroxine-injected rats exhibited usual signs of hyperthyroidism when used in experiments. All animals were given 0.4 mc of $P^{32}O_4$ in dilute HCl/kilo body weight. Specific activity of the $P^{32}O_4$ was approximately 100,000 mc/g. Control and Vit. E-deficient rabbits were given P^{32} intravenously and killed 30 minutes later by large intravenous doses of pentobarbital. Immediately after death the animals were transferred to the cold room where samples of blood and skeletal muscle were obtained for analysis. The rats were handled in the same manner except that injections were given intraperitoneally. Control and Vit. E-deficient rats were killed 30 minutes after injection of P^{32} and control and hyperthyroid rats were killed one hour following injection of P^{32}. The scheme of fractional hydrolysis and precipitation of tissue phosphates devised by Sacks(7) was used to isolate serum inorganic phosphate, skeletal muscle inorganic phosphate, and the phosphate of creatine phosphate and adenosinetriphosphate (ATP). Portions of each specimen were used for phosphorus determination by the method of Fiske and SubbaRow and for counting with an end window Geiger counter.

Results. The skeletal muscle inorganic phosphate concentrations were in good agreement with the values given by Threlfall(8) and no differences were observed between the

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TABLE I. Mean Specific Activities (c.p.m./μmole P) of Plasma and Skeletal Muscle Phosphates.

Exp. group	No. of animals	Time after inj., min.	Plasma inorg. PO_4	Skeletal muscle inorg. PO_4	Creatine PO_4	ATP PO
Control rabbits	5*	30	49,410	1,559	586	689
Vit. E-def. rabbits	5*	30	50,057	5,023	1,425	2,265
P value†				<.01	<.05	<.01
Control rats (chow diet)	7	60	39,024	2,511	770	860
Hyperthyroid rats	5	60	27,215	2,828	1,312	1,510
P value				>.05	>.05	<.01
Control rats (purified diet)	4	30	85,225	2,528		
Vit. E-def. rats	4	30	93,033	5,495		
P value				<.01		

* Only 3 animals were used for isolation of plasma inorganic phosphate in these rabbits.
† Probability that differences observed were due to chance as determined by the t test.

various experimental groups.

Table I gives specific activities (S.A.) of the various phosphates. Plasma inorganic phosphate S. A. was altered appreciably only by hyperthyroidism and in each instance S. A. was considerably greater than that of tissue phosphates. This indicates that an equilibrium with tissue phosphates had not been reached.

Skeletal muscle inorganic phosphate S. A. was significantly increased in both Vit. E-deficient rabbits and rats, and unchanged in the hyperthyroid rats. S. A. of phosphate of ATP and creatine phosphate was significantly increased in hyperthyroid rats as well as in Vit. E-deficient rabbits.

Discussion. Perhaps the most important finding is the high skeletal muscle inorganic phosphate S. A. in Vit. E-deficient rabbits and rats, with no change in serum inorganic phosphate specific activities. The difficulty with interpreting this observation has been generally recognized in similar studies involving isolation of tissue phosphates(9.10). It should be noted that the usual correction for skeletal muscle extracellular space has not been made in recording our data. The validity of such a correction is doubtful, but if applied, it would accentuate the differences between control and Vit. E-deficient animals. Assuming that rate of transfer of inorganic phosphate from blood to the extracellular fluid is not affected by Vit. E deficiency and that size of extracellular space is unchanged, the finding of a high skeletal muscle inorganic phosphate S. A. indicates a high rate of turnover between extracellular and intracellular phosphate since the rate of esterification of phosphate was not reduced.

The high S. A. of the phosphates of creatine phosphate and ATP phosphate in Vit. E-deficient rabbits agrees with the report of Ferdman(11), but probably only reflects a high intracellular inorganic phosphate S. A. If the skeletal muscle inorganic phosphate S. A. is accepted without correction for the extracellular inorganic phosphate, there is no decrease in turnover of creatine phosphate or ATP. On the other hand if a correction were made for extracellular phosphate it would be possible to conclude that there is a decreased turnover of creatine phosphate and ATP. It is therefore necessary to state that rate of phosphorylation of creatine and ATP in skeletal muscle is unchanged or decreased by Vit. E deficiency in the rabbit.

The phosphates of creatine phosphate and ATP also have a high S. A. in hyperthyroid rats. In this case interpretation of the data is less difficult because, even after correction for the extracellular phosphate, the turnover of these phosphates still appears increased. From this, it is concluded that there is no impairment of the phosphorylation of creatine and ATP in skeletal muscles of hyperthyroid rats.

Summary. 1) Rabbits and rats were made Vit. E-deficient by feeding a purified diet. Control animals received the same diet supplemented with Vit. E. Hyperthyroidism was

induced in rats by giving subcutaneous injections of sodium thyroxinate. Control and experimental animals from each group were injected with P^{32} in dilute HCl and specific activities of plasma inorganic phosphate, and skeletal muscle inorganic phosphate, creatine phosphate, and ATP phosphate determined. The S. A. of the plasma inorganic phosphate was unchanged by Vit. E deficiency and was decreased by hyperthyroidism. Skeletal muscle inorganic phosphate S. A. was increased by Vit. E deficiency and the difficulties in interpreting these data were discussed. The S. A. of phosphates of creatine phosphate and ATP phosphate were high in both Vit. E deficient rabbits and hyperthyroid rats. The significance of these findings was discussed in relation to turnover of creatine phosphate and ATP. 2) These experiments indicate that there is an elevated turnover of skeletal muscle intracellular inorganic phosphate and a normal or decreased turnover of skeletal muscle creatine phosphate and ATP in Vit. E deficiency. In contrast there appears to be no depression in turnover of creatine phosphate and ATP in hyperthyroid rats.

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Experimental Colloid Goiter in the Hamster.* (24573)

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Fifty years have passed since David Marine demonstrated the relationship of iodine to goiter and expressed the belief "that goiter must be classed with nutritional disturbances (diseases) and that as such it will take its place in nosology along with chlorosis, rachitis, osteomalacia, etc.,"(1). This statement was based on morphologic and chemical studies of various types of goiter occurring naturally in man and other species, together with effects produced by administration of iodine to dogs with hyperplastic or colloid types of goiter(2,3). Thus, in a very short time Marine and his collaborators were able to lay the foundations for rational treatment and prophylaxis of endemic goiter, which he and Kimball had conclusively demonstrated by 1920(4). In his experimental studies Marine depended on dogs in which endemic goiters had already developed. He formulated the pathogenesis of goiter as follows: iodine deficiency acting on a normal gland leads to hyperplasia, which continues unless iodine is administered; whereupon colloid accumulates in the thyroid, a state which represents "the nearest approach to normal glands that active hyperplasias can become"(2). Colloid goiter, with varying degrees of nodular hyperplasia and scarring, is the form which the endemic disease takes in man. This morphologic picture has, to our knowledge, never been produced in laboratory animals whose thyroid glands were normal initially. To be sure, thyroid enlargement has been observed: 1) in puppies born to females whose thyroids had been extirpated before

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