Effect of Vitamin E-Deficiency on Protein Synthesis in Skeletal Muscle of the Rabbit.* (27085)

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The acute wasting of skeletal muscle in advanced vitamin E-deficiency of the rabbit has interested several workers in studying protein metabolism in this condition with the aid of radioactively labeled amino acids(1). After injection of glycine-1-C^{14}, the specific activity of total muscle proteins of Vit. E-deficient rabbits is much higher than that of control animals(1). Several observations suggested a study of glycine-C^{14} incorporation into the proteins of subcellular structures. Bouman and Slater(2) have shown that essentially all of the α-tocopherol in beef heart is in the mitochondria. The same has been reported for other tissues and other species(3,4). Proponents of the antioxidant theory of Vit. E-function have expressed the opinion that cellular damage produced by Vit. E-deficiency takes place primarily in the mitochondria, where most of the unsaturated lipid is found(5). In view of the important role played by muscle mitochondria in protein synthesis(6) it appeared interesting to test whether Vit. E-deficiency has a greater effect on mitochondrial protein synthesis than on protein synthesis in other subcellular fractions.

Methods. Vit. E-deficiency was produced in White New Zealand rabbits of mixed sex. The animals were approximately 4 weeks old when they were placed on a semi-synthetic Vit. E-deficient diet which contained 2.25% stripped lard and 2.25% cod liver oil. Composition of the diet, and procedure for addition of sodium sulfaquinoxaline to the drinking water were the same as previously described(1). Control animals received oral supplements of 12 mg α-tocopherol acetate per kilo body weight 3 times weekly. Vit. E-deficient animals were injected intraperitoneally with 10 μc of glycine-1-C^{14} per 100 g body weight (specific activity 5 mc per mmol) when they showed symptoms of advanced muscular dystrophy. Control animals were injected after they had been on the purified diet for approximately 3 weeks.

The animals were sacrificed at 0.5, 1, 2, 4 and 12 hours after injection. A 5 g portion of muscle from the left hamstring group was rapidly removed, weighed, minced and homogenized at 0-4°C in 0.25 M sucrose in a conical-pestle all-glass homogenizer. Sufficient 0.25 M sucrose was then added to give a final 10% homogenate. The homogenate was divided into a nucleo-myofibrillar (N-M), mitochondrial, microsomal and supernatant fraction by differential centrifugation. The procedure of Kitiyakara and Harman(7) was used except that the 125 $\times$ g centrifugation employed by these authors to sediment whole cells and debris was eliminated. The microsomal fraction was obtained by spinning at 105,000 $\times$ g for one hour in a Spinco model L centrifuge. The particulate fractions were washed twice with 0.25 M sucrose. The 4 subcellular fractions were mixed with equal volumes of 10% trichloroacetic acid (TCA) and heated at 90°C for 25 minutes. The resulting precipitates were washed with cold 5% TCA, hot methanol, cold methanol-ether 1:1 and twice with ether. The proteins were distributed to nickel-plated planchets and counted in a windowless gas-flow Geiger counter. All counts were corrected to infinite thinness.

The supernatant obtained by treating the microsomal supernatant with TCA contained the free amino acids of the cytoplasm. Aliquots of this supernatant were evaporated in planchets and counted.

Two dystrophic and 2 control animals were used at each time interval except the 4-hour interval, when 4 animals in each group were used. This permitted calculation of standard

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TABLE I. Effect of Nutritional Muscular Dystrophy on Specific Activity of Subcellular Protein Fractions and Protein-Free Supernatant Fraction of Rabbit Skeletal Muscle at Different Time Intervals after Injection of C^{14}-Glycine.

Time after inj., hr	Treatment	N-M protein	Mitochondrial protein	Microsomal protein	Supernatant protein	Protein-free supernatant
.5	Control	4.7*	15.7	15.3	7.3	14.4†
	Dystrophic	14.9	39.8	56.1	26.5	31.0
	Ratio, dystr./contr.	3.2	2.5	3.6	3.6	2.2
1	Control	4.8	12.3	12.7	9.5	10.9
	Dystrophic	44.5	127.0	102.9	75.3	14.7
	Ratio, dystr./contr.	9.2	10.3	8.1	8.0	1.4
2	Control	14.1	42.7	31.7	23.3	13.4
	Dystrophic	63.5	131.0	163.1	122.0	14.1
	Ratio, dystr./contr.	4.5	3.1	5.1	5.2	1.0
4	Control	17.7 ± 7.6	40.8 ± 32.2	43.2 ± 16.6	29.2 ± 11.9	21.1 ± 8.6
	Dystrophic	76.9 ±20.9	171.6 ± 83.3	199.2 ± 34.5	142.0 ± 19.8	11.8 ± 4.4
	Ratio, dystr./contr.	4.3	4.2	4.6	4.9	.6
	P	< .05	< .05	< .05	< .05	.1
12	Control	63.6	195.8	158.9	137.9	9.7
	Dystrophic	125.3	423.4	381.1	317.0	3.8
	Ratio, dystr./contr.	2.0	2.2	2.4	2.3	.4

* Specific activity of tissue protein fractions = counts/min./mg protein.
† Protein-free supernatant = counts/min./mg tissue.

deviations and statistical analysis of the 4-hour data by the Student t test(8).

Results and discussion. Results are summarized in Table I. Specific activity (S.A.) of proteins from Vit. E-deficient animals was higher than that of control animals at all time intervals after injection of the labeled glycine, and in all 4 subcellular fractions. The factor by which the S.A. of proteins from deficient animals was increased over that of the controls was highest at one hour after injection and was approximately the same in the 4 fractions. There is no indication in these data that mitochondrial protein synthesis is more strongly affected by Vit. E-deficiency than is protein synthesis in the other subcellular structures. Specific activity of mitochondrial proteins, both from Vit. E-deficient and control animals, was as high as that of microsomal proteins. This is in agreement with the work of Simpson and McLean(9) who have presented evidence that mitochondria are a major site of protein synthesis in rat muscle. The data on non-protein radioactivity, presented in the last column of Table I, indicate that the higher specific activity of proteins of Vit. E-deficient animals may not be due to a higher rate of protein synthesis. At 30 minutes after injection of the labeled glycine the non-protein radioactivity per mg of dystrophic muscle was twice as high as that of normal muscle. The difference between the 2 groups of animals was smaller at one and 2 hours after injection, and at 4 and 12 hours non-protein radioactivity was lower in dystrophic muscle. Assuming that the radioactivity of the TCA-supernatant solution reflects the radioactivity of free amino acids in the muscle cell, it appears that at least part of the increased specific activity of muscle proteins of Vit. E-deficient animals is due to a higher activity of the free amino acid pool at the early time after injection. Without knowledge of the actual S.A. of the free glycine in the muscle cell at various intervals of time after injection no decision can be made as to the turnover rate of proteins in dystrophic muscle. Ferdman(10) and Grigoreva(11) have measured the S.A. of protein-bound and free methionine-S^{35} in rabbit skeletal muscle at 24 hours after injection of the labeled amino acid. They found the S.A. of the free amino acid unaffected by Vit. E-deficiency and concluded that the higher S.A. of protein-bound methionine-S^{35} in the deficient animals re-

flected a higher renewal rate of the muscle proteins in Vit. E-deficiency. However, the rapid change of non-protein radioactivity demonstrated in the present study raises doubts as to the significance of free amino acid specific activity determinations made 24 hours after injection.

Statistical analysis shows that the difference between the S.A. of proteins of control and of dystrophic animals at 4 hours after injection was significant in all 4 subcellular fractions ($P<0.05$). The possibility that the difference between non-protein radioactivity of control and of dystrophic muscle at 4 hours after injection was due to chance cannot be excluded ($P = 0.1$).

Summary. Normal and Vit. E-deficient rabbits were injected with glycine-1-C^{14} and sacrificed at time intervals ranging from 0.5 to 12 hours. Homogenates of skeletal muscle were fractionated by differential centrifugation, and specific activity of the subcellular protein fractions determined. In all subcellular fractions and at all time intervals the proteins of Vit. E-deficient animals had a much higher specific activity. The effect of Vit. E-deficiency on incorporation of glycine-C^{14} was approximately the same in all subcellular protein fractions. Compared with control animals the radioactivity of the non-protein supernatant fraction rose and fell faster in muscle of Vit. E-deficient animals.

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Pituitary Inhibitory Effects of Digitoxin and Hydrocortisone.* (27086)

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It is well known that adrenal cortical steroids depress the secretion of ACTH by the anterior pituitary gland(1,2,3). In addition, numerous other steroids, for example testosterone(4) and the synthetic corticosteroids (5) have been shown to have similar pituitary inhibitory properties. It has often been suggested that the cardiac glycosides, owing to their structural similarity to the adrenal corticosteroids might possess similar pharmacological properties. Since the cardiotonic structure-activity relationships of adrenal corticoids have been studied extensively in recent years(6,7,8), it became of interest to compare the effects of digitoxin and hydrocortisone on the pituitary-adrenal axis. The experiments described here demonstrate that chronic administration of digitoxin significantly inhibits both synthesis and stress-induced release of pituitary ACTH.

Materials and methods. The experiments were performed on male albino Wistar rats fed on a diet of commercial rat chow and water *ad lib.* and maintained at a constant temperature of 24°C.

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