Nitrogen Metabolism of Isolated Muscle of Normal and Vitamin E-Deficient Hamsters.* (20442)

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In muscular dystrophy resulting from Vit. E-deprivation, there is a loss of muscle substance from the animal body suggesting that the rate of protein breakdown is considerably accelerated. The purpose of this paper is to report the results of experiments with isolated tissues in which the rate of protein catabolism, as judged by the rate of accumulation of amino nitrogen, was compared in normal and in dystrophic tissues. The present findings suggesting actual diminution in protein breakdown are of interest.

Materials and methods. For this purpose, the method devised by Kline(1) for studying nitrogen metabolism in isolated tissue slices incubated in an artificial medium was used. This method is based on the estimation of the loss of nitrogen from tissue slices during incubation by determination of the increase of nitrogen in the bicarbonate buffer medium. During incubation there is always a loss of nitrogen from the tissues, and Frantz, Loftfield and Miller(2) using labelled nitrogen found that under *in vitro* conditions the breakdown of protein exceeds protein synthesis. However, these authors concluded, as had Bernheim and Bernheim(3) previously, that the rate of loss of nitrogen gives important information regarding the nitrogen metabolism of the tissues. Kline studied slices of liver and diaphragm from normal, hypophysectomized, thyroidectomized and adrenalectomized rats, and found alterations in the rates of nitrogen metabolism that were reproducible and compatible with the changes which the removal of these glands is known to induce in the intact animal. Moreover, the validity of the method as an index of active metabolism rather than merely a measure of diffusion or other purely physical phenomena, was demonstrated further by the study of the restorative effects of administration of the hormones of the thyroid and adrenals on the metabolism of tissues of animals deprived of these glands.

Young hamsters weighing between 90 and 125 g were used. One group of animals (normal group) were fed the deficient diet† plus an adequate supplement of Vit. E. A second group were maintained on the Vit. E-deficient diet for periods of from 4 to 6 weeks, after which time, microscopic studies of the skeletal muscles from a representative number of animals showed structural changes typical of muscular dystrophy. After the animals had been anesthetized with nembutal, the gastrocnemii and diaphragm were removed and chilled immediately. Slices weighing from 30 to 100 mg were placed in test tubes containing 4 ml of Krebs-Ringer bicarbonate buffer and 0.2% glucose. The solution was saturated with O_2 just before and after the addition of the tissue. The tubes were placed in a mechanical shaker immersed in a water bath at 37°C and shaken at a rate of about 120 times per minute.

At the end of a 15 minute equilibration period, some of the tubes were removed to serve for zero value determinations as described by Kline. Triplicates of diaphragm and of skeletal muscle were used for establishing this base line and another 3 samples of each were used for comparison at the end of one hour of incubation. Proteins were precipitated from the medium with tungstic acid. Free amino nitrogen was then determined colorimetrically(4). The slices varied considerably in weight because they were prepared and weighed rapidly. However, differences within the range used in these experiments did not influence the results.

Results and discussion. The results of these experiments are summarized in the table, and

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† % content of diet: sucrose 20; casein 7; rolled oats 40; skimmed milk powder 29; sodium chloride 1; lard 1; cod liver oil 2.

TABLE I. Release of Amino Nitrogen from Isolated Skeletal Muscle and Diaphragm of Normal and Dystrophic Hamsters.

Tissue	No.	Amino-N in medium (μg/100 mg wet wt) 15 min.	60 min.	Difference
Skeletal muscle, normal	15	17.8	50.7	33.0 ± 1.7
" " vit. E-def.	12	13.3	41.1	27.9 ± 2.8
Diaphragm, normal	15	20.2	52.2	32.0 ± 2.2
" vit. E-def.	12	17.3	42.9	25.8 ± 3.1

are expressed as μg of amino nitrogen per 100 mg of tissue (wet weight). Observations on a representative number of samples, showed no significant difference in the water content of the tissues of normal and Vit. E-deficient animals. It was found that both the "initial" and final amounts of amino nitrogen in the medium were greater in the experiments with tissues of normal animals than with tissues of Vit. E-deficient animals. The differences are statistically significant although not numerically large. It should be pointed out, however, that they have been calculated on Kline's assumption that the differences between the 15 min. values and those obtained later (*i.e.* 60 min.) are more significant than are the absolute values at the end of the incubation. If these final values were equally significant, it can be seen that the differences would be greater. In the opinion of Kline, the amount of nitrogen which enters the medium during the first 15 minutes is determined largely by the effects of slicing, tissue damage and the surface area of the slice, and may not be a good indication of the metabolic processes of the tissue. For this reason, the end of this 15-minute incubation period is taken as "zero time." However, this does not explain our observation that dystrophic tissues invariably yielded lower 15 min. values than did the normal tissues.

In 2 experiments in which skeletal muscle of normal and of Vit. E-deficient rabbits were compared, essentially similar results were obtained.

If the metabolic activity of these surviving slices actually is an indication of the relative metabolic rates of the tissues of these animals *in vivo,* these results would suggest that the rate of protein breakdown in the dystrophic animal may not be enhanced. On the contrary, it is possible that the rate of protein catabolism may be reduced, and that this reduction is secondary to a decreased rate of protein synthesis. Obviously, this possibility should be investigated further by other methods, since it offers an entirely new concept in our consideration of the mechanism of the loss of muscle that occurs in muscular dystrophy.

Summary. A significant decrease was observed in the rate of release of amino nitrogen by the slices of surviving diaphragm and skeletal muscle from Vit. E-deficient hamsters when compared with tissues of normal controls.

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