ANALYSIS OF CERTAIN COMPONENTS OF SKELETAL MUSCLE DURING VITAMIN E DEFICIENCY*

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Within the past few years, the varied effects of vitamin E deficiency were reviewed by Pappenheimer (1), Mason (2), Hickman and Harris (3), and Mattill (4). Further studies of the profound changes which its absence produces in the structure, composition, and particularly in the functional activity of muscle tissue have not yet led to an understanding of its rôle. The biochemical approach has been from two points of view which are not mutually exclusive. Tocopherol may inhibit oxidation in vivo as it does in vitro; or it may modify, possibly even participate directly in, cellular reactions, especially in those concerned with oxidation.

The antioxygenic aspect is represented by the sparing action of tocopherol on vitamin A and carotene which has been observed repeatedly, by the increased efficiency imparted to suboptimal doses of essential fatty acids (5), and the greater stability of the body fats when tocopherol is present in the diet (6). Furthermore, in its absence, peroxides have been demonstrated in the tissues (7); the brown pigment which appears in certain organs and tissues is believed to consist of polymerized substances originating in unusual products of oxidation (8, 9).

The relation of tocopherol to cellular oxidation is suggested by the higher rate of oxygen consumption by muscle strips from animals on vitamin E-deficient diets. Slices demonstrate this increase less markedly (10), and homogenates not at all (11). Tocopherol phosphate reduces the activity of the succinic dehydrogenase system in preparations from normal and dystrophic tissues alike (12). Its action may be direct or indirect; by precipitating calcium below the level of concentration necessary to activate diphosphopyridine nucleotidase, it would preserve diphosphopyridine nucleotide (DPN) which inhibits succinic dehydrogenase (13), or it may inhibit DPNase directly (14, 15), thus protecting DPN, as has been shown to happen in the lactic acid dehydrogenase system in heart muscle (16).

* The content of this and the following paper was presented in part at the International Conference on Vitamin E, held under the auspices of the New York Academy of Sciences, April 15 and 16, 1949.
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In either case, the supply of DPN would be reduced in vitamin E deficiency.

Perhaps one of the functions of DPN is the transfer of phosphate (17). A diminished rate of phosphorylation of creatine by homogenates of muscles from certain species when deprived of vitamin E has been demonstrated (18); but normal phosphorylation was not restored by the presence of tocopherol phosphate.

The nature of the substrate undergoing increased combustion in dystrophic muscles is not known but the diminished creatine content of such muscles and the creatinuria, together with a considerable increase of creatine in the liver of dystrophic animals (19), tend to confirm the view that vitamin E may be linked with the metabolism of protein (8).

This paper records further attempts to explore the rôle of vitamin E as a biological antioxidant and as an agent in the regulation of cellular oxidation.

**EXPERIMENTAL**

**Biotin**—The observation that biotin is destroyed by autoxidizing fats and is protected by α-tocopherol (20) prompted a study of the biotin content of normal and dystrophic muscles. On diets containing subnormal amounts of biotin, dystrophy due to lack of vitamin E might appear earlier than otherwise.

Hamsters, guinea pigs, and rabbits were placed on the dystrophy-producing diet heretofore used (21). Control animals on the same diet were given α-tocopherol acetate\(^1\) in olive oil by mouth, the guinea pigs and rabbits 15 mg. twice weekly, and the hamsters 7.5 mg.; the guinea pigs were also given 15 mg. of ascorbic acid every 3rd day.

Biotin, both free and combined, was determined microbiologically\(^2\) by the method of Coryell *et al.* (22), turbidity after 24 hours being measured in a Coleman No. 11 spectrophotometer at 650 µm.

Immediately after the animals were stunned and bled, distilled water homogenates were prepared as 1:20 dilutions. Tendinous material was removed by filtering through cheese-cloth, and aliquots were acidified, autoclaved, adjusted to pH 4.5, diluted to volume, and filtered. Because of the high content of sodium sulfate in the total biotin samples, a like amount was added to the standard biotin solution.\(^3\)

The results of the biotin study (Table I) were disappointing in that the differences between control and dystrophic animals were not significant.

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\(^1\) Kindly supplied by Hoffman-La Roche, Inc., Nutley, New Jersey.

\(^2\) For the original culture of *Lactobacillus arabinosus*, we are indebted to Dr. John R. Porter and the Department of Bacteriology.

\(^3\) Kindly supplied by Merck and Company, Inc., Rahway, New Jersey.
In the muscles of hamsters on biotin-low diet, free biotin was considerably reduced, total biotin only slightly. Reduction of the biotin intake by inclusion of egg albumin in the diet did not affect the rapidity with which dystrophy appeared. If fatty acid peroxides are present in dystrophic animals, they do not destroy biotin in vivo, perhaps because of lack of contact or because biotin is combined with stabilizing substances.

**Table I**

**Biotin Content of Muscle from Animals with and without Vitamin E Supplement**

The values are given in micrograms per gm. $\times 10^3$.

<table>
<thead>
<tr>
<th></th>
<th>Total biotin</th>
<th>Free biotin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ vitamin E</td>
<td>- vitamin E</td>
</tr>
<tr>
<td>Hamsters (5, 5)*</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>&quot; (5, 5) (low biotin)</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>Rabbits (7, 6)</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Guinea pigs (3, 2)</td>
<td>17</td>
<td>28</td>
</tr>
</tbody>
</table>

* Number of animals.

**Table II**

**$O_2$ of Muscle Strips from Animals with and without Vitamin E Supplement**

<table>
<thead>
<tr>
<th></th>
<th>Ringer</th>
<th>Ringer + glucose</th>
<th>Ringer + glucose + DPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pigs, - vitamin E (5)*</td>
<td>2.0</td>
<td>2.0</td>
<td>2.4</td>
</tr>
<tr>
<td>&quot; &quot; + &quot; &quot; (5)</td>
<td>1.5</td>
<td>1.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Rabbits, - vitamin E (7)</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>&quot; + &quot; &quot; (5)</td>
<td>1.3</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Hamsters, - vitamin E (5) (but no dystrophy)</td>
<td>3.1</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>&quot; + &quot; &quot; (4)</td>
<td>2.3</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

* Number of animals.

**Oxygen Uptake and DPN**—The increased rate of oxygen consumption by dystrophic muscle strips was again confirmed in the case of all three species (Table II). This was most pronounced in rabbit muscle. It was also evident in hamsters which showed no external signs of dystrophy, although they had been maintained on the deficient diet for a longer time than is usually necessary to produce it. Age was not a factor, since all the hamsters were within a few days of the same age.

The addition of various freely diffusible substances to the nutrient medium produced insignificant changes in the oxygen uptake, which implies that, even in dystrophic muscles, adequate amounts of substrate are
present. Any influence which DPN may have cannot be revealed merely by adding it to the medium in which the muscle is respiring, perhaps because it does not penetrate.

**Glutamine**—If there is an abnormal oxidation of muscle protein in vitamin E deficiency, this might be reflected in the level of free amino acids in muscle tissue. An amino aciduria has been demonstrated in patients with progressive muscular dystrophy (23). Since skeletal muscle contains a large proportion of its free amino acids as glutamine (24), changes in the level of this storehouse of labile amino groups might be a sensitive index to an altered oxidative deamination.

Glutamine was determined by the method of Hamilton (25), which depends on the fact that heating glutamine produces pyrrolidonecarboxylic acid which no longer reacts with ninhydrin to release carbon dioxide.

<table>
<thead>
<tr>
<th>Table III</th>
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<tbody>
<tr>
<td><strong>Glutamine in Muscle Carboxyl N from Animals with and without Vitamin E Supplement</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Guinea pigs, + vitamin E (6)*</td>
</tr>
<tr>
<td>“ “ − “ “ (7)</td>
</tr>
<tr>
<td>Rabbits, + vitamin E (6)</td>
</tr>
<tr>
<td>“ − “ (5)</td>
</tr>
</tbody>
</table>

* Number of animals.

From 2 to 4 gm. of muscle tissue were homogenized with appropriate volumes of picric acid, and the CO₂ from the amino acids present was measured in the Van Slyke manometric apparatus. Some of the samples of muscle were stored at −40° before analysis.

As shown in Table III, the skeletal muscle of dystrophic guinea pigs showed a striking decrease in glutamine content; in rabbits, the decrease from normal was less marked, and perhaps not significant. This species difference may be due to the fact that on the vitamin E-deficient diet rabbits become dystrophic within 2 to 4 weeks, whereas guinea pigs require 6 to 7 weeks.

The total non-glutamine amino acid content of the muscles was influenced little, if at all, by vitamin E deficiency. A study of the distribution of other amino acids in this condition might be revealing.

The function of glutamine appears to be different from that of glutamic acid (26). Glutamine serves as a neutral storage (27) and transport (28) form of labile amino groups. Its synthesis is an endothermic reaction (29) for which respiration or glycolysis (30) supplies the necessary energy.
Homogenates of rat and guinea pig livers synthesize glutamine if adenosine triphosphate, Mg++, and ammonia are present (31); with pigeon liver preparations (32) and with an enzyme system obtained from sheep brain (33), liberation of inorganic phosphate parallels the reaction.

The observed reduction in glutamine in the muscles of dystrophic animals may well be an immediate and direct result of the loss, through combustion, of energy that is normally stored in high energy phosphate bonds. A significant decrease in the glutamine level of muscle has also been demonstrated in guinea pigs deficient in ascorbic acid (34). Whether the function of vitamin E is specific and how it contributes to the normal economy of muscle metabolism in different species remain to be determined.

The author is indebted to Dr. George Kalnitsky and Dr. H. A. Mattill for their suggestions.

SUMMARY

In nutritional muscular dystrophy resulting from a deficiency in vitamin E, the free and total biotin content of skeletal muscle from hamsters, rabbits, and guinea pigs did not vary from that of control animals. When the biotin content of the diet was lowered (hamsters), that of the muscles was also decreased, irrespective of the adequacy of the vitamin E intake. It is therefore unlikely that vitamin E acts as an antioxidant in tissue to preserve biotin, although this relationship has been observed in the autoxidative destruction of biotin by unsaturated fats in vitro.

The heightened consumption of oxygen by dystrophic muscle from guinea pigs and rabbits is again confirmed; muscle from hamsters that had not yet developed dystrophy showed an increased uptake. The addition of various substrates and of DPN to the nutrient medium produced no alteration in the utilization of oxygen.

The glutamine content of skeletal muscles of dystrophic guinea pigs was about one-third that of muscle from control animals, but the average content of non-glutamine amino acids was unchanged. In vitamin E-deficient rabbits, the difference was less marked.

The possible significance of the decreased glutamine content is briefly discussed with reference to the energy relationships. Whether and how vitamin E participates directly or indirectly in normal muscle economy remain to be determined.

BIBLIOGRAPHY

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