RESPIRATION AND GLYCOLYSIS OF RABBIT MUSCLE
IN VITAMIN E DEFICIENCY*

BY J. P. HUMMEL AND ROBERT S. MELVILLE
(From the Department of Biochemistry, State University of Iowa, College of Medicine, Iowa City, Iowa)
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Accompanying the paralysis produced by deprivation of vitamin E, the respiration of skeletal muscle is significantly accelerated. The activity of some tissue enzymes has also been shown to be altered, but none of these observations has as yet provided any basis for interpreting the physiological rôle of α-tocopherol.

Although the excessive rate of respiration of muscle from dystrophic animals has been repeatedly demonstrated (1–3), no studies seem to have been made of the respiratory quotient or the rate of anaerobic glycolysis in such muscle. The purpose of this investigation was to ascertain which substrates, if any, are burned preferentially, and whether the accelerated respiration is accompanied by increased anaerobic glycolysis.

EXPERIMENTAL

The same experimental animals were used in this and in the preceding study (4), and the basis for judging the degree of dystrophy was the same. After blood and liver specimens had been taken for creatine and glycocyanine analyses, the entire left and right psoas muscles were quickly removed and placed in cold Ringer's phosphate solution. The psoas was chosen because the long parallel fibers are almost free of connective tissue and are therefore particularly suitable for the preparation of strips. The tissue, constantly bathed in cold Ringer's phosphate, was dissected with corneal scissors into strips approximately 5 cm. long and 0.5 mm. in diameter. The collected strips were drained and washed with fresh Ringer's phosphate.

Suitable amounts of the muscle strips (approximately 300 mg. for respiration and 100 mg. for glycolysis experiments) were blotted dry on filter paper, weighed, and placed in chilled manometric vessels containing the proper medium. All vessels were equilibrated for 10 minutes at 37° before the manometric readings were begun. The time elapsed between the removal of the muscle and the first manometer readings was about 75 minutes.

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Respiration Experiments—Dixon-Keilin vessels were used, containing 2 ml. of Krebs-Ringer phosphate solution with or without 100 mg. per cent of glucose. The side arm contained 0.2 ml. of 4 N H$_2$SO$_4$. The center well contained a glass plunger and a filter paper roll. The stop-cock well was filled with 2 N NaOH and kept closed.

Immediately following the first manometer reading, the acid was tipped in from the side arm of one of the vessels, liberating the initially bound CO$_2$. After this reading was taken, the stop-cock of the center well was opened to the alkali. Following CO$_2$ absorption, the manometer was again read, giving the correction for oxygen uptake. The tissue in the remaining flasks was allowed to respire for 60 minutes. The acid was then tipped in and the liberated CO$_2$ was measured as before. The CO$_2$ was then absorbed by opening the center well to the alkali. The difference between the corrected initial reading and the final reading after absorption of alkali represented the oxygen uptake.

After each experiment, the tissue samples were rinsed in distilled water and dried to constant weight at 105°. Each determination was carried out in triplicate.

Glycolysis Experiments—Standard Warburg vessels were used containing 3 ml. of Krebs-Ringer bicarbonate solution, with or without 100 mg. per cent glucose. After being attached in the bath, the flasks and manometers were flushed for 7 minutes with a vigorous stream of carbon dioxide (5 per cent)-nitrogen (95 per cent) mixture which had passed over hot copper wire. The evolution of CO$_2$ was measured for a period of 30 minutes after equilibration. The tissues were then washed and dried as before. These determinations were also carried out in triplicate.

RESULTS AND DISCUSSION

The data support the view that metabolic changes appear well in advance of the morphological degeneration during the course of avitaminosis E. The oxygen uptake was abnormally rapid during the incipient stage when the animals were still gaining weight. These results recall those of Friedman and Mattill (5), who showed that isolated gastrocnemius strips from rats in a predystrophic condition exhibited an excessive rate of oxygen consumption. When the dystrophic symptoms became severe, the oxygen consumption increased to 2 to 3 times the normal.

During the preparation of the muscle strips, it was noted that moderately and severely dystrophic muscle was more readily dissected than normal muscle, and of course appreciably less contractile. Although the increased rate of respiration of dystrophic muscle is usually considered to be true acceleration of metabolism, it could conceivably be an artifact due to the smaller amount of cellular damage produced as compared with that
suffered by normal tissue during dissection. Disruption of cellular integrity of muscle by mincing or homogenizing lowers the rate of respiration markedly. It also abolishes the differences between the QO_2 of normal and of dystrophic muscle (6). A decision between these two points of view is difficult from the evidence at hand (Table I).

The respiratory quotient was not significantly altered during the course of dystrophy until severe muscle degeneration was observed. In the earlier stages it was approximately 0.9, suggesting that carbohydrate continues to be the principal foodstuff burned in incipient and mild dystrophy. The lowered respiratory quotient of muscle from severely dystrophic rabbits is probably caused by fasting of the almost paralyzed animals. The presence of glucose in the medium affected neither the oxygen uptake nor the respiratory quotient appreciably.

Muscle from moderately and severely dystrophic rabbits showed a greater rate of glycolysis than muscle from normal animals or from those in the incipient condition, but only if glucose was added to the medium; without glucose the differences were small. The increase in glycolysis (50 per cent) was proportionately less than the increase in respiration (250 per cent) in the same preparations.

These data would seem to indicate that vitamin E deficiency fundamentally affects the oxidative metabolism of the muscle; the changes in the respiratory quotient and in the rate of glycolysis may be secondary effects of cellular degeneration. Unfortunately, no information was ob-

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**Table I**

Respiration, Respiratory Quotient, and Anaerobic Glycolysis of Muscle Strips from Rabbits in Various Stages of Dystrophy*

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of animals</th>
<th>QO_2</th>
<th>R. Q.</th>
<th>QO_2Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No glucose</td>
<td>Glucose</td>
<td>No glucose</td>
<td>Glucose</td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>1.10 (0.50–1.70)</td>
<td>1.11 (0.49–1.88)</td>
<td>0.92</td>
</tr>
<tr>
<td>Incipient</td>
<td>4</td>
<td>2.03 (1.59–2.37)</td>
<td>1.86 (1.74–1.94)</td>
<td>0.94</td>
</tr>
<tr>
<td>Mild</td>
<td>5</td>
<td>1.86 (1.75–2.12)</td>
<td>1.86 (1.74–1.94)</td>
<td>0.94</td>
</tr>
<tr>
<td>Severe</td>
<td>4</td>
<td>2.91 (2.37–3.48)</td>
<td>2.69 (2.30–2.90)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

* Krebs-Ringer phosphate solution used in the respiration experiments was prepared by mixing 12 parts of 0.154 M phosphate buffer, pH 7.4, with 100 parts of cold Krebs-Ringer solution and gassing the mixture with oxygen for 10 minutes. The Krebs-Ringer bicarbonate solution used in the glycolysis experiments was similarly prepared, except that 21 parts of 0.154 M sodium bicarbonate were added in place of the phosphate buffer. This solution was gassed with CO_2 (5 per cent)-N_2 (95 per cent) for 15 minutes.
tained on possible alterations in the rate of aerobic glycolysis. A study of the Pasteur reaction of dystrophic muscle may possibly bear upon the nature of the metabolic disturbance in avitaminosis E.

**SUMMARY**

Skeletal muscle strips from rabbits on a vitamin E-deficient diet respired at an abnormally rapid rate even before symptoms of muscle dystrophy were noted. The rate of oxygen uptake increased as muscular dystrophy progressed.

The respiratory quotient of skeletal muscle was not altered during the course of vitamin E deficiency until the animals became moribund.

The rate of glycolysis remained normal until symptoms of dystrophy became evident, after which glycolysis was increased.

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J. P. Hummel and Robert S. Melville


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