Respiration of mitochondria of red and white skeletal muscle

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Mitochondria of guinea pig red and white skeletal muscle were isolated in a medium containing 0.25 M sucrose, 1 mM ethylenediamine tetraacetate, and 1% fraction V bovine albumin. Oxidative phosphorylation with pyruvate-malate as substrate was tightly coupled in both types of mitochondria when oxygen uptake was measured over a 2- to 4-min period with a platinum electrode. The P/O ratios approached the theoretical value of 3. Oxygen consumption was also determined manometrically with the substrates 10 mM pyruvate—1 mM malate, 10 mM succinate, 6 mM reduced nicotinamide adenine dinucleotide (NADH2), 20 mM dl-lactate, and 12-20 mM dl-α-glycerophosphate. White muscle mitochondria had a higher rate of oxygen consumption with α-glycerophosphate than with lactate, succinate, and NADH2. Those of red muscle were less active with α-glycerophosphate than with the other substrates. These results indicate that an α-glycerophosphate shuttle may couple the reactions generating NADH2 in the cytoplasmic mitochondrial respiratory chain. The properties of the red muscle mitochondria suggest that the direct oxidation of NADH2 may be more important in this tissue than the α-glycerophosphate shuttle.

Oxidative phosphorylation α-glycerophosphate shuttle nicotinamide adenine dinucleotide oxygen consumption muscle mitochondria

In the recovery phase that follows anaerobic muscular work, a portion of the lactate formed in the cytoplasm during contraction is reoxidized to pyruvate with the simultaneous production of reduced nicotinamide adenine dinucleotide (NADH2). Until recently it was assumed that the resulting NADH2 and pyruvate both freely enter the mitochondria where their oxidation is linked to the formation of adenosine triphosphate. However, the direct coupling of NADH2 generated in the cytoplasm with the mitochondrial respiratory chain now seems in doubt because of evidence that carefully isolated liver mitochondria are impermeable to NADH2 (6, 19, 25). To circumvent this apparently normal block to the direct entrance of reductive hydrogen into mitochondria, an α-glycerophosphate cycle or shuttle across the mitochondrial membrane has been proposed (9, 12, 33). In the first step of the shuttle, a catalytic amount of l-α-glycerophosphate is formed in the cytoplasm by the NADH2-coupled reduction of dihydroxyacetone phosphate and enters the mitochondria. There the reoxidation of the α-glycerophosphate is linked to the respiratory chain by a flavoprotein enzyme (13, 14, 18, 26) and the product dihydroxyacetone phosphate, diffusing back into the cytoplasm, again becomes available for reduction to continue the hydrogen shuttle into the mitochondria. It appears that such a shuttle may function in insect flight muscle (7, 9, 33) and its occurrence in mammalian skeletal muscle is suggested by the presence of the two enzymes necessary, a mitochondrial l-α-glycerophosphate oxidase and a cytoplasmic NAD-linked l-α-glycerophosphate dehydrogenase (13, 14, 31). However, the distribution of these enzymes is not uniform, white skeletal muscle being richer in both than red (5). This difference can also be demonstrated in individual red and white muscle fibers histochemically (32). Red fibers, although poorer in both l-α-glycerophosphate oxidizing enzymes, contain a more active NADH2 dehydrogenase (diaphorase) than white muscle fibers.

In the present work it was found that α-glycerophosphate induced a greater oxygen uptake by white muscle mitochondria than most other substrates tested including NADH2. In contrast, red muscle mitochondria showed a larger oxygen uptake with NADH2 than with α-glycerophosphate. This and other findings suggest that the direct oxidation of NADH2 by the mitochondria of red muscle may be more important physiologically in this tissue than the indirect entrance of reductive hydrogen by way of the α-glycerophosphate shuttle. Conversely, the direct mitochondrial oxidation of NADH2 appears to be less active in white muscle than the α-glycerophosphate shuttle.

Received for publication 8 July 1963.

This work was supported by grants from the Muscular Dystrophy Association of Canada and the Medical Research Council of Canada.
METHODS

Adult guinea pigs of both sexes were lightly anesthetized with ether and exsanguinated. A total of 4-6 g red muscle was obtained from the musculatures and from the deeper layers of the quadriceps, adductor femoris group, and triceps. From 6 to 8 g white muscle was collected from the superficial layers of the triceps, quadriceps, adductor femoris, iliacon, and psoas, as well as from the lateral portions of the longissimus dorsi. Even in the purest samples of red and white muscle the predominant cell type was accompanied by 5-10% of fibers of the contrasting variety. However, with experience it proved possible to select relatively pure samples of red and white muscle on the basis of color and texture (3-5, 32). Occasionally it was necessary to reject an entire animal when differentiation was poor.

The excised muscle was trimmed and minced with scissors in an ice-cooled stainless steel dish. All subsequent steps were carried out at 0°C. The minced muscle was homogenized in 10 volumes of a solution containing 0.25M sucrose, 1mM ethylenediamine tetraacetate (EDTA), and 1% fraction V bovine plasma albumin (Armour) adjusted to pH 7.4. During the early part of the study glass-Teflon homogenizers of the Potter type (A. H. Thomas, Philadelphia, Pa.) were used. With this apparatus 3-4 min homogenization was required for each 4-10 5-8 batch of muscle, with frequent interruptions to prevent the temperature rising above 3°C. The homogenate was centrifuged for 7 min at 360 g, the supernatant decanted through a pad of glass wool and recentrifuged for 10 min. The resulting supernatant was centrifuged for 15 min at 10,000 g and the pellet suspended with the aid of a Vortex Jr. mixer (Scientific Industries, Springfield, Mass.) in half the volume of solution initially used for homogenization. The suspension was recentrifuged and the resulting pellets washed once more in the centrifuge as described. The final working suspension in 0.25M sucrose containing 1% crystalline bovine albumin (Armour) had a concentration of 2-6 mg mitochondrial protein per milliliter. Electron photomicrographs showed the mitochondria to be intact, resembling myocardial mitochondria protected by albumin during isolation (10). However, there was some contamination by cellular debris.

In about half the experiments 4-8 g muscle was homogenized in 10 volumes of the sucrose-EDTA-albumin solution for 40 sec in a microcup (Eberbach, Ann Arbor, Mich.) attached to a Waring Blendor. At the end of the first 360 g centrifugation described above the supernatant was reserved. The foam lying on the supernatant, together with the pellet, was resuspended in 5 volumes of the homogenizing solution with the Vortex mixer and centrifuged at 360 g again. The combined supernatants were filtered through glass wool and then treated as described above for glass-Teflon homogenates.

To determine the mitochondrial protein concentration in the final suspensions used in the respiration experiments, triplicate aliquots were each washed three times in the centrifuge at 10,000 × g for 10 min with 100 volumes 0.25 M sucrose and the protein in the pellets determined by the method of Lowry et al. (21). To determine whether the sucrose washing removed from the mitochondria the albumin adsorbed from the homogenizing solution, albumin labeled with 14C was added to this solution before homogenizing a pair of red and white muscle samples. Comparison of the initial specific activity of the albumin in the homogenizing medium with that of the protein of the washed mitochondria showed that about 0% of the protein in both the red and the white mitochondrial preparations consisted of adsorbed albumin. No correction was made for this error when calculating the oxygen uptake per milligram protein. Unless indicated otherwise, in each experiment red and white muscle mitochondria were prepared simultaneously from the same animal and tested under identical conditions to permit analysis of the results as paired data.

Oxygen consumption was determined with standard manometric techniques at 30°C using flasks with a total volume of 4.5 ml and differential manometers (R. Gilmont, Great Neck, N. Y.). Carbon dioxide was absorbed with 5 n potassium hydroxide. A mitochondrial suspension containing 2-6 mg protein was added to flasks containing 0.5% crystalline bovine albumin, 1.5 mM adenosine triphosphate, 10 mM orthophosphate, 4 mM magnesium chloride, and 116 mM potassium chloride. After 5-8 min equilibration, substrate was tipped in from a sac to give a final fluid volume of 1.0 ml at pH 7.0. When lactate was the substrate, the flasks also contained 1 μmole NAD and 0.3 mg crystalline rabbit muscle lactate dehydrogenase (type 1, Sigma, St. Louis, Mo.). In the manometric oxidative phosphorylation experiments the mitochondria were equilibrated for 5 min in paired flasks containing substrate as well as the usual medium. Then 50 μg crystalline yeast hexokinase (Sigma) and glucose to a final concentration of 20 mM were tipped in from a sac. The control flask was immediately deprenitized with 0.1 ml cold 5% perchloric acid. Oxygen consumption was followed for 25 min in the other flask.

### TABLE I. Oxygen uptake by mitochondria of red and white skeletal muscle, μmoles per milligram mitochondrial protein per hour

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rm</th>
<th>Wm</th>
<th>Rm</th>
<th>Wm</th>
<th>Rm</th>
<th>Wm</th>
<th>Rm</th>
<th>Wm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>2.33</td>
<td>2.37</td>
<td>2.05</td>
<td>0.86</td>
<td>1.75</td>
<td>0.62</td>
<td>4.44</td>
<td>1.45</td>
</tr>
<tr>
<td>Malate</td>
<td>1.00</td>
<td>2.75</td>
<td>1.59</td>
<td>1.59</td>
<td>0.72</td>
<td>1.59</td>
<td>0.41</td>
<td>0.29</td>
</tr>
<tr>
<td>Succinate</td>
<td>0.63</td>
<td>0.42</td>
<td>0.21</td>
<td>0.17</td>
<td>0.33</td>
<td>0.36</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.35</td>
<td>0.42</td>
<td>0.18</td>
<td>0.18</td>
<td>0.49</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.50</td>
<td>0.50</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Pyruvate + ADP</td>
<td>0.75</td>
<td>0.75</td>
<td>0.49</td>
<td>0.49</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Pyruvate + ATP</td>
<td>0.75</td>
<td>0.75</td>
<td>0.49</td>
<td>0.49</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Rm = red muscle mitochondria. Wm = white muscle mitochondria. *t Test based on the difference between the Rm and Wm mean values and the standard error of the difference (SEM) between these means as described by Snedecor (30).
after which its contents were deproteinized. The decrease in orthophosphate was determined by the method of Lowry and Lopez (20).

RESULTS

The level of respiratory activity was similar to that reported for skeletal muscle mitochondria by previous workers (1, 17, 23). Respiration usually began promptly after substrate addition but with dL-a-glycerophosphate there was occasionally a delay of 5-10 min. With most of the substrates tested oxygen uptake was essentially linear for an hour and frequently for 2 hr. However, red muscle mitochondria were exceptionally active with NADH and their uptake plateaued abruptly within 30 min coincident with substrate exhaustion.

It is apparent from Table 1 that the oxygen uptakes of the white muscle mitochondria and red muscle mitochondria were similar with pyruvate-malate. The mean uptake by the red muscle mitochondria with succinate was more than double that of the white muscle mitochondria with this substrate, but the difference was not significant because of the variability in response. However, with lactate and NADH the red muscle mitochondria were consistently more active than the white muscle mitochondria. Conversely, the uptake of the white muscle mitochondria was more than twice that of the red muscle mitochondria with a-glycerophosphate. The two mitochondrial types thus appeared to have different patterns of response to the substrates tested. This was demonstrated in those experiments in which the uptake with a-glycerophosphate was determined together with that produced by one or more of the other substrates. In such experiments the activity with each substrate could be expressed as a ratio to that found with a-glycerophosphate in the same mitochondrial sample. From these ratios, summarized in Fig. 1, it is apparent that with the red muscle mitochondria pyruvate-malate, lactate, succinate, and NADH produced a greater oxygen uptake than a-glycerophosphate. In contrast, the white muscle mitochondria were more active with a-glycerophosphate than with lactate, succinate, or NADH. These patterns were not affected by washing the preparations with a heparin-containing solution to remove mitochondrial fragments (10).

The greater response of the white muscle mitochondria to a-glycerophosphate was not unexpected since quantitative (5) as well as histochemical methods (24, 32) have shown white muscle to be richer in mitochondrial a-glycerophosphate oxidase. The observation that NADH stimulated a higher rate of oxygen consumption in red than in white muscle mitochondria suggested that the latter might be more resistant to the trauma of the fractionation process. To examine their relative osmotic resistance, red and white muscle mitochondria were suspended in glass redistilled water for 20 min at room temperature. Normal working tonicity was then restored by the addition of 0.5 volumes of 0.75 M sucrose-3% albumin solution before measuring the oxygen uptake. Such treatment did in fact increase the oxygen consumption of both red and white muscle mitochondria with NADH as substrate (Table 2). Although the increase was proportionately somewhat larger with the white muscle mitochondria, it is clear that the activity of the treated white muscle mitochondria was still less than that of the untreated red muscle mitochondria. Selective osmotic damage therefore did not account entirely for the lesser response of the white muscle mitochondria to NADH shown in Table 1. This differential response of red and white muscle mitochondria to NADH also survives the damaging effect of freezing in the preparation of muscle sections for histochemical examination (11). The tendency for hypotonic treatment to depress uptake with a-glycerophosphate, seen in experiments 3 and 4 of Table 2, has been ascribed to cytochrome c loss.

![FIG. 1. Oxygen uptake relative to that found simultaneously in the same mitochondrial preparations with a-glycerophosphate as substrate. Mean ratios ± SEM are shown. The number of paired Rm and Wm experiments is indicated in each panel. Substrate concentrations were those shown in Table 1. All means, except for that of Wm with pyruvate-malate, differ significantly from 1.0 by the t test, with P < 0.02.](http://ajplegacy.physiology.org/doi/10.1152/ajplegacy.1977.234.1.1017)
from the mitochondria (12). That a differential cytochrome c loss might also explain the difference in response of the two mitochondrial types to NADH might be due in part to a preferential extraction of cytochrome c from the white muscle mitochondria during the isolation procedure. It is clear from Table 3 that the addition of cytochrome c produced a smaller stimulation than that reported by Hedman et al. This discrepancy probably was due to the tendency of potassium chloride-containing media such as used by these workers to extract more cytochrome c from mitochondria than the sucrose-EDTA solution employed in the present study (17). It may be seen from experiment 1 in Table 3 that cytochrome c stimulated the red muscle mitochondria more than the white muscle mitochondria, indicating that the higher uptake of the red muscle mitochondria with NADH in the unsupplemented preparations was not due to a relative cytochrome c deficiency in the white muscle mitochondria that could be corrected by its addition. Amytal produced the expected (16) suppression of uptake when NADH was the substrate, whether cytochrome c was added or not. The minimal effect of amytal on α-glycerophosphate oxidation was taken as further evidence (5) that the l-α-glycerophosphate oxidase of both red and white muscle mitochondria, like that of the mitochondria of brain (27) and of insect flight muscle (8), is not NAD-linked.

The results of the manometric oxidative phosphorylation experiments are shown in Table 4. The mean P/O ratios of 2.3 and 2.4 with pyruvate-malate were similar to those reported for human muscle mitochondria by Azzzone et al. (1). The NADH and pyruvate generated from lactate yielded lower values than pyruvate-malate but the lowest ratios were obtained with α-glycerophosphate. In the latter case the values were considerably below the 1.0–1.7 ratios found by Azzzone et al. Better results were obtained with all three substrates when phosphorylation was measured over a 2- to 4-min period while following the oxygen consumption amperometrically in a closed cell with a rotating platinum electrode (15). These experiments, which will be described in detail elsewhere, yielded P/O values closer to the theoretical ratio of 3 with pyruvate-malate. Oxygen consumption was tightly coupled to phosphorylation with this substrate and decreased sharply by a factor of 4–6 when the added adenosine diphosphate was exhausted, indicating good respiratory control. Although these may be considered characteristics of functionally intact muscle mitochondria (28), the active oxidation of NADH by our red muscle mitochondria preparations can nevertheless be interpreted as reflecting damage to the limiting membrane of the mitochondria. As shown in Table 2, a difference in membrane permeability probably does not account for the dissimilar response of the isolated red and white muscle mitochondria to NADH, but these experiments did not eliminate prior damage during the isolation procedure. An attempt was therefore made to protect the mitochondria during their preparation by increasing the sucrose concentration in the homogenizing medium from 0.25 to 0.88 M. The latter concentration has been found by Ziegler and Linnane (34) to preserve the structure of heart mitochondria better than 0.25 M sucrose, although Deshpande et al. (10) have since reported that 0.25 M sucrose supplemented with 1% albumin is equally satisfactory. It was decided to compare these two types of media in the preparation of mitochondria. Mixed muscle was used in order to obtain sufficient mitochondria. Oxygen uptake of the mitochondria was measured with a platinum electrode for 2 min after substrate addition. The closed cell contained 2 mg mitochondrial protein, 1 M EDTA, 5 mM orthophosphate, 12.5 mM tri(hydroxymethyl)aminomethane, 2 mM magnesium, and 0.25 M or 0.5 M sucrose, all at pH 7.0. For the mitochondria prepared in the usual 0.25 M sucrose-1 M EDTA-1% albumin solution, the sucrose concentration in the cell was 0.25 M. For those prepared in 0.88 M sucrose-1 M EDTA-1% albumin, the sucrose in the cell was 0.5 M (34). It may be seen in Table 5 that with NADH as substrate, the mitochondria prepared with 0.88 M sucrose had about half the uptake rate of those isolated in the solution containing 0.25 M sucrose. This finding and the decreased uptake with other substrates apparent in Table 5 have been noted previously and ascribed to an intramitochondrial inhibitory effect of sucrose (29). Ziegler and Linnane (34) prefer to inter-

### Table 3. Effect of cytochrome c and amytal on oxygen uptake, μmoles O₂ per milligram mitochondrial protein per hour

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Substrate</th>
<th>Control</th>
<th>2 μM Amytal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rm</td>
<td>Wm</td>
</tr>
<tr>
<td>1</td>
<td>10 mM NADH</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>10 mM NADH + 10 μM cytochr. c</td>
<td>10.0</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>50 mM dl-α-Glycerophosphate</td>
<td>0.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Rm and Wm same as in Table 1.

### Table 4. Mitochondrial oxidative phosphorylation: P/O ratios*

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Substrate</th>
<th>10 mM Pyruvate + 1 mM Malate</th>
<th>20 mM dl-Lactate</th>
<th>20 mM dl-α-Glycerophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rm</td>
<td>Wm</td>
<td>Rm</td>
</tr>
<tr>
<td>1</td>
<td>10 mM NADH</td>
<td>5</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>10 mM NADH + 10 μM cytochr. c</td>
<td>2.3</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>50 mM dl-α-Glycerophosphate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Rm and Wm same as in Table 1. *μMoles phosphorus esterified divided by μatoms oxygen consumed.

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DISCUSSION

Electron photomicrographs showed the mitochondria to be intact but accompanied by cellular debris. The protein of this material together with the albumin adsorbed on the mitochondria from the homogenizing medium lowered the oxygen consumption rates since these were based on protein content. For this reason the intersubstrate activity ratios for each mitochondrial type in Fig. 1 are considered more reliable than the oxygen uptake rates based on protein content (Table 1). The presence of cellular debris also raises the possibility that mitochondrial differences suggestive of a metabolic specialization were in fact associated with the nonmitochondrial material in our preparations. This appears unlikely because it can be shown histochemically that the oxidation of the present substrates in muscle under conditions comparable to those used here is limited almost entirely to the mitochondria. The activity of the red and white muscle mitochondria toward NADH₂, succinate, lactate, and α-glycerophosphate demonstrable histochemically (11, 32) is similar to that reported here, but the differences in the response of the two mitochondrial types are more striking in tissue sections than the present results would suggest. This discrepancy is most likely due to a partial suppression of such differences in the present work by an admixture of mitochondria from muscle fibers of the contrasting variety that are found in the purest samples of red and white muscle.

Pyruvate with a catalytic amount of malate seemed to be an equally satisfactory fuel for red and white muscle mitochondria. Good P/O ratios and the finding that the respiration with these substrates was tightly coupled to phosphorylation indicated an acceptable degree of biochemical integrity. Assuming that the other metabolic features of the two mitochondrial types approximate their normal characteristics in vivo, it would appear that red and white muscle fibers favor different modes of coupling the respiratory chain to the glycolytic reactions generating NADH₂ in the cytoplasm. Thus, the direct oxidation of NADH₂ is much more active in red than in the white muscle mitochondria. However, this is offset by the greater activity in white muscle of both the cytoplasmic NAD-linked l-α-glycerophosphate dehydrogenase and the mitochondrial l-α-glycerophosphate oxidase (5, 24, 32). These two enzymes with a catalytic amount of l-α-glycerophosphate could form a shuttle in white muscle coupling the oxidation of l-lactate and glyceraldehyde 3 phosphate in the cytoplasm to the mitochondrial respiratory chain. In contrast, red muscle mitochondria and heart mitochondria oxidize succinate, lactate, and NADH₂ directly but both are deficient in the oxidase necessary for the operation of the α-glycerophosphate shuttle (10, 18). Because of this deficiency, it is difficult to avoid the conclusion that the direct oxidation of NADH₂ by carefully prepared myocardial mitochondria (10) is at least in part physiological, especially since lactate may form a considerable portion of the heart's fuel (2). For the same reason it seems likely that intact red muscle mitochondria, like those of the heart, normally utilize NADH₂ directly since red muscle mitochondria also are relatively poor in the oxidase needed for the α-glycerophosphate shuttle to operate. The similarity of red muscle and myocardium in this and other features (11) probably is related to their common aerobic orientation and many of the characteristics of red muscle mitochondria described here attain their full expression in heart mitochondria (10, 18, 38).

The foregoing interpretation of the reciprocal relation between the rate of oxygen uptake with α-glycerophosphate and NADH₂ in red and white muscle mitochondria is based on the generally held view (6, 7) that the difficulty of coupling the extramitochondrial NADH₂-generating reactions to the respiratory chain arises from the limited permeability of the normal mitochondrial membrane to NADH₂. However, the persistence of the reciprocal response to these substrates after osmotic swelling has increased the permeability to NADH₂ (6, 19, 22) suggests that the two types of skeletal muscle mitochondria may differ fundamentally in their enzymatic constitution and that permeability to NADH₂ and l-α-glycerophosphate may be of secondary importance.

It seems probable that the substrate response patterns of red and white skeletal muscle mitochondria may well depend on the substrate levels. In the present work these were in most cases sufficient to saturate the enzyme systems concerned. Further studies at substrate concentrations approximating those in resting and active red and white muscle may necessitate some revision of the mitochondrial response patterns reported here.

### Table 5. Effect of sucrose concentration on oxygen uptake, μmoles per milligram mitochondrial protein per hour

<table>
<thead>
<tr>
<th>Sucrose Concentration</th>
<th>Homogenizing solution medium</th>
<th>Incubation medium</th>
<th>5 mM Pyruvate + 0.25 mM Malate</th>
<th>2 mM NADH₂</th>
<th>4 mM Succinate</th>
<th>5 mM dr-α-Glycerophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 M</td>
<td>0.25 M</td>
<td>0.5 M</td>
<td>3.6</td>
<td>9.0</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>0.80 M</td>
<td>0.3 M</td>
<td>0.4</td>
<td>1.2</td>
<td>1.0</td>
<td>0.1</td>
<td>0.2</td>
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</table>

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CONCLUSIONS

The oxygen consumption of mitochondria isolated from red and white muscle of guinea pigs has been determined with pyruvate-malate, dl-α-glycerophosphate, succinate, d-lactate, and reduced nicotinamide adenine dinucleotide (NADH) as substrates. Although they showed similar rates of oxygen uptake with pyruvate-malate, the mitochondria from the two muscle types demonstrated a different pattern of response to the other substrates. White muscle mitochondria had a higher rate of oxygen consumption with α-glycerophosphate than with lactate, succinate, and NADH. In contrast, the mitochondria from red muscle were less active with α-glycerophosphate than with the other substrates. These results are compatible with earlier evidence that an α-glycerophosphate shuttle may couple the reactions generating NADH in the cytoplasm of white muscle with the mitochondrial respiratory chain. The properties of the red muscle mitochondria suggest that the direct oxidation of NADH may be more important in this tissue than the α-glycerophosphate shuttle.

Various treatments aimed at altering permeability failed to change the reciprocal ability of α-glycerophosphate and NADH to stimulate the oxygen consumption of the two mitochondrial types. It therefore appears that the difference in the response of the mitochondria of red and white skeletal muscle to these two substrates reflects a difference in enzymatic constitution.

I wish to thank Donna Pownall, Florence Zawislak, and Lawrence Juskow for their skillful technical assistance.

REFERENCES