Biochemical Properties of Subsarcolemmal and Interfibrillar Mitochondria Isolated from Rat Cardiac Muscle*

(June 29, 1977)

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Two populations of mitochondria were observed upon ultrastructural examination of cardiac muscle tissue, one located directly beneath the sarcolemma (subsarcolemmal mitochondria) and another between the myofibrils (interfibrillar mitochondria). Subsarcolemmal mitochondria were released by treatment of heart muscle with a Polytron tissue processor, while interfibrillar mitochondria were released by nagaarse digestion of the remaining tissue. These results were supported by electron microscopy of Polytron-treated heart tissue showing rupture and loss of sarcolemma with release of the underlying mitochondria but with retention of intact mitochondria between the myofilaments. Electron microscopy of the isolated mitochondria indicated that both mitochondrial types maintained their structural integrity throughout the isolation procedure.

Specific activities of succinate dehydrogenase and citrate synthase were higher in the interfibrillar mitochondria as compared to the subsarcolemmal mitochondria, while those of carnitine palmitoyltransferase and a-glycerophosphate dehydrogenase were nearly the same in both. Interfibrillar mitochondria oxidized all substrates tested approximately 1.5 times faster than did the subsarcolemmal mitochondria. Thus the two mitochondrial types differed not only in their respective locations in the cell, but also in certain biochemical properties.

The abundance of fibrillar material in heart muscle makes isolation of intact cardiac mitochondria difficult. Grinding minces of heart tissue with a mortar and pestle in sand (2, 3) or with a Potter-Elvehjem homogenizer (4–6) results in incomplete homogenization and low yields of mitochondrial protein (8 to 11 mg/g of heart). While use of the Polytron tissue processor (7–9), which disrupts tissue by mechanical shearing and localized sonication, results in a greater percentage of intact mitochondria than does simple grinding, the yields (approximately 8 mg/g of heart) cannot be enhanced by prolonging either the duration or intensity of homogenization without damaging the mitochondria.

Greatly improved yields of cardiac mitochondrial protein are obtained by exposure of tissue minces to the protease, nagaarse (10, 11). This treatment results in a 5-fold increase in mitochondrial protein (to approximately 30 mg/g of heart). Thus it would appear that nagaarse is able to tap a source of mitochondria that is ordinarily unavailable to simple grinding techniques or to sonication. Ultrastructural examination of mammalian cardiac muscle cells reveals that the cardiac mitochondria are grouped in two relatively distinct populations—one directly beneath the sarcolemma (subsarcolemmal mitochondria), the other between the myofibrils (interfibrillar mitochondria) (12–14). Based on the disposition of these cardiac mitochondria, we propose that grinding or sonication will release the subsarcolemmal population, while the bulk of the interfibrillar population will remain trapped by the surrounding fibrils. It is only when the nagaarse is applied that the latter mitochondria are also released.

The present study was designed to test this proposition. The effects of both Polytron treatment and nagaarse exposure on minced cardiac muscle and all subsequent fractions were monitored by electron microscopy. It was found that Polytron treatment does in fact release subsarcolemmal mitochondria, and that the interfibrillar mitochondria are released only after nagaarse digestion. Moreover, the two populations of mitochondria so isolated are metabolically different.

MATERIALS AND METHODS

Cardiac Muscle Mitochondrial Fractionation—Male Sprague-Dawley rats (from Zivic Miller, Allison Park, Penn., or Carworth Farms, Wilmington, Mass.) were housed in plastic cages (in bedding) for 7 to 10 days before use. They received Purina Rat Chow (Ralston Purina Co.) ad libitum. For each experiment, from three to five animals (250 to 300 g) were decapitated and bled for 10 to 20 s. Their hearts were removed and immediately placed in cold Buffer A (220 mM mannitol, 70 mM sucrose, 5 mM Mops, pH 7.4). Basically

1. The abbreviations used are: Mops, 4-morpholinopropanesulfonic
the procedure of Sordahl et al. (8) was used for preparation of Polytron mitochondria. After removal of extracellular tissue, the hearts were rinsed 5 to 10 times, blotted dry, and weighed. The tissue was finely minced and brought to a final concentration of 10 g of tissue/100 ml of Buffer A plus 2 mM EGTA and 0.2% bovine serum albumin. This tissue suspension was homogenized with a Polytron tissue processor (Brinkmann Instruments) for exactly 2.4 s at a rheostat setting of 6.5. The time from decapitation to initial homogenization never exceeded 15 min.

The Polytron homogenate was centrifuged in a Sorvall RC-5 (SS-34 head) according to the protocol outlined in Fig. 1. In order to ensure complete recovery of Polytron mitochondria from the Polytron homogenate, the original Polytron pellet was resuspended to the original volume by use of a Potter-Elvehjem homogenizer in Buffer A (plus 2 mM EGTA and 0.2% albumin) and recentrifuged at 300 × g. The resultant supernatant was combined with the original and centrifuged at 8000 × g for 10 min to obtain the mitochondrial fraction, which was washed twice. The final Polytron mitochondrial pellet was resuspended in Buffer A (0.5 mM EGTA) to give a final protein concentration of approximately 25 mg/ml. All supernatants from these washes were combined to yield the total Polytron supernatant.

The nagarse mitochondrial fraction was derived from nagarse treatment of the washed Polytron pellet (See Fig. 1). The pellet was resuspended in Buffer B (100 mM KCl, 50 mM Mops, pH 7.4) plus 2 mM EGTA and 0.2% albumin and nagarse mitochondria prepared according to the procedure of Chance and Hagihara (10) as modified by Tomec and Hoppel (15). Nagarse was added to a final concentration of 5 mg/g wet weight of tissue and the homogenate immediately homogenized with a Potter-Elvehjem homogenizer, diluted 2-fold with Buffer B, and immediately centrifuged at 5000 × g for 5 min. The supernatant containing the nagarse was stored and analyzed separately from other nagarse supernatants. Enzyme activities and total protein found in this fraction were added to the values obtained from the remaining nagarse supernatants, as reported in Table 1.

The high speed pellet remaining after the nagarse treatment was resuspended in the original volume of Buffer B and sedimented at high speed to yield the nuclear pellet. In order to quantitate recovery of mitochondria from this pellet, we washed it twice in Buffer B (2 mM EGTA; 0.2% albumin) and added the supernatants back to the original nuclear supernatant. These were centrifuged at 3000 × g for 10 min. The resulting pellet was washed twice and then resuspended in Buffer B (0.5 mM EGTA) to a final concentration of 25 mg/ml. The supernatants were combined to yield the remaining nagarse supernatant.

**Enzyme Assays**—All spectrophotometric assays were carried out at 30°C in a total volume of 1 ml. Subcellular fractions were diluted to 1.0 mg/ml in 0.1% cholic acid and 0.05 M phosphate buffer, pH 7.4, except as noted. Succinate dehydrogenase (16), glutamate dehydrogenase (17), and citrate synthase (18) were assayed by published methods. Carnitine palmitoyltransferase was assayed either by the DTNB method of Fritz et al. (19) as modified by Bieber et al. (20) or by the [14C]palmitoylcarnitine assay of Hoppel and Tomec (17). Enzyme activities and total protein found in this fraction were added to the values obtained from the remaining nagarse supernatants, as reported in Table 1.

**RESULTS**

Morphological examination of cardiac muscle demonstrates two mitochondrial populations, one located directly beneath the sarcolemma and the other between the myofibrils (Fig. 2, also Refs. 12-14). The distribution of protein and mitochondrial marker enzymes in cardiac muscle tissue is shown in Table I and Fig. 3. Polytron treatment of the cardiac muscle mince released only 9.2 ± 0.6 mg of mitochondrial protein/g of heart in 10 separate isolations. This yield was not increased by increasing either the time or the intensity of homogenization. Such treatment merely damaged the initially released mitochondria, as judged by decreased respiratory control (data not shown).

Figs. 4 and 5 show that the cells in Polytron-treated cardiac muscle are separated at their intercalated disks and have disrupted sarcolemmas. Subsarcolemmal mitochondria are absent, while interfibrillar mitochondria remain “trapped” between the filaments. The latter mitochondria are for the most part morphologically unaltered. Nagarse treatment of the Polytron pellet released a large portion of the interfibrillar

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**Subcellular Fractionation of Polytron and Nagarse Mitochondria**

Morphological examination of cardiac muscle tissue is shown in Table I and Fig. 3. Polytron treatment of the cardiac muscle mince released only 9.2 ± 0.6 mg of mitochondrial protein/g of heart in 10 separate isolations. This yield was not increased by increasing either the time or the intensity of homogenization. Such treatment merely damaged the initially released mitochondria, as judged by decreased respiratory control (data not shown).

Figs. 4 and 5 show that the cells in Polytron-treated cardiac muscle are separated at their intercalated disks and have disrupted sarcolemmas. Subsarcolemmal mitochondria are absent, while interfibrillar mitochondria remain “trapped” between the filaments. The latter mitochondria are for the most part morphologically unaltered. Nagarse treatment of the Polytron pellet released a large portion of the interfibrillar
Recovery of protein and mitochondrial marker enzyme activity during subcellular fractionation

Enzymes and protein were assayed as described under "Materials and Methods." Protein concentration was expressed in milligrams/g wet weight of heart. Enzyme activity in the total homogenate is given as micromoles of product formed/min/g of heart tissue. The percentage of the total protein or of individual enzyme activities in the various subcellular fractions (as described under "Materials and Methods" and shown in Fig. 1) were added to obtain the reported recoveries.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Total homogenate</th>
<th>Nuclear fraction (N)</th>
<th>Polytron mitochondria</th>
<th>Nagarse mitochondria</th>
<th>Polytron supernatant (S1)</th>
<th>Nagarse supernatant (S1)</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>202</td>
<td>32.8</td>
<td>4.4</td>
<td>10.9</td>
<td>33.5</td>
<td>14.3</td>
<td>95.9</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>1.50</td>
<td>24.2</td>
<td>13.8</td>
<td>45.9</td>
<td>9.3</td>
<td>9.9</td>
<td>103.1</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>1.51</td>
<td>26.1</td>
<td>13.1</td>
<td>50.6</td>
<td>4.3</td>
<td>5.5</td>
<td>99.6</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>75.6</td>
<td>27.3</td>
<td>12.4</td>
<td>47.5</td>
<td>5.9</td>
<td>8.8</td>
<td>101.9</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase</td>
<td>1.66</td>
<td>23.4</td>
<td>20.3</td>
<td>55.5</td>
<td>3.6</td>
<td>3.3</td>
<td>106.1</td>
</tr>
<tr>
<td>α-Glycerophosphate dehydrogenase</td>
<td>0.37</td>
<td>24.4</td>
<td>23.2</td>
<td>63.3</td>
<td>10.6</td>
<td>17.1</td>
<td>111.0</td>
</tr>
<tr>
<td>NADPH cytochrome c reductase</td>
<td>0.44</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;1.3</td>
<td>23.3</td>
<td>92.0</td>
<td>115.3</td>
</tr>
</tbody>
</table>

FIG. 2. Electron micrograph of a slightly oblique section of an in situ cardiac muscle cell. A cluster of mitochondria is situated directly beneath the sarcolemma (arrows), while other mitochondria are arranged in rows between myofibrils, x 16,000.

Mitochondria, constituting 20.6 ± 1.6 mg of mitochondrial protein/g of heart for seven separate isolations. Careful washing of the remaining pellet left only about 20 to 25% of the mitochondrial marker enzyme activity in this fraction (N) (Table I and Fig. 3). Viewed in the electron microscope, this washed pellet consisted primarily of fragmented bundles of myofilaments, microsomes, reticular fibers, nuclei, and segments of capillaries, with only a relatively few intact muscle mitochondria remaining (Figs. 6 and 7). It is of interest to note that nagarse completely removed the dense material that characterizes the Z band of the myofibrils.

Thus the total amount of mitochondrial protein/g of heart was about 30 mg, comparable to the amount obtained solely by nagarse treatment of minced cardiac muscle. It appears that there are two distinct populations of cardiac muscle mitochondria, one obtainable by Polytron treatment, which disrupts the sarcolemma, and the other requiring proteolytic digestion of the myofibrils for its liberation.

Properties of Isolated Polytron and Nagarse Mitochondria

Degree of Integrity—Previous studies of mitochondria isolated by either the Polytron or nagarse demonstrated that either method used alone resulted in the isolation of tightly coupled mitochondria (7–11). This was also true in our prepa-
Damage to mitochondrial membranes may be detected by the appearance of mitochondrial enzymes in the supernatants during subcellular fractionation. These include matrix enzymes such as glutamate dehydrogenase and citrate synthase or mitochondrial inner membrane enzymes such as succinate dehydrogenase and α-glycerophosphate dehydrogenase. Since 60 to 70% of these mitochondrial enzyme activities were accounted for in the combined Polytron and nagarse mitochondrial fractions (Table I), with little of these enzymes detectable in the respective supernatants, it may be deduced that neither group of mitochondria was significantly damaged.

Integrity of isolated mitochondria may also be determined by polarographically measuring the extent of their coupling. Respiratory control ratios were high and not significantly different for seven separate mitochondrial preparations (Table II). The ADP/oxygen ratio approximated the theoretical value for both α-ketoglutarate and glutamate as substrates.

Since intact mitochondrial membranes are impermeable to exogenous NADH, the ability of isolated mitochondria to oxidize added NADH was also used as a measure of integrity. The rate of NADH oxidation was 10 ng atoms of oxygen/min/mg in both nagarse and Polytron mitochondria as compared to the total potential for NADH oxidation in disrupted mitochondria of 300 ng atoms/min/mg.

These biochemical parameters of integrity are supported by electron micrographs of the isolated mitochondria (Figs. 8 and 9). The vast majority of the mitochondria in both Polytron and nagarse pellets were quite well preserved. Relatively few disrupted mitochondria were present in either preparation. Minor differences were apparent between the Polytron and nagarse mitochondria in the configuration of the cristae.

Nonmitochondrial Contamination—Electron microscopy showed that both mitochondrial pellets were devoid of contaminating cytoplasmic material (Figs. 8 and 9). NADPH cytochrome c reductase, an enzyme located in microsomes (22), was assayed in order to determine possible contamination of either mitochondrial type with microsomal material (Table I and Fig. 3). There was no activity detectable in either nuclear or mitochondrial fractions; the combined supernatants accounted for all activity in the total homogenate.

Relative Enzyme-specific Activities—Table III and Fig. 3 compare relative specific activities of Polytron and nagarse mitochondria. Since recoveries of enzyme activities were complete in all cases, it may be concluded that any differences in specific activity of Polytron or nagarse mitochondria were real and not due to selective loss of activity from one fraction or to selective activation of the other. Relative specific activities of citrate synthase and succinate dehydrogenase were higher in the nagarse mitochondria (1.53 and 1.50 times higher, respectively), but carnitine palmitoyltransferase and α-glycerophosphate dehydrogenase were nearly the same in both. The similarities in the specific activity of carnitine palmitoyltransferase were verified by using the [14C]palmitoylcarnitine assay of Hoppel and Tomec (17).

Oxidative Metabolism of Nagarse and Polytron Mitochondria

![Fig. 4 (upper left). The portion of cardiac muscle cell which is brillar mitochondria, which have a normal appearance, × 9,500.](http://www.jbc.org/)

![Fig. 6 (lower left). Fragments of myofibrils sedimented by low speed centrifugation of the nagarse-treated Polytron pellet (N in Fig. 1). Few mitochondria remain, × 11,000.](http://www.jbc.org/)

![Fig. 7 (lower right). A longitudinal section of a myofibrillar fragment in pellet N showing the complete extraction of Z band material, × 23,000.](http://www.jbc.org/)
**Biochemistry of Subsarcolemmal and Interfibrillar Mitochondria**

**TABLE II**

*Rates of oxidative phosphorylation of Polytron and nagarse mitochondria*

Polytron and nagarse mitochondria were prepared as described under "Materials and Methods" and shown in Fig. 1. Rates of ADP-dependent oxygen consumption were expressed as nanoatoms of oxygen/min/mg of mitochondrial protein (mean ± S.E.), where n = number of mitochondrial preparations. The respiratory control indices and ADP/oxygen ratios were calculated as described previously (23, 24).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Polytron mitochondria</th>
<th>Nagarse mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate (20 mM) (n = 7)</td>
<td>129 ± 10.5</td>
<td>230 ± 14.8</td>
</tr>
<tr>
<td>α-Ketoglutarate (10 mM) + malonate (10 mM) (n = 5)</td>
<td>106 ± 8.4</td>
<td>194 ± 4.9</td>
</tr>
<tr>
<td>Glutamate (20 mM) (n = 7)</td>
<td>163 ± 10.4</td>
<td>29.7 ± 3.1</td>
</tr>
<tr>
<td>α-Ketoglutarate (10 mM) + malonate (10 mM) (n = 5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE III**

*Specific activities of mitochondrial marker enzymes*

Polytron and nagarse mitochondria were prepared as described under "Materials and Methods" and shown in Fig. 1. Mitochondrial enzyme specific activities (mean ± S.E.) are expressed as nanomoles of product formed/min/mg of mitochondrial protein, n = number of mitochondrial preparations. Carnitine palmitoyltransferase "DTNB assay" refers to the method which measures release of coenzyme A from palmitoyl-CoA by using DTNB (19, 20). CPT II assay measures conversion of [14C]palmitoylcarnitine to [14C]palmitoyl-CoA (17).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Polytron mitochondria</th>
<th>Nagarse mitochondria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate dehydrogenase (n = 8)</td>
<td>34.3 ± 5.8</td>
<td>45.7 ± 6.1</td>
</tr>
<tr>
<td>Citrate synthase (n = 6)</td>
<td>749 ± 89</td>
<td>1380 ± 155</td>
</tr>
<tr>
<td>Carnitine palmitoyltransferase</td>
<td>28.5 ± 3.5</td>
<td>32.4 ± 3.8</td>
</tr>
<tr>
<td>DTNB assay (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPT II assay (n = 4)</td>
<td>262 ± 15</td>
<td>301 ± 22</td>
</tr>
<tr>
<td>α-Glycerophosphate dehydrogenase (n = 6)</td>
<td>7.8 ± 0.94</td>
<td>7.8 ± 1.2</td>
</tr>
</tbody>
</table>

**TABLE IV**

*Rates of oxidative metabolism (State III; ADP-stimulated) with lipid substrates*

Polytron and nagarse mitochondria were prepared as described under "Materials and Methods" and shown in Fig. 1. Rates of oxidative metabolism were expressed as in Table II. They were measured with a variety of both lipid (Table IV) and nonlipid (Table V) substrates in both Polytron and nagarse mitochondria. In all cases, the metabolic rates were significantly higher in nagarse mitochondria than in Polytron mitochondria. To exclude the possibility that the lower oxidation rates of the Polytron mitochondria were caused by adhering myofilaments, sarcolemmal, or other coating material, which had been removed from the nagarse mitochondria by the proteolytic digestion, the Polytron mitochondria were treated with nagarse. Such treatment did not increase the rates of oxidation of the Polytron mitochondria for substrates tested to those rates found in the nagarse mitochondria, nor did it alter morphology (data not shown).

**TABLE V**

*Rates of oxidative metabolism (State III; ADP-stimulated) with nonlipid substrates*

Data are expressed as described in Table IV.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Polytron mitochondria</th>
<th>Nagarse mitochondria</th>
<th>Rate (nagarse)/Rate (polytron)</th>
<th>p &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate (20 mM) (n = 7)</td>
<td>129 ± 10.5</td>
<td>230 ± 14.8</td>
<td>1.54</td>
<td>0.0005</td>
</tr>
<tr>
<td>α-Ketoglutarate (10 mM) + malonate (10 mM) (n = 5)</td>
<td>106 ± 8.4</td>
<td>163 ± 10.4</td>
<td>1.54</td>
<td>0.0025</td>
</tr>
<tr>
<td>Pyruvate (10 mM) + L-malate (2.5 mM) (n = 6)</td>
<td>175 ± 12.4</td>
<td>239 ± 23.7</td>
<td>1.44</td>
<td>0.01</td>
</tr>
<tr>
<td>Succinate (10 mM) + cyanide (4 mM) (n = 4)</td>
<td>147 ± 4.7</td>
<td>239 ± 19.7</td>
<td>1.60</td>
<td>0.0025</td>
</tr>
<tr>
<td>β-Hydroxybutyrate (20 mM)</td>
<td>75.3 ± 7.6</td>
<td>125 ± 11.7</td>
<td>1.67</td>
<td>0.0025</td>
</tr>
</tbody>
</table>

**DISCUSSION**

We found that Polytron treatment of cardiac muscle released only those mitochondria directly beneath the sarcolemma. A second group of mitochondria situated between the sarcolemma and the myofibrils was released when the Polytron pellet was exposed to nagarse. Sequential isolation of the two classes of mitochondria permitted their separate analysis, in contrast to previous studies which were based either on admixtures of the two types of mitochondria (10, 33) or on the subsarcolemmal mitochondria alone (7-9, 34, 35).

**Fig. 6 (upper).** A representative field of mitochondria released by Polytron treatment (Polytron mitochondrial fraction of Fig. 1). Note the absence of cytoplasmic contaminants, × 4,700.

**Fig. 9 (lower).** A representative field of the nagarse mitochondrial fraction released from heart muscle cells by nagarse treatment of the Polytron pellet, × 4,700.
We found that these two mitochondrial types are not only located in different places in the cell, but also differ biochemically. The oxidative rates of interfibrillar mitochondria were 1.4 to 1.7 times higher than those of the subsarcolemmal mitochondria with all substrates tested. Ratios of specific activities of citrate synthase, glutamate dehydrogenase, and succinate dehydrogenase in interfibrillar mitochondria versus subsarcolemmal mitochondria were approximately 1.5, but activities of total carnitine palmitoyltransferase and α-glycerophosphate dehydrogenase were nearly identical. The biochemical differences found probably represent the minimal extent to which any true biochemical differences exist because some cross-contamination of these mitochondrial types might be expected to occur.

While interfibrillar mitochondria oxidized all substrates tested at significantly faster rates than did subsarcolemmal mitochondria, the pattern of substrate utilization was nearly the same in both mitochondrial types. Pyruvate (+L-malate) was oxidized more rapidly than the other substrates tested, while the oxidation of either β-hydroxybutyrate or octanoate (+L-malate) was very low. Octanoyl-l-carnitine (+L-malate) was oxidized more rapidly than octanoate, even though octanoate does not require carnitine for movement across the mitochondrial membrane (36). This suggests that the activity of the carnitine-palmitoyltransferase system which converts octanoyl carnitine to octanoyl-CoA is significantly higher than either the diffusion of octanoate across the inner mitochondrial membrane or the activation of octanoyl-CoA to octanoyl-Carnitine. Pyruvate (+L-malate) was oxidized more rapidly than the other substrates tested, while the oxidation of either β-hydroxybutyrate or octanoate was nearly identical. The biochemical differences found probably represent the minimal extent to which any true biochemical differences exist because some cross-contamination of these mitochondrial types might be expected to occur.

Using marker enzyme activities to follow subcellular fractionation, we were able to show that 60 to 70% of the mitochondria in the tissue were recovered in the combined mitochondrial pellet. That 25% of the activity remained in the nuclear pellet and only 10 to 15% in the combined supernatants attests to the gentleness with which these mitochondria were handled during the isolation procedure. The rather low centrifugal force used to sedimt the mitochondria was designed to minimize cytoplasmic contamination of the pellet, but probably left some intact mitochondria in the supernatant. It may thus be concluded from these enzymic recoveries that subcellular fractionation was complete, with no significant contamination or disruption of either mitochondrial type.

It has been suggested that heart mitochondria prepared by use of the Polytron tissue processor are "superior" to the mitochondria obtained by nagarse digestion of minced tissue, since nagarse has been shown to lead to the loss of long chain fatty acid activating enzyme (33, 37). While it is true that nagarse mitochondria are not able to oxidize free palmitic acid, they do not appear to be damaged in any other manner; in fact they are able to oxidize most substrates more rapidly than the Polytron mitochondria.

Alternatively, it may be argued that the Polytron damages those mitochondria which it releases from heart muscle. Our studies show no damage to these mitochondria by established criteria. Furthermore, it has recently been shown that liver mitochondria may also be isolated by using the Polytron under conditions nearly identical with ours (38). Respiratory control ratios, ultrastructural appearances, and the distribution of marker enzymes were the same as those found when liver mitochondria were isolated by using the Dounce homogenizer.

Differences between interfibrillar mitochondria and subsarcolemmal mitochondria reported here suggest that these two mitochondrial populations play a somewhat different metabolic role in the economy of the cell. Previous work has shown that in certain physiological and pathological states, one of the two populations of muscle mitochondria may increase in number (21, 39-43). This phenomenon may reflect a compensatory mechanism at the organelle level in response to a specific cell lesion. This hypothesis is now testable, since the observations reported here demonstrate that the two mitochondrial populations can be independently studied.

Acknowledgments - We wish to thank Ms. Barbara Good for editing and Ms. Jan Williams and Ms. Sandra Player for typing this manuscript.

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