Reevaluation of Oxidative Phosphorylation in Cardiac Mitochondria from Normal Animals and Animals in Heart Failure

By George E. Lindenmayer, M.D., Louis A. Sordahl, Ph.D., and Arnold Schwartz, Ph.D.

ABSTRACT

For an adequate evaluation of mitochondria from diseased hearts, basic characteristics of isolation, storage, media, ultrastructure and type of assay were first determined using mitochondria from normal animals. A proteinase procedure yielded mitochondria from small laboratory animals, with low respiratory control and marked permeability changes. The isolation medium yielding the most stable mitochondria with the highest respiratory control contained 0.18M KCl, 10mM EDTA, and 0.5% to 1% bovine serum albumin at pH 7.2. Heart failure in guinea pigs and rabbits was produced by varying degrees of stenosis of the ascending aorta. An aberration in respiratory control was found in mitochondria from hearts in severe failure. The quantitative differences between normal and experimental respiratory control values were greatest when the highest possible normal respiratory control levels were obtained. The difference between mitochondria prepared by a proteinase method from control and failing hearts was minimal. No changes in oxidative phosphorylation were noted in mitochondria from hearts arrested by nitrogen, suggesting that acute hypoxia does not irreversibly damage energy-liberating reactions. It is concluded that severe heart failure is characterized by defects in mitochondrial oxidative phosphorylation, and that techniques of isolation or assay or both are probably not causing the abnormalities.

ADDITIONAL KEY WORDS

cardiac mitochondria respiratory control hypoxia energy production cardiac arrest guinea pig rabbit

The cellular and molecular events characteristic of congestive heart failure are still largely unknown. During the past 10 years, two general and opposing concepts of energetics in heart failure have evolved, one implicating an aberration in energy-liberating reactions catalyzed by mitochondria (1-8) and the other describing normal energy production (9-11). The importance of mitochondria in normal cardiac function is exemplified by the fact that the cardiac muscle cell consists of at least 20% by dry weight of these organelles (12). The conflicting data may be explained in one or both of two ways: (a) The experimental models or human tissue employed were in different degrees or states of failure. (b) Isolation and assay conditions of subcellular components varied significantly in different laboratories. In a recent assessment of the problem, Sobel et al. (13) reported no differences from controls in oxidative phosphorylation of mitochondria from guinea pig hearts that failed after partial stenosis of the ascending aorta. Their experimental model was the same as that previously employed in two laboratories.
(2, 3) where significant alterations in mitochondria were observed. Sobel et al. suggested that the Warburg manometric technique, used for the above studies (2, 3), results in mitochondrial alterations because of damage during the relatively prolonged incubation period in fortified media. The use of the more rapid oxygen electrode method revealed no differences between mitochondria from failing hearts and those from controls (13). Since previous manometric studies were carried out under controlled conditions (2, 3, 5) the explanation by these authors appears doubtful. When properly conducted and using fortified media, manometry is an adequate and useful method for estimating several important aspects of mitochondrial activity (14).

Some of the difficulties involved in assaying activities of heart mitochondria (e.g., retention of endogenous substrate, ion content) have been discussed by others (15-19). To resolve the problem of whether isolation or assay techniques or conditions are responsible for the reported mitochondrial defects in heart failure, we have investigated, in depth, for the reported mitochondrial defects in heart failure, we have investigated, in depth, for mitochondrial intactness and activity were used. Considering this information, a reexamination of mitochondria derived from the previously employed "failing" model was then undertaken. To avoid complex assay conditions, a polarographic procedure using an oxygen electrode apparatus (Oxygraph, Gilson Medical Electronics) using either a vibrating platinum electrode or a Clark oxygen electrode (Yellow Springs Instruments), in an assay medium containing 0.25M sucrose, 10mM tris-HCl, or tris-succinate and mitochondria (1.0 to 1.5 mg/ml). The procedure and definitions have been described (3). Calculation of respiratory control and ADP-O ratios were determined from data derived after the first addition of ADP. The fortified assay medium in the manometric experiments was the same as previously used (3).

Protein determinations were done by a biuret method (21). Assays using equivalent (+10%) amounts of mitochondrial protein were compared.

Congestive Heart Failure.—Albino guinea pigs (400 to 700 g) were anesthetized with 25 mg/kg pentobarbital ip and ventilated by an intratracheal cannula and a Phillips Bird respirator. The gas used was 95% O₂-5% CO₂ mixture. The ascending aorta was narrowed as previously described (3). After recovery, the animals were closely observed until the previously described signs (3) were manifest. At this time the guinea pig with heart failure and a control or sham-operated guinea pig were anesthetized with 25 mg/kg (control) or 15 mg/kg (experimental) pentobarbital, the trachea was intubated and the lungs were ventilated. The thoracic and peritoneal cavities were examined prior to removal of the heart.

Acute Heart Failure.—This was induced in anesthetized, open-chest guinea pigs by narrowing the ascending aorta 50% to 60% with 00 silk (3). The animal with partial aortic occlusion and a control animal were simultaneously ventilated with 95% O₂-5% CO₂. The hearts were removed.
Figure 1

Oxidative phosphorylation in heart failure

Results

Mitochondria isolated in the KCl medium had a much higher initial respiratory control index, indicative of "tighter" coupling of phosphorylation to respiration (25), than mitochondria isolated in the other two media (Fig. 1). Mitochondria from all species prepared by the Nagarse method always oxidized reduced nicotinamide adenine dinucleotide (NADH), at high respiratory rates. Mitochondria isolated in the KCl medium did not utilize NADH, while the sucrose-isolated mitochondria oxidized NADH at a rate lower than the Nagarse-preparation.

These data suggest that the KCl-EDTA-albumin medium is ideal for isolation of tightly coupled heart mitochondria from all species tested (data not presented). In general, respiratory control values of 12 to 15 (glutamate) and 3 to 7 (succinate) were characteristic of mitochondria isolated in this medium.

Bovine serum albumin appeared to be necessary when heart mitochondria were to be stored for protracted periods at 0° to 4°C; high respiratory control was maintained for 18 hours. Mitochondria isolated by the other procedures, including KCl-EDTA in the absence of albumin, could not be stored for more than 8 hours without a severe loss of respiratory control. Respiratory control was completely lost after prolonged (18 hours) storage.
MINUTES

Oxygen electrode tracings of rat heart mitochondria prepared in KCl-EDTA-albumin as described in Methods. Tracings A are mitochondria assayed for 20 minutes at 25°C and tracings B the same preparation assayed at 37°C. Arrows indicate additions of 268 nanomoles of ADP. Numbers to left of state 3 "bursts" are QO2 values expressed in nanoatoms O2/min/mg mitochondrial protein. Glutamate is the substrate. Mw = washed mitochondria; RCI = respiratory control index.

storage, when mitochondria were isolated in 0.3M sucrose, 10mM EDTA, 0.5% albumin at pH 7.2, indicating the importance of KCl.

Figure 2 illustrates the stability of appropriately isolated mitochondria from various species at an elevated temperature and after repeated additions of ADP. When a fortified medium of the Warburg type was used in a manometric device during prolonged incubation (50 minutes at 37°C) no decrease in oxygen consumption (state 3) occurred. Magnesium chloride produced a stimulation of oxygen consumption (state 4) in the oxygen electrode system. Subsequent addition of oligomycin totally inhibited the Mg2+ stimulated respiration. High respiration due to an uncoupled state of oxidative phosphorylation is not inhibited by these concentrations of oligomycin (26).

The guinea pigs, 3 to 10 days after partial constriction of the ascending aorta, developed tachycardia, dyspnea, rales, hypothermia, lethargy, and congested "nutmeg" liver (3). Gross pathological examination revealed an increase in the (heart weight/body weight) X 106 from 2.57 ± 0.09 (SEM) to 3.74 ± 0.25; (liver weight/body weight) X 105 from 5.57 ± 0.20 to 8.34 ± 0.45; and (lung weight/body weight) X 105 from 3.27 ± 0.13 to 4.19 ± 0.25. Many animals had pleural effusion and ascites. Three out of ten animals, however, had significant cardiac hypertrophy without obvious failure.

Figure 3 shows representative traces of oxygen consumption from mitochondria isolated from normal and experimental guinea pigs.

### TABLE 1

<table>
<thead>
<tr>
<th>Expt</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RCI</td>
<td>ADP/O QO2</td>
</tr>
<tr>
<td>1</td>
<td>3.4</td>
<td>110</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>103</td>
</tr>
<tr>
<td>3</td>
<td>4.2</td>
<td>200</td>
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<tr>
<td>4</td>
<td>5.1</td>
<td>125</td>
</tr>
<tr>
<td>5</td>
<td>3.6</td>
<td>105</td>
</tr>
<tr>
<td>Mean</td>
<td>5.0</td>
<td>141</td>
</tr>
<tr>
<td>± se</td>
<td>0.8</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>100</td>
</tr>
</tbody>
</table>

The mitochondria were isolated in 0.25M sucrose, 10mM tris buffer and 1mM EDTA and assayed in the sucrose-tris medium (see Methods) at 30°C. Control refers to cardiac mitochondria of normal guinea pigs and experimental refers to mitochondria from hearts of guinea pigs in congestive heart failure. Paired experiments (control and experimental) were carried out on the same day. RCI = respiratory control index; QO2 = oxygen consumption in nanoatoms O2/min/mg mitochondrial protein.

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OXIDATIVE PHOSPHORYLATION IN HEART FAILURE

**Figure 3**
Representative oxygen electrode tracings of mitochondria from a normal guinea pig heart and one in congestive heart failure. The mitochondria were isolated in 0.25M sucrose, 10mM tris, 1mM EDTA. Glutamate or succinate was used as the substrate (5mM-final concentration); assays were carried out in the sucrose-tris medium at 30°C. ADP, 550 nanomoles, was added where indicated. RCI = respiratory control index; \( \text{QO}_2 \) = oxygen consumption in nanomoles \( \text{O}_2/\min/\text{mg mitochondrial protein} \).

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate Substrate</td>
<td>RCI=2.2</td>
<td>RCI=1.7</td>
</tr>
<tr>
<td>Succinate Substrate</td>
<td>RCI=2.8</td>
<td>RCI=1.5</td>
</tr>
</tbody>
</table>

**Figure 4**
Representative oxygen electrode tracings of mitochondria from a normal rabbit heart and one in acute heart failure. The mitochondria were isolated in 0.18M KCl-10mM EDTA. Rotenone, 4 \( \mu \text{g} \), was added to inhibit NADH-linked oxidation, after which ADP was not utilized until succinate (5mM final concentration) was added. Assay conditions were as described in Figure 3. RCI = respiratory control index; \( \text{QO}_2 \) = nanomoles \( \text{O}_2/\min/\text{mg mitochondrial protein} \).

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**Diagram:**

- **OXIDATIVE PHOSPHORYLATION IN HEART FAILURE**
- **Figure 3:** Oxygen electrode tracings of mitochondria from a normal guinea pig heart and one in congestive heart failure. Glutamate or succinate was used as the substrate. Assays were carried out in sucrose-tris medium at 30°C. ADP, 550 nanomoles, was added where indicated. RCI = respiratory control index; \( \text{QO}_2 \) = oxygen consumption in nanomoles \( \text{O}_2/\min/\text{mg mitochondrial protein} \).
- **Figure 4:** Oxygen electrode tracings of mitochondria from a normal rabbit heart and one in acute heart failure. The mitochondria were isolated in KCl-10mM EDTA. Rotenone, 4 \( \mu \text{g} \), was added to inhibit NADH-linked oxidation, after which ADP was not utilized until succinate (5mM final concentration) was added. Assay conditions were as described in Figure 3. RCI = respiratory control index; \( \text{QO}_2 \) = nanomoles \( \text{O}_2/\min/\text{mg mitochondrial protein} \).

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**Legend:**
- **OXIDATIVE PHOSPHORYLATION IN HEART FAILURE**
- **Figure 3:** Oxygen electrode tracings of mitochondria from a normal guinea pig heart and one in congestive heart failure. Glutamate or succinate was used as the substrate. Assays were carried out in sucrose-tris medium at 30°C. ADP, 550 nanomoles, was added where indicated. RCI = respiratory control index; \( \text{QO}_2 \) = oxygen consumption in nanomoles \( \text{O}_2/\min/\text{mg mitochondrial protein} \).
- **Figure 4:** Oxygen electrode tracings of mitochondria from a normal rabbit heart and one in acute heart failure. The mitochondria were isolated in KCl-10mM EDTA. Rotenone, 4 \( \mu \text{g} \), was added to inhibit NADH-linked oxidation, after which ADP was not utilized until succinate (5mM final concentration) was added. Assay conditions were as described in Figure 3. RCI = respiratory control index; \( \text{QO}_2 \) = nanomoles \( \text{O}_2/\min/\text{mg mitochondrial protein} \).
TABLE 2
Oxidative Phosphorylation in Acute Heart Failure

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>16.0</td>
<td>11.7</td>
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<tr>
<td>Succinate</td>
<td>3.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Glutamate</td>
<td>165</td>
<td>182</td>
</tr>
<tr>
<td>Succinate</td>
<td>192</td>
<td>167</td>
</tr>
</tbody>
</table>

The mitochondria were isolated in 0.18M KCl-10mM EDTA and assayed in the sucrose-tris medium at 30°C. The values for guinea pigs are the averages of 2 experiments and the values for rabbits are the means (± SE) of 6 experiments. See Methods for details. RCI = respiratory control index; QO₂ = oxygen consumption in nanoatoms O₂/min/mg mitochondrial protein.

TABLE 3
Quantitative Evaluation of Oxidative Phosphorylation in Guinea Pig by Use of Different Isolation Media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>STE</td>
<td>5.0 ± 0.6</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>KE</td>
<td>15.8 ± 1.3</td>
<td>67 ± 5</td>
</tr>
<tr>
<td>KEA</td>
<td>18.5</td>
<td>96</td>
</tr>
<tr>
<td>CE</td>
<td>5.9</td>
<td>101</td>
</tr>
<tr>
<td>NP</td>
<td>6.3</td>
<td>131</td>
</tr>
</tbody>
</table>

Control refers to normal cardiac mitochondria. Experimental refers to mitochondria isolated from guinea pig hearts in congestive failure. The mitochondria were assayed at 30°C with glutamate as substrate. Values are means (± SE) of 5 experiments (STE) or 4 experiments (KE) or the average of 2 experiments (KEA, CE, NP). STE = 0.25M sucrose, 10mM tris, 1mM EDTA; KE = 0.18M KCl, 10mM EDTA; KEA = 0.18M KCl, 10mM EDTA, 0.5% albumin; CE = 0.18M choline Cl, 10mM EDTA; NP = Nagarse-containing medium. RCI = respiratory control index; QO₂ = nanoatoms O₂/min/mg mitochondrial protein. An experiment at room temperature (about 23°C) in KE medium gave results similar to the above.

Pigs. Table 1 presents complete data for the experiments carried out in the sucrose medium, under the defined conditions. ADP-O ratios, respiratory control, and oxygen consumption values were calculated, in each experiment, after the first addition of ADP. Significant depressions of respiratory control, ADP-O, and oxygen consumption were found when either glutamate or succinate was used as substrate. There were some changes in oxygen consumption in mitochondria from acutely failed hearts of guinea pigs and rabbits. Although these were not as great as in chronic congestive failure, significant differences were found between mitochondria of normal and experimental animals in respiratory control and oxygen consumption in the presence of glutamate (Table 2). A representative experiment (Figure 4) describes the type of evaluation carried out in this laboratory using mitochondria isolated in the KCl-EDTA-albumin medium. Rotenone, a specific inhibitor of the NADH dehydrogenase-associated flavoprotein of electron transport (28), is usually added, after a scan with glutamate, to block NADH-dependent oxidation, after which succinate-supported electron transport can be examined.

Preliminary studies on mitochondria from hearts of guinea pigs with cardiac hypertrophy but not in congestive heart failure show minimal or no changes in oxidative phos-
TABLE 4

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Glutamate</th>
<th>Succinate</th>
<th>Glutamate-malate</th>
<th>Beta-hydroxybutyrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RCI</td>
<td>ADP:O</td>
<td>RCI</td>
<td>ADP:O</td>
</tr>
<tr>
<td>Glutamate</td>
<td>5.0 ± 0.6</td>
<td>2.9 ± 0.1</td>
<td>141 ± 19</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Succinate</td>
<td>2.2 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>148 ± 20</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Glutamate-malate</td>
<td>4.8 ± 0.3</td>
<td>3.3 ± 0.1</td>
<td>229 ± 30</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Beta-hydroxybutyrate</td>
<td>2.5 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>115 ± 10</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>

Control and experimental refer, respectively, to mitochondria from normal and failing guinea pig hearts. Mitochondria were isolated in 0.25M sucrose, 10mM tris, 1mM EDTA and assayed in the sucrose-tris medium at 30°C. Values are means of 5 experiments (glutamate and succinate) or averages of 2 experiments (glutamate-malate and beta-hydroxybutyrate). The guinea pigs were in chronic congestive heart failure. RCI = respiratory control index; QO₂ = oxygen consumption in nanoatoms O₂/min/mg mitochondrial protein.

TABLE 5

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Glutamate</th>
<th>Succinate</th>
<th>Glutamate</th>
<th>Succinate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RCI</td>
<td>ADP:O</td>
<td>RCI</td>
<td>ADP:O</td>
</tr>
<tr>
<td>Glutamate</td>
<td>5.0 ± 0.6</td>
<td>2.9 ± 0.1</td>
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<td>148 ± 20</td>
<td>1.4 ± 0.1</td>
</tr>
</tbody>
</table>

Control refers to normal cardiac mitochondria. Experimental refers to cardiac mitochondria of guinea pigs in chronic congestive heart failure and rabbits in acute heart failure. Values are means (±SE) of 4 experiments (guinea pigs) or 6 experiments (rabbits). Guineapig cardiac mitochondria were isolated in 0.25M sucrose, 10mM tris, 1mM EDTA and rabbit cardiac mitochondria were isolated in 0.18M KCl, 10mM EDTA medium. RCI = respiratory control index; QO₂ = oxygen consumption in nanoatoms O₂/min/mg mitochondrial protein.

The accurately determined quantitative differences noted between mitochondria from normal and failing hearts can be further defined by isolating the mitochondria in different media. When a KCl-EDTA medium with or without albumin was used, for example, maximal differences were found between control and experimental mitochondria (Ta-
ble 3). The choline or Nagarse-containing media were the poorest for detecting differences between control and experimental mitochondria. Interestingly, no difference in NADH utilization was found between mitochondria from normal and failing hearts.

The ability of mitochondria from normal and failing hearts to use different substrates for oxidative phosphorylation is shown in Table 4. Glutamate, glutamate-malate, and beta-hydroxybutyrate are all NADH-linked substrates. Glutamate appears to be the best substrate in this group for detecting possible differences in oxidative phosphorylation. The data also show that although significant differences were observed in succinate-supported oxidative phosphorylation, the magnitude was not as great as that found with glutamate.

In an effort to exaggerate differences between mitochondria from normal and failing hearts, comparative assays were carried out at 30° and at 38°C. The data do not indicate any significant differences induced by temperature (Table 5), nor do they implicate any damage to mitochondria by the higher incubation temperatures. As would be expected, increasing the temperature from 30° to 38°C caused an increase in oxygen consumption. Exaggerated differences between mitochondria from normal and failing hearts were found, however, when the mitochondria were incubated for 60 to 180 minutes at 30°C prior to assay (data not shown).

The data in Table 6 indicate the possible error of averaging repetitive bursts in estimation of respiratory control and ADP:O values. Respiratory control and other indices of mitochondria isolated in a sucrose medium tend to decrease with repetitive additions of ADP and the normals tend to reach that of the mitochondria from failing hearts. ADP:O values remain essentially constant after repetitive additions of ADP for both control and experimental mitochondria. However, when mitochondria are isolated in the KC1-EDTA-albumin medium, multiple additions of ADP may be made without significant respiratory control changes (Fig. 2).

Mitochondria from guinea-pig hearts are not so rigorously dependent upon adequate in vivo oxygenation for the maintenance of functional integrity as one might suspect. Five to 6 minutes of hypoxia in guinea pigs (severe enough to cause cardiac arrest) had no significant effect on oxidative phosphorylation (respiratory control = 12.0 for control, 10.7 for hypoxia; ADP:O = 2.5 for control, 2.8 for hypoxia; oxygen consumption [nanoatoms O2/min/mg mitochondrial protein] = 214 for control, 218 for hypoxia; these values were obtained with glutamate as substrate at 30°C; similar results were noted when succinate was employed).

**Discussion**

In an appraisal of molecular events existing in or leading to a pathologic state, one must usually disrupt the cell and study subcellular systems. In doing this, the organelles are placed in at least two artificial environments, the isolation and assay media. If any estimate of in vivo function is to be made, the isolation and assay methods at least, should be as non-destructive and as "physiological" as possible.
The obvious conclusion is that the probability of finding a difference between a normal and diseased state is decreased if the subcellular systems are damaged or altered during isolation and assay. The present study supports this reasoning as applied to study of mitochondria from normal and failing hearts.

Initial respiratory control of mitochondria from normal hearts prepared by the KCl-EDTA medium with or without albumin (or with choline-EDTA-albumin medium) was higher than that obtained using other published methods (9, 22, 23, 27). Mitochondria prepared by this method also maintain their biochemical integrity (as determined by respiratory control) during protracted periods of storage. A relatively high level of EDTA (10mM) should be used when isolating cardiac mitochondria because of the large amounts of calcium present in cardiac tissue and because Ca$^{2+}$ can cause uncoupling of mitochondria (26). The role of albumin in maintaining the biochemical integrity of cardiac mitochondria is unknown, although the evidence is in favor of mechanisms more complex than free fatty acid binding (28-31).

Using the conditions defined for mitochondrial isolation and assay, it is clear that defects in oxidative phosphorylation do exist in both chronically and acutely failing, but not hypoxic, hearts of the experimental models employed. How can this conclusion be resolved with those studies indicating no changes, using the same experimental model (9, 13)? The simplest explanation is that there is variance in the degree of failure in animals used in different laboratories (3, 32). More important, however, is the possibility that varying aspects of technique are relevant in explaining the divergent data. The present study directs attention to the following points:

1. Averaging of values for respiratory control or selection of values for $Q_{O_2}$ after repetitive additions of ADP to mitochondria that are isolated in certain media (13) may lead to erroneous conclusions.

2. In terms of isolation, the proteinase procedure for small laboratory animals often yields mitochondria with significant permeability changes which might make comparisons difficult. Thus Nagarse-prepared mitochondria (both normal and experimental) rapidly utilized NADH, which implies "leaky membranes," i.e., an increase in permeability of the external mitochondrial membrane (33). Differences between control and experimental mitochondria in the present study were much less apparent when the Nagarse procedure was used than when other isolation methods were used.

3. Properly prepared cardiac mitochondria function adequately at 25°, 30°, and 37°C with minimal or no deterioration of activity or structure for at least 20 to 30 minutes. No information on possible NADH oxidation in previous studies was reported (9-11, 13). If a significant loss of respiratory control does occur when mitochondria are incubated at 37°C in the presence or absence of fortified media, the isolation technique should be questioned (13).

Sobel et al. (13) have presented three suggestions to explain how differences in oxidative phosphorylation between control and experimental animals might be artifacts. (a) The release of free fatty acids (FFA) during prolonged incubation at 37°C may have deleterious effects on mitochondrial function. This is improbable since the present data clearly show that inclusion of albumin and EDTA, both inhibitors of FFA-induced uncoupling of mitochondria (31, 34), resulted in maximal differences in respiratory control between mitochondria from normal and failing hearts. (b) The use of Warburg manometry, which requires relatively long incubation periods, may result in deterioration of mitochondria. There is no evidence to support this contention. Magnesium does not uncouple or damage oxidative phosphorylation but, rather, maintains a high rate of ADP production. Manometry, as an adequate and...
important technique, has recently been dis-
cussed by Slater (14). Furthermore, since
control and experimental mitochondria were
studied concomitantly (2, 3, 5), it is doubtful
that prolonged incubation alone would se-
lectively damage only the mitochondria from
failing hearts. If this were in fact true, it
would reflect a significant difference (perhaps
fragility) between the control and experimen-
tal preparations. In any case the present
report vitiates the incubation argument by
employing an oxygen electrode system and
a relatively simple assay medium, and the
results obtained were similar to those pre-
viously reported (1, 3, 5). The results of
prolonged storage at 0°C of control and
experimental mitochondria, presented in this
communication, suggest that the experimental
mitochondria are perhaps more fragile than
normal. (c) The reported changes might be
due to acute hypoxia resulting from pro-
longed time between death of the animal
and removal of the heart. The present data
make this possibility remote, since acute in
vivo hypoxia does not effect significant
changes in respiratory control of the mito-
chondria. These data are consistent with
previous studies showing recovery of the con-
tractility of the acutely anoxic heart concomi-
tant with a partial restoration of energy-rich
phosphate compounds and oxidative phos-
phorylation (35). Currently, this information
is of primary importance in evaluation of car-
diac transplantation procedures. However, these
data do not preclude the interesting possibility
that the observed mitochondrial differences
in failure may be secondary to a chronic in
vivo hypoxia.

Summarizing our findings, the isolated car-
diac mitochondria used in the present study,
to our knowledge, are in the best currently
known in vitro biochemical state. This was
determined by several established criteria:
very high respiratory control, high oxygen
consumption, stability at various temperatures
and after repeated additions of ADP, limited
NADH utilization indicating intact outer
membranes, and maintenance of activity af-
ter prolonged storage. These observations are
further substantiated by electronmicroscopic
studies showing no significant ultrastructural
changes of isolated normal mitochondria even
after 1 hour of incubation (state 4) at 30°C
or 20 minutes of incubation at 37°C (data
not presented). The biochemical integrity of
cardiac mitochondria has not been so docu-
mented in all the previously reported studies
(1-11, 13). Considering this information on
normal mitochondria, the changes reported
in this study have added meaning. It is highly
doubtful that the observations are artifacts
of either isolation or assay (13). This study
was specifically undertaken to eliminate such
an argument by using as wide a range of
isolation and assay techniques as possible.

Chidsey et al. have presented an interest-
ing discussion on divergencies in data (11).
They found no mitochondrial changes in
human papillary muscle from failing hearts.
These authors suggest that the following
reasons could apply. No changes exist in
human heart failure; their patients were on
maintenance doses of digoxin and may not
have been in the same severe state of failure
as that in which changes have been reported.
Humans typically develop hypertrophy and
failure much more slowly than most experi-
mental models. Papillary muscle might not
be representative of the ventricular wall. We
would like to add that data obtained from
isolated ventricular muscle obtained by biop-
sy may not represent the average state of
energy-liberating mechanisms either in the
total ventricle or in the whole heart. The
heterogeneity of heart muscle may preclude
the validity of extrapolating data obtained
from small segments to total tissue. For this
reason we studied energy liberation in the
entire heart. The possibility that defective
mitochondria were diluted with normal mito-
chondria only serves to make the differences
between the average values obtained from
normal and diseased hearts that much more
meaningful. Obviously, an examination of
this type yields only minimal information
concerning the development of a pathological
state in individual areas of the heart.
OXIDATIVE PHOSPHORYLATION IN HEART FAILURE

We wish to stress the possible importance of the stage of heart failure. Meerson (36) has defined three significant developmental stages in the failing heart: (1) transient breakdown stage, characterized by contractile insufficiency with a deficiency of certain enzymes, (2) protracted stage of relatively stable hyperfunction, characterized by the absence of cardiac insufficiency, by hypertrophy, and by adequate oxidative phosphorylation; and (3) protracted stage of progressing cardiocclerosis and gradual exhaustion of the hypertrophied heart, characterized by disturbances in protein synthesis and sustained hypoxia. We suggest that stage 3 might be characterized by or caused by an aberration of mitochondrial function(s). It is of interest that Syrian hamsters in severe congestive heart failure, secondary to a genetically linked cardiomyopathic process, were not in a preterminal condition but did show significant differences between control and experimental mitochondria (37, 38). Hamsters that were in the hypertrophic phase (2, above) but did not exhibit congestive heart failure, however, showed normal mitochondrial activity (39). Mitochondria isolated from hearts of animals or humans with hypertrophy in the absence of severe failure might be expected, therefore, to show little or no abnormality.

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Reevaluation of Oxidative Phosphorylation in Cardiac Mitochondria from Normal Animals and Animals in Heart Failure
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