There have been relatively few biochemical studies on experimentally induced congestive heart failure of left ventricular origin. Most of the conclusions concerning metabolism in heart failure have been based on studies on human beings, using techniques of coronary sinus catheterization on experimentally induced chronic right heart failure in the dog or on isolated failing preparations from animals. Thus, Benson et al. and Olson studied experimental heart failure in the dog, employing the technique of Barger et al. In this procedure, the failure is induced by a double surgical intervention wherein tricuspid insufficiency and pulmonary stenosis are produced in two stages. Measurements of energy-rich compounds, as well as arteriovenous (A-V) difference of oxygen and substrate in these dogs with cardiac failure, revealed no significant alteration of these parameters as compared with control animals. In acute failing preparations, Wollenberger, Furchgott and de Gubareff, and Lee et al. studying, respectively, a dog heart-lung preparation, isolated failing atria from guinea pigs, and isolated failing cat papillary muscles, found no depression of either adenosine triphosphate (ATP) or of phosphocreatine (PC) content as compared with control tissues.

Since studies by Benson et al. and by Ellenbogen et al. have revealed abnormalities both in glycerol extracted actomyosin and in myosin of the failed heart, the suggestion was made that there existed a deficiency of energy utilization rather than energy liberation or storage mechanisms in heart failure. Bing arrived at similar conclusions from his clinical studies which involved measurements of oxygen and substrate uptake. On the other hand, Yankapoulos et al. and Davis et al. found no evidence of changes in either actomyosin or myosin of hearts failed by the Barger procedure. In addition, Szekeres and Schein found a diminution in levels of ATP, PC, oxygen consumption, and glycolysis in hearts obtained from rats in experimentally induced acute cardiac failure. A similar depression of ATP and PC content in failing cat papillary muscles was reported by Greiner.

Since the concentration of energy-rich compounds represents a balance between energy-producing and energy-utilizing reactions, the steady state levels of these substances may not reflect the potentials or capabilities of energy-liberating or storage mechanisms within the tissue. For example, a normal level of high energy phosphate may exist in the face of reduced rate of energy liberation and/or storage if the rate of energy utilization is also decreased. Such a situation may exist in hearts of reserpinized cats and guinea pigs which contain normal concentrations of high energy phosphates, yet show a definite impairment of mitochondrial oxidative phosphorylation and contractile force. Moreover, because of individual variation, the reliability of measurements of either oxygen or substrate uptake by A-V difference has recently been...
criticized by Katz. Consequence, the use either of levels of energy-rich compounds or of oxygen and substrate uptake as indices of the rate of energy liberation may be only approximate at best.

These considerations, together with the apparent variation in results of the aforementioned investigators, suggested the need for a more direct approach to the problem of heart failure and metabolism. Since, in heart, mitochondria are known to play a major role in the liberation and storage of energy, possible alterations in mitochondrial function during chronic and acute heart failure were studied in the present work. In addition, other minor energy-liberating reactions in heart, associated with glycolysis, were examined in both the normal and failing preparations.

Methods

INDUCTION OF "CHRONIC" CONGESTIVE HEART FAILURE (CHF)

Guinea pigs of either sex weighing between 700 and 900 Gm. were used. After anesthesia was induced with sodium pentobarbital (intraperitoneally, 30 mg./Kg.), a tracheotomy was performed and a polyethylene tube (PE 50) inserted into the trachea, via a small incision. A gas mixture (95 per cent O$_2$ + 5 per cent CO$_2$) was administered through this tube. During the surgical procedure, the rate of gas inflow was increased and the lungs inflated every 30 seconds by pressing down on the tracheal opening. The chest was opened in the region of the left third intercostal space. After the pericardium in the area of the aorta was separated, a ligature of 2-0 black silk (about 20 per cent of normal size, in order to induce heart failure in 1.5 to 2 hours. The criterion of failure was a diminution of both ventricular pressure pulses and heart rate to approximately 50 per cent of control values as indicated in the text.

MEASUREMENT OF CARDIAC CONTRACTILITY AND VENTRICULAR PRESSURES

The animals were anesthetized, the chests opened under artificial respiration and the hearts exposed. Ventricular pressure pulses were recorded continuously on a Sanborn Twin Viso through a Statham P23Da pressure transducer, which was connected to the ventricular chamber through a number 18-gauge needle. When a steady state level of left ventricular pressure was reached, a hemostat was placed on a proximal portion of the ascending aorta and the vessel completely occluded for about 5 to 15 seconds. The peak systolic pressure recorded during occlusion was taken as a measure of "peak isometric pressure" (P.I.P.) of the heart. The validity of this measurement as an index of cardiac contractility has been discussed by Siebens et al. and by Woske et al. 22

MEASUREMENT OF P:O RATIOS IN MITOCHONDRIA

Heart mitochondria were prepared and P:O ratios (µatoms of phosphate esterified per µatom of O$_2$ used) were measured as previously described by Lee et al. Protein was determined by the biuret procedure. Inorganic phosphate (IP) was measured by the method of Fiske and Subbarow.

HISTOLOGICAL STUDY

At the time of sacrifice, portions of the liver, spleen, heart, lungs, and kidney were removed, washed in normal saline, and immersed in neutral 10 per cent formalin solution. The tissues were fixed, stained, and mounted for histological examination. In some of the animals, the organs were weighed before sampling and fixing.

MEASUREMENT OF DEGREE OF COUPLING OF OXIDATIVE PHOSPHORYLATION BY THE RESPONSE TO ACCEPTOR SYSTEM

Briefly, this involves comparing the rate of respiration of mitochondria before and after addition of a phosphate acceptor system (glucose and hexokinase). If oxidation is tightly coupled to phosphorylation, oxygen consumption cannot proceed without phosphorylation which requires a phosphate acceptor. Thus, the oxygen uptake of tightly coupled mitochondria is very low in

"The animals in congestive failure received 15 mg./Kg. of pentobarbital which was sufficient to maintain light anesthesia."
GLYCOLYTIC METABOLISM IN HEART FAILURE

Table 1

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of experiments</th>
<th>Heart rate (beats/min.)</th>
<th>Mean left ventricular pressure (systolic/diastolic) (mm./Hg)</th>
<th>Mean right ventricular pressure (systolic/diastolic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>246 ± 12.5</td>
<td>75 ± 2.8</td>
<td>18 ± 3.8</td>
</tr>
<tr>
<td>CHF</td>
<td>17</td>
<td>317 ± 21.3</td>
<td>60 ± 3.6</td>
<td>32 ± 4.1</td>
</tr>
</tbody>
</table>

*In this and subsequent tables, the values are accompanied, after the ± sign, by the standard error of the mean (S.E.).

the absence of the phosphate acceptor. After addition of a phosphate “trapping” system such as glucose and hexokinase, however, the oxygen consumption increases from two- to threefold. On the other hand, if a degree of uncoupling of oxidative-phosphorylation exists, the rate of oxygen uptake without the phosphate acceptor would be higher than that of the tightly coupled mitochondria under the same conditions, and the rate of increase of oxygen uptake of uncoupled mitochondria after the addition of the acceptor system would be less than that observed in tightly coupled mitochondria. Thus, any coupling which may not be great enough to be manifested in a lowered P:O ratio may be reflected in the acceptor response. In experiments for determining acceptor response, hexokinase and glucose (in 0.2 ml.) were placed in the side arm, and oxygen uptake before and after the addition of the acceptor system in the reaction mixture was measured.

EXPERIMENTS ON THE ELECTRON TRANSPORT CHAIN IN CARDIAC MITOCHONDRIA

The phosphorylation associated with the transfer of electrons in discreet portions of the electron transport chain in mitochondria were studied using an artificial electron acceptor and a donor. For the study of phosphorylation occurring between DPNH* and cytochrome C, glutamate and ferricyanide were used as an electron donor and acceptor, respectively, according to the method of Pressman.20 These experiments were carried out under anaerobic conditions using a Dubnoff metabolic shaker and 95 per cent N₂-5 per cent CO₂ as the gassing mixture and P:2e ratios were calculated by dividing the amount of IP esterified by one-half the amount of ferricyanide reduced.

To study the phosphorylation accompanying the electron transfer between cytochrome C and oxygen, potassium ascorbate and oxygen were used as electron donor and acceptor, respectively, according to the method of Lehninger et al.27 Ascorbate purportedly donate electrons nonenzymatically directly to cytochrome C. These experiments were carried out in the Warburg apparatus with air as the gas phase and with ascorbate as a substrate.

EXPERIMENTS ON MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE (ATPASE) ACTIVITY

The ATPase activity associated with cardiac mitochondria was determined by the procedure of Racker and Gatt.28 In this method, small concentrations of ADP and an ATP generating system (phosphatepyruvate + pyruvate kinase) were used to insure a steady state level of ATP without excess concentration of ADP.

EXPERIMENTS ON HOMOGENATES PREPARED FROM HEARTS OF GUINEA PIGS IN CHRONIC CONGESTIVE HEART FAILURE

Measurement of Oxygen Consumption

When the symptoms of chronic congestive heart failure (CHF) were manifest, hearts were removed and homogenized in cold 0.16M KCl. Warburg flasks were prepared as follows: phosphate buffer, pH 7.4, 100 mM; glucose, 10 mM; 20 per cent homogenate, 2.0 ml; 0.16M KCl, to make 3.0 ml. The homogenates were prepared from normal animals in the same manner. After the measurement of oxygen uptake of these homogenates, the dry weight of the homogenates was obtained and the oxygen consumption expressed in microliters of oxygen consumed per milligram dry weight per hour (QO₂).

Measurement of Glycolysis

After homogenization of the heart muscle, the suspension was centrifuged at 600 × g for 10 minutes at 0° C., and glycolysis of the cell-free homogenate was measured, utilizing Ehrlemeyer flasks (25 ml.) containing the following additions: glucose, 7.0 mM; ATP (Sigma), 0.7 mM; DPN (Sigma), 0.1 mM; nicotinamide, 40.0 mM; KHCO₃, 25.0 mM; MgCl₂, 4.0 mM; cell-free homogenate, 1.0 ml; 0.16 M KCl, pH 7.4 to make 3.0 ml. The flasks were incubated in a Dubnoff metabolic shaker at 38° C. for 30 minutes with a gas phase...
of 95 per cent N₂-5 per cent CO₂. At the end of the incubation period, the reaction was terminated by the addition of 1 ml. of cold 50 per cent trichloroacetic acid (TCA). A protein content of each flask was determined according to the method of Jacobs et al. Glucose uptake and lactic acid formation were determined by the glucose oxidase method and the Barker-Summerson procedure, respectively, on an aliquot of the TCA supernatant. The results are expressed in micromoles of glucose consumed or lactate formed per milligram of protein per hour.

Results

Experimental Induced "Chronic" Congestive Heart Failure

Pathophysiological Changes

The animals surviving surgery (about 70 per cent) generally began to exhibit external signs of failure in from 2 to 10 days. These included: cyanosis around lips and mouth, hypothermia, transudation from nose and eyes, loss of weight, distinct dyspnea, bilateral rales, and hyperpnea. The appearance of the organs was as follows: heart, dilated and cyanotic; liver, spleen, and kidneys, congested and enlarged; lungs, generally markedly edematous and congested. Marked hydrothorax was almost always evident upon opening of the chest. Those guinea pigs not exhibiting most of the above signs almost invariably revealed an improperly tied aortic ligature.

Histological examination* revealed changes similar to those observed in human congestive heart failure. Some of the findings are as follows:

The myocardium showed a loss of striation, as well as disassociation and desegmentation.

Ventricular pressure pulses and heart rates of these animals are shown in table 1. It can be seen that the characteristic findings in the

*Kindly performed by Dr. S. Y. Paik, Meadowbrook Hospital, Long Island, New York, and by Dr. G. Kaufman, Veterans Administration Hospital, Brooklyn, New York.
GLYCOLYTIC METABOLISM IN HEART FAILURE

TABLE 3

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of animals</th>
<th>Body weight (Gm.)</th>
<th>Heart weight (Gm.)</th>
<th>HW/BW × 10^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>738 ± 12.6</td>
<td>2.06 ± 0.017</td>
<td>2.73</td>
</tr>
<tr>
<td>CHF</td>
<td>12</td>
<td>708 ± 39.9</td>
<td>3.10 ± 0.030</td>
<td>4.38</td>
</tr>
</tbody>
</table>

TABLE 4

P:O Ratios and Oxygen Consumption of Mitochondria from Control and CHF Guinea Pig Hearts

<table>
<thead>
<tr>
<th>Type</th>
<th>Substrate</th>
<th>No. of experiments</th>
<th>P:O</th>
<th>Q_{O_2} (μatoms O_2/mg. protein/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Glutamate</td>
<td>20</td>
<td>3.13 ± 0.05</td>
<td>9.53 ± 0.05</td>
</tr>
<tr>
<td>CHF</td>
<td>Glutamate</td>
<td>19</td>
<td>1.78 ± 0.05</td>
<td>6.86 ± 0.40</td>
</tr>
<tr>
<td>Control</td>
<td>Succinate</td>
<td>10</td>
<td>1.27 ± 0.06</td>
<td>8.60 ± 2.7</td>
</tr>
<tr>
<td>CHF</td>
<td>Succinate</td>
<td>11</td>
<td>1.12 ± 0.23</td>
<td>4.52 ± 0.70</td>
</tr>
</tbody>
</table>

*The main compartment of each flask contained the following: MgCl₂, 8mM; KCl, 50 mM; ATP (disodium salt, Sigma), 2.5 mM; cytochrome C (from beef heart, Sigma), 0.017 mM; DPN (Sigma), 0.5 mM; nicotinic acid, 0.52 mM; NaF, 12 mM; substrate, 13 mM; glucose, 56 mM, and hexokinase (Sigma, type III), 20 units; K-phosphate buffer, pH 7.4, 20 mM. 0.5M sucrose containing 0.001 M EDTA, at pH 7.4 to make a total flask volume of 3.0 ml. The temperature was 37.5°C; reaction time was 20 minutes; each determination was run in triplicate. Each flask received approximately the same amount of mitochondrial protein (an average of 5 mg. per flask) in each experiment. The gas phase was air.

CHF animal were a marked elevation of right ventricular systolic and diastolic pressures and tachycardia.

The results in table 2 indicate that the failing heart in this preparation definitely exhibited a significant loss of contractility as manifested by a decrease in "peak isometric pressure" attained during temporary complete occlusion of the aorta. Figure 1 depicts a typical record. It may be seen that in the case of the control, after occlusion (at the first arrow) the left ventricular systolic pressure rose to a high level and maintained this level until the clamp was released (at the second arrow). On the other hand, the ventricular pressure in the case of the CHF animal increased only slightly after aortic occlusion and began to drop immediately. The venous pressure of the cardiac failure animals as determined in the left internal jugular vein was significantly higher than the control values.

The heart to body weight ratios of the control and CHF guinea pigs, as shown in table 3, indicate that marked cardiac hypertrophy occurred in the case of the CHF animal. Histological examination of the heart tissue revealed little edema, so that the increase in weight was probably due mostly to increased tissue mass.

Mitochondrial Changes

P:O Ratio and Acceptor Response. Generally, the hearts were removed for biochemical studies after the contractility and other physiological parameters were obtained. Several groups of guinea pigs in failure, however, were sacrificed without the use of anesthesia and without having received any surgical manipulation. The biochemical results were identical with those obtained from hearts which had been subjected to anesthesia and surgery. The results shown in table 4 indicate clearly that mitochondria isolated from the "chronic" failing animals were uncoupled with respect to oxidative phosphorylation. Thus, with glutamate as substrate, the P:O ratio of the failed mitochondria was 43 per cent of that of the controls. The per cent depression of the P:O ratio with succinate as substrate was of the same order. In a number of experiments utilizing α-ketoglutarate as substrate with or without malonate, the P:O ratio was lowered to the same extent as with the other substrates. It is of significance...
TABLE 5

Acceptor Response of Control and CHF Guinea Pig Heart Mitochondria

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of experiments</th>
<th>Q10 (μatoms O2/mg/hr.)</th>
<th>% Increase with acceptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>4.20 ± 0.63</td>
<td>195</td>
</tr>
<tr>
<td>CHF</td>
<td>9</td>
<td>6.56 ± 0.33</td>
<td>8.5</td>
</tr>
</tbody>
</table>

The acceptor system (glucose and hexokinase) in the side arm was tipped into the main compartment after the first 10-minute reading. The flask contents and incubation conditions were the same as indicated in table 4. The substrate used was glutamate.

that the mitochondria isolated from the failing animal exhibited diminished oxygen consumption in the presence of glutamate, succinate, or a-ketoglutarate. This indicates a defect in the electron transport system of the mitochondria. Uncoupling of the oxidative phosphorylation mechanisms was further substantiated by the results obtained in the study of the acceptor response as shown in table 5. While the control mitochondria exhibited almost a threefold increase in oxygen consumption after addition of the glucose and hexokinase, the mitochondria obtained from the failing animal responded only insignificantly to a similar addition of the phosphate acceptor system.

ATPase Activity. In view of the uncoupling of oxidative phosphorylation in mitochondria isolated from the failing heart, the ATPase activity of these mitochondria was studied, and the results are shown in table 6. It is of significance that even the severely uncoupled mitochondria isolated from failed hearts, exhibited the same ATPase activity as the control mitochondria under the conditions studied.

Studies on Sites of Uncoupling of Oxidative Phosphorylation. The phosphorylation associated with the electron transfer between DPNH and cytochrome C was studied, employing glutamate and ferricyanide as electron donor and acceptor, respectively. The ratio, esterified IP:2 electron transferred (P:2e), measured the efficiency of phosphorylation. The ratios of the “failed” mitochondria were not significantly different from those of the control mitochondria as shown in table 7. This indicates that the electron transfer and phosphorylation occurring between DPNH and cytochrome C were not significantly impaired in the “failed” mitochondria.

The phosphorylation associated with the electron transfer between cytochrome C and O2 was studied, using the system in which ascorbate and oxygen served as electron donor and acceptor, respectively. The P:O ratio of the “failed” mitochondria was markedly depressed compared with that of the control preparations, as shown in table 8. The above data suggest that the uncoupling of oxidative phosphorylation in the “failed” mitochondria is mainly localized at the site of electron transfer between cytochrome C and O2.

Effect of Ouabain on the CHF Guinea Pig

Ouabain was administered to the failing animal, to determine if a characteristic positive inotropic response could be elicited in spite of the severe depression of mitochondrial metabolism. Figure 2 represents a typical response and indicates a very rapid and significant improvement in cardiac contractility. In addition, many animals exhibited a diminution of the elevated right ventricular pressures and heart rate following ouabain treatment. Examination of the mitochondria isolated from the “ouabainized” failed hearts at the peak of inotropic response showed no alteration of the P:O ratios, oxygen consumption or acceptor response effect, as compared with the mitochondria from untreated failed hearts. Digitoxin or ouabain administered either directly in vitro to the mitochondria isolated from CHF animals or preincubated with a mitochondrial suspension from the failing hearts (concentrations used were from 10−7 to 10−5M) was without effect either on the diminished phosphorylation or on the
GLYCOLYTIC METABOLISM IN HEART FAILURE

**FIGURE 2**

Effect of ouabain on a CHF guinea pig. Ouabain was injected as indicated by the arrow, in a dose of 15 µg/Kg, into the right ventricle. The tracing depicts left ventricle pressure pulses recorded as described in the text. The paper speed was changed from a fast (50 mm/sec.) to a slow speed (5 mm/sec.), as indicated. On vertical scale, distance between heavy line represents 25 mm Hg pressure. The zero pressure line is the first heavy line from the bottom of the tracing. The CHF animal was 10 days postoperative and weighed 800 Gm. Note that two minutes after the administration of the drug, the pressure has increased significantly.

Oxygen consumption of the "uncoupled" mitochondria.

**EXPERIMENTALLY INDUCED ACUTE HEART FAILURE IN THE GUINEA PIG**

Acute heart failure as induced by severe aortic constriction was studied to determine the effect of duration of aortic stenosis on mitochondrial alteration. For this purpose, the heart was removed and mitochondria isolated at various time intervals after constriction. The hearts were considered failed when the left ventricular pressure pulse and heart rate diminished to approximately 50 per cent of the control levels. The results in table 9 show the P:O ratios and oxygen consumption of mitochondria isolated from hearts failed after two hours of aortic constriction. It is significant that no alteration of either P:O ratio or oxygen consumption is apparent even after severe failure. However, utilizing the more sensitive acceptor response, these mitochondria show a very definite loss of respiratory control mechanisms. This alteration occurs as early as 15 minutes after aortic constriction, as shown in figure 3. The depression of acceptor response to mitochondria obtained from the acutely failed heart appears to be graded, increasing with increasing duration of constriction.

**TABLE 6**

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of experiments</th>
<th>Inorganic phosphate liberated (mM/mg. protein/5 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>1.70 ± 0.196</td>
</tr>
<tr>
<td>CHF</td>
<td>9</td>
<td>1.72 ± 0.240</td>
</tr>
</tbody>
</table>

*Each 2-ml. tube contained: D,L-histidine buffer, pH 7.4, 20 mM; MgCl₂, 2.5 mM; KCl, 25 mM; phosphoenolpyruvic acid, 3.0 mM; ATP, 1.5 mM; pyruvate kinase (Boeringer), 1.5 units; mitochondrial suspension (1 ml of 0.16 M KCl-tissue suspension equivalent to 1 Gm. of original heart), 0.1 ml; KCl, 0.16M, was added to make a total volume of 1.0 ml. The temperature was 37.5 C, incubation time, 5 minutes.*

**TABLE 7**

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of experiments</th>
<th>IP esterified (mM)</th>
<th>Fe(CN)₆ reduced (mM)</th>
<th>P:O ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>16.8</td>
<td>24.3</td>
<td>1.55 ± 0.08</td>
</tr>
<tr>
<td>CHF</td>
<td>5</td>
<td>13.4</td>
<td>25.2</td>
<td>1.12 ± 0.08</td>
</tr>
</tbody>
</table>

*Each flask contained KHCO₃, 10 mM; and K-ferricyanide, 13 mM, in addition to the constituents indicated in table 4. The mitochondrial suspension (1 ml which was equivalent to 1 Gm. of original heart) was added to each vessel in a volume of 1.0 ml. Sucrose, 0.35 M, containing 0.001 M EDTA, pH 7.4, was added to make a total flask volume of 3.0 ml. The flasks were gassed with a mixture of 95 per cent nitrogen and 5 per cent CO₂ and incubation was carried out in a Dubnoff metabolic shaker for 40 minutes at a temperature of 37.5 C.*

**STUDIES OF HOMOGENATES PREPARED FROM CHF GUINEA PIG HEARTS**

Figure 4 follows the rate of respiration of both control and CHF homogenates with respect to time. It is evident that there is a fall off of oxygen consumption in both homogenates, but in the case of the homogenate prepared from failing animals, the oxygen uptake starts at a significantly lower level. On the basis of QO₂, i.e., µl. oxygen/mg. dry weight/hr., the control and experimental preparations were 6.22 and 2.87, respectively. In figure 5, it is apparent that both glucose consumption and lactic acid formation of the cell-free homogenate derived from the chron-
Effect of duration of severe aortic constriction on the P:O ratio and acceptor response. The dotted curve represents the P:O ratio in per cent of control values, while the solid curve represents the increase in oxygen consumption after addition of the phosphate acceptor system (glucose and hexokinase) in per cent of control values. Each point on the curves represents the average of three experiments. The control heart mitochondria were obtained from guinea pigs which were anesthetized, placed under artificial respiration with open chest, and kept in this condition for the same time intervals as were the animals with aortic constriction.

Discussion

The changes accompanying the "chronic" heart failure, as observed in the present study, are those which are frequently seen in clinical congestive heart failure of left ventricular origin. The marked increase in right ventricular systolic pressure, for example, is indicative of pulmonary hypertension probably resulting secondarily from the left ventricular failure. Histological study revealed severe pulmonary congestion with edema, as well as evidence of passive congestion of the liver and kidneys. The cardiac myofibrillar disorganization was also typical of passive congestion. The diminished ventricular contractility and the marked cardiac dilatation and hypertrophy, together with the aforementioned characteristics, appear to be suggestive of congestive heart failure probably approximating the acute type in man. Similar findings in experimentally induced congestive failure have been observed by Gertler.20

The results obtained indicate that mitochondria isolated from these failing hearts were clearly depressed with respect to oxidative phosphorylation. In the case of the chronic animal, the uncoupling of phosphorylation from oxidation was accompanied by

---

TABLE 8
Phosphorylation Associated with the Aerobic Oxidation of Ascorbate by Control and CHF Guinea Pig Heart Mitochondria

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of experiments</th>
<th>P:O ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>CHF</td>
<td>6</td>
<td>0.15 ± 0.01</td>
</tr>
</tbody>
</table>

Each flask contained the same constituents as in table 4. The substrate was K-ascorbate, at pH5.3, 13 mM. Reaction time was 40 minutes. Temperature was 38 C. A blank flask containing boiled mitochondria was run with each experiment to correct for auto-oxidation of ascorbate. The mitochondrial suspension was added to each flask as indicated in table 7. The gas phase was air.

TABLE 9
P:O Ratios and Oxygen Consumption of Mitochondria from Control and Acutely Failed Guinea Pig Hearts (AHF)

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of experiments</th>
<th>P:O ratio</th>
<th>QO2 (μatoms O2/mg./hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>2.94 ± 0.03</td>
<td>9.96 ± 0.80</td>
</tr>
<tr>
<td>AHF</td>
<td>15</td>
<td>3.04 ± 0.03</td>
<td>9.37 ± 0.57</td>
</tr>
</tbody>
</table>

Reactants and incubation conditions were the same as in table 4.

The aortic ligation was maintained in place for two hours.
a depressed mitochondrial oxygen consumption.

The results and conclusions presented here are contrary to those of Plaut and Gertler.\textsuperscript{31} These authors found no uncoupling of oxidative phosphorylation in mitochondria of failing guinea pig hearts except when α-ketoglutarate was used as a substrate in the presence of malonate. The present authors, on the other hand, observed mitochondrial alteration with all the substrates studied. The differences between the results of the two studies are not immediately explicable. Plaut and Gertler pooled the hearts of four to six failing guinea pigs in order to isolate enough sarcosomes for their experiments. In the present study, however, one animal was used for each experiment. The acceptor response, moreover, is considered to be a more sensitive index of oxidative phosphorylation.\textsuperscript{17} In the present study, results obtained in the measurement of both P:O ratios and the acceptor response indicated that the mitochondria obtained from the failing hearts were uncoupled with respect to oxidative phosphorylation.

Energy-rich phosphate compounds and IP were measured by M. Feinstein\textsuperscript{33} on many of the same "chronic" animals used in the present study. Feinstein found a significant depression of the ATP, PC, and free creatine content as well as elevated IP when the mitochondrial function was impaired. These results, together with the mitochondrial changes, strongly suggest a deficiency of major-energy-liberating or storage reactions in the chronically failing guinea pig heart. The impairment of energy-liberating mechanisms found in the present investigation is not in accord with the results obtained in several other types of studies, principally of the type involving isolated cardiac failure preparations.\textsuperscript{3,4,5,9} As we have suggested previously,\textsuperscript{17} however, steady state levels of the high energy phosphate compounds do not necessarily reflect energy generative or storage efficiency.

Another factor which may be of importance in considering the difference in results of these and other studies is the type of cardiac failure preparation employed. Thus the iso-
lated failing heart may not represent biochemically the same situation found in situ cardiac failure. In connection with this, it is of interest to note that Wollenberger (personal communication) has recently observed extensive in situ morphological changes in heart mitochondria from dogs in chronic congestive left ventricular failure, yet the same author, as well as Brody et al., reported no evidence of a deficiency of energy liberating reactions in isolated failing dog heart-lung preparations. Herrmann and Dechard, and Myers and Mangun some time ago reported evidence of depressed energy liberating mechanisms in rabbit hearts following the induction of "chronic" heart failure by aortic regurgitation.

On the other hand, the "chronic" preparation, as used by Olson, in which no depression of energy liberation has been found, may, as has been pointed out, represent primarily right ventricular involvement and may, therefore, present a different biochemical as well as physiological situation than that found in cardiac failure chiefly of left ventricular origin.

With regard to the site of uncoupling of oxidative phosphorylation in mitochondria from failed hearts, the phosphorylation accompanying the transfer of electrons from glutamate to ferricyanide was not affected. On the other hand, the phosphorylation measured in the system using ascorbate and O2 as an electron donor and acceptor, respectively, was considered depressed in mitochondria obtained from failed heart, as compared with those from the control heart. Since this latter phosphorylation presumably represents that accompanying the electron transfer from cytochrome C to oxygen, it appears that the site of uncoupling of oxidative phosphorylation in failed heart mitochondria resides mainly in this terminal chain of electron transfer, namely, between cytochrome C and oxygen.

Mitochondria isolated from the acutely failed hearts showed normal P:O ratios and QO2. However, use of the acceptor system response provided evidence of a mild degree of uncoupling of oxidative phosphorylation. Furthermore, the uncoupling in mitochondria from the acutely failed heart appeared to be graded, i.e., the degree of uncoupling increased with increasing duration of aortic stenosis. It is possible that even more prolonged constriction would finally be reflected in a lowered P:O ratio.

It appears from these results that the mitochondrial alteration in failure produced by left ventricular loading may occur in an early stage of failure, and with increasing severity of failure probably the alteration becomes manifested as a significant depression of P:O ratios and oxygen consumption of the mitochondria. These data are in accord with the observations and conclusions of Szekeres and Schein who studied acute heart failure in the rat. They found depressed P:O ratios and oxygen consumption of homogenates prepared from failing hearts as well as significantly low levels of ATP and PC as compared with control values.

In the present study, the oxygen uptake of homogenates obtained from the "chronic" failing heart was also markedly lower than control values. This may be explained on the basis of the depressed mitochondrial respiratory activity.

In addition to the altered mitochondrial activity, other energy yielding mechanisms appear to be affected during the "chronic" heart failure. Utilizing glucose uptake and lactic acid formation as measurements of anaerobic glycolytic activity, the present authors found a significant depression of these parameters in cell-free homogenates obtained from the "chronically" failing hearts, as compared with control tissue preparations. Apparently then, a depression of all known energy-liberating reactions in the heart occurs in the experimental preparation employed in the present study.

No evidence is available concerning the causal relationship in heart failure between the impairment of mitochondrial and glycolytic function and the development of heart failure. This impairment of energy-liberating mechanisms may not be the primary cause of heart failure. However, it may play an important role in the further development
of the heart failure in a certain stage of the disease.

The results of the experiments concerning the effect of ouabain on the failing preparations, as described in this paper and in the paper by Feinstein, lend support to the conclusions of the present authors and of others that the cardiac glycosides do not exert their inotropic effect through the improvement of energy supply or through a direct action on mitochondrial systems. Ouabain can effect an improvement in the function of a failing heart, apparently in the presence of markedly depressed levels of energy rich compounds and severely impaired energy-liberating mechanisms.

Summary

Studies were made on the biochemical activity of mitochondria and of homogenates obtained from normal hearts and from hearts after experimentally induced failure. The principal types of failure investigated were: "chronic" congestive failure and acute failure in the guinea pig. The "chronic" failure was induced in from 2 to 10 days by a partial constriction of the ascending aorta, while the acute type of failure was induced by a more severe aortic constriction of a similar type. Various physiological parameters were studied. The "chronic" animals exhibited tachycardia, elevated right ventricular systolic pressure and a significant depression of myocardial contractility. In addition, a highly significant increase in cardiac tissue mass was observed. Passive congestion of the liver, spleen, kidneys, and lungs was evident.

Mitochondria isolated from the hearts of guinea pigs in "chronic" failure exhibited a significant depression of metabolic activity. Thus phosphorylation associated with the oxidation of glutamate, succinate, or α-ketoglutarate was uncoupled in mitochondria isolated from the "failed" heart. Cardiac glycosides administered to animals with experimental cardiac failure did not alter the uncoupled state of the mitochondria, although these agents effected a significant improvement in cardiac contractility. The ATPase activity of the "failed" mitochondria was normal. Experiments concerning the localization of the site or sites of uncoupling of oxidative phosphorylation in the "failed" mitochondria revealed that the defect probably resided in the phosphorylation step associated with the electron transfer between cytochrome C to oxygen.

Homogenates prepared from hearts of chronically failing animals were markedly depressed with respect to oxygen and glucose consumption and lactic acid formation.

Mitochondria isolated from acutely failed guinea pig hearts exhibited a "mild" uncoupling of oxidative phosphorylation manifested as a decrease in responsiveness to a phosphate acceptor system. This was a graded effect, increasing in severity with increasing duration of aortic constriction.

On the basis of the present study, it is possible that uncoupling of oxidative phosphorylation in heart mitochondria may play a role in the development of congestive cardiac failure.

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References


Appendix: Abbreviations Used

ATP = Adenosine triphosphate.
ADP = Adenosine diphosphate.
AMP = Adenosine monophosphate.
IP = Inorganic phosphate.
DPNH = Diphosphopyridine nucleotide, reduced.
DPN = Diphosphopyridine nucleotide.
CHF = "Chronic" congestive heart failure.
AHF = Acute heart failure.
PC = Phosphocreatine.

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