Effects of Experimental Congestive Heart Failure, Ouabain, and Asphyxia on the High-Energy Phosphate and Creatine Content of the Guinea Pig Heart

By Maurice B. Feinstein, Ph.D.

Investigations concerning possible biochemical abnormalities in relation to mechanical failure of the heart have been carried out both in humans and experimental animals. Evidence indicating deficiencies in the creatine and adenine nucleotide stores of the failing human heart was presented by Cowan, Herrmann, Decherd and Oliver, and Mangun and Myers. Experiments on cardiac tissue obtained from experimental animals by Weicker, and Greiner indicated that high-energy phosphate stores were reduced after spontaneous failure. A detailed criticism of these observations was made by Wollenberger, and his own very careful experiments demonstrated that, in the dog heart-lung preparation, spontaneous failure was not associated with any loss of high-energy phosphate stores. The same result was obtained in experiments utilizing isolated guinea pig atria and cat papillary muscle. The only investigation of high-energy phosphate stores relating to cardiac failure in an intact chronic animal was that of Olson and Piatnek, who found no loss of adenosine triphosphate (ATP) or phosphorylcreatine (PC) in dogs with chronic congestive heart failure. However, in view of the recent work of Wollenberger, Krause, and Wahler on the content of high-energy phosphates in dog heart, it appears that the values reported by Olson and Piatnek for PC are only about 25 per cent of the true concentration of this compound.

Previous experimentation in this field has involved the study of failure in cardiac tissue isolated from the other organs of the body, as well as its normal innervation, or in intact animals by virtue of an increased mechanical load on the right ventricle. It, therefore, seemed advisable to reinvestigate this problem in an intact animal by inducing failure in a manner more closely related to human congestive failure, that is, by mechanical overloading of the left ventricle. This investigation is also concerned with the effects of the cardiac glycoside ouabain and acute periods of asphyxia on the mechanical function of the heart in relation to its high-energy phosphate stores.

Methods

PREPARATION OF TISSUE EXTRACTS

Guinea pigs were anesthetized with sodium pentobarbital (35 mg./Kg. intraperitoneally) and artificially respired with a respirator for 15 minutes. Their chests were opened by cutting through two or three ribs on both sides of the sternum and then cutting across the sternum just anterior to the diaphragm. The sternum was lifted upwards to reveal the heart below. Bleeding points were immediately clamped with hemostats. Right and left ventricular pressure pulses were recorded via an 18- or 20-gauge hypodermic needle placed into the ventricular cavity by direct puncture. The hypodermic needle was connected to a Statham P23Da transducer by about 12 inches of polyethylene tubing. After suitable intraventricular pressure pulses were recorded with a Sanborn Twin-Viso, the left ventricular apex was cut out and immediately plunged into liquid nitrogen. In a cold room, at —20 C, the frozen tissue was scraped.
with a knife to remove any adhering blood. Then utilizing a mortar and pestle, precooled with liquid nitrogen, the frozen portion of ventricle was pulverized to a fine powder, under a layer of liquid nitrogen. A portion of the finely ground material, the clear supernatant was treated as follows: (a) 1.0 ml. was added to 5.0 ml. of ice-cold 0.3N perchloric acid contained 2.0 ml. of ice-cold 0.3N perchloric acid. This solution was used for the enzymatic spectrophotometric method of Kalckar. The adenine nucleotides were determined by the enzymatic spectrophotometric method of Furchgott and deGubareff. The adenine nucleotides were determined by the enzymatic spectrophotometric method of Furchgott and deGubareff. The enzyme preparation utilized for assay purposes was the potassium phosphate eluate from the calcium phosphate gel (Fraction 7). This preparation had no detectable myokinase or ATPase activity. An aliquot of the stock enzyme solution was diluted 10 to 20 times with ice-cold water just prior to use. Myokinase was prepared from rabbit skeletal muscle by the method of Lee. The enzyme preparation utilized for assay purposes was the potassium phosphate eluate from the calcium phosphate gel (Fraction 7). This preparation had no detectable myokinase or ATPase activity. An aliquot of the stock enzyme solution was diluted 10 to 20 times with ice-cold water just prior to use. Myokinase was prepared from rabbit skeletal muscle by the method of Lee. The enzyme preparation utilized for assay purposes was the potassium phosphate eluate from the calcium phosphate gel (Fraction 7). This preparation had no detectable myokinase or ATPase activity. An aliquot of the stock enzyme solution was diluted 10 to 20 times with ice-cold water just prior to use. Myokinase was prepared from rabbit skeletal muscle by the method of Lee. The enzyme preparation utilized for assay purposes was the potassium phosphate eluate from the calcium phosphate gel (Fraction 7). This preparation had no detectable myokinase or ATPase activity. An aliquot of the stock enzyme solution was diluted 10 to 20 times with ice-cold water just prior to use. Myokinase was prepared from rabbit skeletal muscle by the method of Lee. The enzyme preparation utilized for assay purposes was the potassium phosphate eluate from the calcium phosphate gel (Fraction 7). This preparation had no detectable myokinase or ATPase activity. An aliquot of the stock enzyme solution was diluted 10 to 20 times with ice-cold water just prior to use. Myokinase was prepared from rabbit skeletal muscle by the method of Lee.
HEART FAILURE AND OUABAIN

adjusted to pH 6.5 to 7.0, was added to 0.4 ml.
of water, 0.1 ml. 1M sodium succinate buffer at
pH 6.4, and 0.01 ml. 1M magnesium chloride, in
a 1 ml. silica cuvette. The changes in O.D., at
 pH 6.4, and 0.01 ml. 1M magnesium chloride, in
a 1 ml. silica cuvette. The changes in O.D., at

Figure 2. The curves show the course of the reactions with
time. It can be seen that by far the major adenine
nucleotide present in fresh ventricle is ATP. Also
the reduced amount of ATP (and total adenine
nucleotide) in the heart of the animal with aortic
coarctation is evident.

The molecular extinction coefficient of ATP
(Pisut) solutions at 265 m\textmu and pH 6.4 was cal-

culated to be 13.04 \times 10^2. The average fall in
O.D. after adenylc deaminase and potato apyrase
action was 57 per cent. From these data the
factor of 7.34 was calculated, which represents
the fall in optical density per micromole of ade-
nine nucleotide deaminated per milliliter of solu-
tion in the cuvette.

ANALYSIS OF ADENOSINE TRIPHOSPHATE
AND PHOSPHORYLCREATINE BY
ENZYMATIC REDUCTION OF
TRIPHOSPHOPYRIDINE NUCLEOTIDE

Kornberg\textsuperscript{20} developed an assay procedure for
the determination of ATP based upon the enzy-

matic reduction of triphosphopyridine nucleotide
(TPN) associated with the oxidation of glucose-
6-phosphate (G-6-P) by the enzyme glucose-6-
phosphate dehydrogenase. G-6-P is generated by
the action of hexokinase in the presence of glucose
and ATP. The reaction sequence is illustrated
by reactions 4 and 5 in figure 1.

PC can be determined by the same system
through the action of the enzyme ATP-creatine
transphosphorylase which in the presence of ADP
will generate ATP (see reaction 6 of figure 1).

The ATP generated by reaction 6 can be deter-

mined by the amount of TPN reduction obtained
through reactions 4 and 5. Reduction of 1 \mu M/ml.
TPN causes an increase in O.D. of 6.22. Such a
system has been used to determine ATP-creatine
transphosphorylase activity in tissues\textsuperscript{23} and PC in
aqueous solutions\textsuperscript{24} but has not been previously
applied to the analysis of PC in tissues.

G-6-P dehydrogenase was prepared by the
method of Kornberg and hoecker\textsuperscript{23} from Brewer's
yeast, and ATP-creatine transphosphorylase by
method B of nod, kny, and lardy\textsuperscript{24} from
rabbit skeletal muscle. Crystalline hexokinase
(prepared by the unpublished method of Darrow
and Colowick) was a gift from Dr. W. D. Wosilait.
The reaction mixture consisted of 100 \muM glucose;
0.5 \muM TPN; 0.5 \muM MgCl\textsubscript{2}; 0.1 ml. K\textsubscript{2}CO\textsubscript{3}
neutralized perichloric acid tissue extract (see solu-
tion (d), under "Preparation of Tissue Extracts"),
and 0.5M glycylglycine buffer pH 8.0 in a total
volume of about 0.5 ml. ATP and PC were deter-

mined by the O.D. change at 340 m\textmu after the
addition of the appropriate enzymes. Analyses of
ATP and PC in tissue extracts by this method
gave values which averaged 80 per cent of those
determined by the Furchgott and deGubareff
method (for PC) and the Kalckar method (for
ATP). The discrepancy in these results was
found to result, primarily from TPNH oxidase
activity which was present in the G-6-P dehydro-
genase preparation. For this reason, the method
was not used very extensively in this investigation.
It was apparent, however, that when correc-
tion for the TPNH oxidase activity was made,
the values obtained for PC by the relatively less
specific chemical method were in good agreement
with the values obtained with the very specific
enzymatic method. This indicates that what is
measured as PC by the chemical method is actu-
ally PC and not other labile phosphate compounds.
Also, the decrease in ATP and PC levels in ani-
mals with aortic coarctation, compared to controls,
was evident with any of the methods used.

ANALYSIS OF CREATINE

Free creatine was determined in tissue extracts
by the method of eggleton, elsden, and goach.\textsuperscript{25}
Sulfhydryl group inhibition of the color reaction
was overcome with 10^{-5}M p-hydroxymercuribenzo-
eate (Sigma), as shown by enor and Rosenber.\textsuperscript{26}
Values about 5 per cent lower were found when
p-hydroxymercuribenzoate was omitted. The rate
of color development was followed in a Beckmann
spectrophotometer at 525 m\textmu. It was observed
that after reaching a peak value in about 20
minutes the optical density decreased with time.
For this reason, the reaction was followed continu-
ously with time and the peak readings taken for
calculation of the free creatine concentration.
The reaction could be carried out in microcuvettes
(Pyrocell) with 0.2 ml. K\textsubscript{2}CO\textsubscript{3}-neutralized extract.
0.05 ml. 1 per cent alpha-naphthol solution, and
0.025 ml. diacetyl (0.05 per cent). The extinction
coefficient under these conditions was 12.76 \times 10^0.

SURGICAL METHOD FOR THE PRODUCTION
OF AORTIC COARCTATION

The basic procedure is essentially the same as
that described by Gertler.\textsuperscript{27} Guinea pigs (usually
females) of 700 to 1,000 Gm. body weight were
anesthetized with 35 mg./Kg. sodium pentobarbi-
tal. After opening the chest through an inter-
costal incision, the ascending aorta was exposed
TABLE 1
Concentrations of Inorganic Phosphate, Phosphorylcreatine, and Adenine Nucleotides in Normal Guinea Pig Left Ventricle

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<th>Animal no.</th>
<th>IP (μM per Gm.)</th>
<th>PC (μM per Gm.)</th>
<th>Creatine (μM per Gm.)</th>
<th>Total creatine (μM per Gm.)</th>
<th>ATP (μM per Gm.)</th>
<th>APP (μM per Gm.)</th>
<th>ADP (μM per Gm.)</th>
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*Inorganic phosphate (IP); phosphorylcreatine (PC); adenosine triphosphate (ATP); adenosine diphosphate (ADP); and adenylic acid (AMP).

APP refers to the sum of ATP and ADP. It corresponds to that fraction of the total acid-soluble phosphate of the tissue, which is determined by the 7- to 10-minute acid-heat hydrolysis method for labile pyrophosphate.

Results

ANALYSES OF HIGH-ENERGY PHOSPHATE COMPOUNDS AND CREATINE IN NORMAL GUINEA PIG VENTRICLE

The analyses of normal guinea pig left ventricle revealed the great lability of the PC content. Care had to be exercised to maintain pulmonary ventilation until the sample of myocardium was removed. If the animal was stunned by a blow on the head and the heart then removed within one minute, or if the chest was opened under pentobarbital anesthesia but without artificial respiration, the values for PC were very low (1.5 to 2.8 μM/Gm.). In addition, the tissue had to be pulverized to a fine powder, while frozen, before extraction with ice-cold acid, so as to avoid possible thawing in large segments of tissue before the acid could penetrate sufficiently to destroy all enzymatic activity. When these precautions were taken, a mean value of 8.35 μM/Gm. wet weight was obtained for PC, as determined by the method of Furchgott and deGubareff (table 1). This is higher than any previous values reported for this substance in mammalian heart, except for those of Wollenberger, Krause, and...
HEART FAILURE AND OUABAIN

Concentrations of Inorganic Phosphate, Phosphorylcreatine and Adenine Nucleotides in Left Ventricles of Guinea Pigs with Experimental Congestive Heart Failure

TABLE 2

Concentrations in μM per Gm. wet weight

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<th>Animal no.</th>
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</tbody>
</table>

*For explanation of abbreviations, see footnote to table 1.
†ADP was not determined separately with myokinase in experiments 2 and 7. In these two experiments, APP was determined from the change in O.D. due to potato adenylpyrophosphatase after determination of AMP with adenylie acid deaminase. Potato adenylpyrophosphatase hydrolyzes both ATP and ADP to AMP and inorganic phosphate. In all other experiments, APP was determined by calculation of the sum of ATP and ADP.
‡The numbers in parentheses refer to the number of days after aortic constriction that the assays were performed.

**Utilizing** a new method for ultra-rapid freezing of tissue, and a new method for phosphate analysis, these workers have reported a value of 10 μM/Gm. in guinea pig ventricle. The amount of ATP found in guinea pig ventricle (5.59 μM/Gm., see table 1), by the Kalckar method, is somewhat higher than most previously reported results in the literature (see review by Furchgott and Lee) for mammalian heart. The differences can probably be ascribed to the different methods of fixation and extraction of the tissue before analysis.

By assaying the tissue for both PC and free creatine, it was determined that about 50 per cent of the total creatine content of the heart is in the phosphorylated form (table 1). Wollenberger, Krause, and Wahler have reported the same proportions for creatine and PC in guinea pig ventricle, using completely different methods for determining free creatine and PC.

**ANALYSES OF HIGH-ENERGY PHOSPHATE COMPOUNDS AND CREATINE IN GUINEA PIGS WITH EXPERIMENTAL CONGESTIVE HEART FAILURE**

Analyses of ventricular tissue obtained from guinea pigs at various periods of time after constriction of the ascending aorta revealed quite striking changes in the content of IP, PC, total creatine, ATP, and total adenine nucleotides (table 2). The greatest changes were in the IP, PC, and total creatine content of the heart. The mean PC...
Analysis of guinea pig left ventricle for adenine nucleotides by the method of Kalckar. Adenine nucleotides were determined in succinate buffered perchloric acid extracts of normal (a) guinea pig left ventricle and left ventricle from an animal (b) with experimental congestive heart failure. The amount of tissue extracted in each case was the same. Adenylic acid deaminase (AAD), myokinase (MYOK.) and potato adenylpyrophosphatase (APYRASE) were added at the times indicated by the arrows. The changes in optical density after the addition of each enzyme are noted on the curves. The increase in optical density immediately after addition of the enzyme solutions is determined from the absorption increase due to the addition of the enzyme to a succinate buffered perchloric acid blank. Note the lower initial optical density of solution b, as well as the lower ATP content. Concentrations in $\mu$M/ml. of solution are obtained as follows: $AMP = (\Delta\text{O.D. after } AAD)/7.34$; $ADP = (2 \times \Delta\text{O.D. after MYOK.})/7.34$; $ATP = (\Delta\text{O.D. after APYRASE} - \Delta\text{O.D. after MYOK.})/7.34$.

concentration was decreased 54 per cent to 3.86 $\mu$M/Gm. in 20 experimental animals. There was a corresponding increase in IP from 3.72 $\mu$M/Gm. to 8.02 $\mu$M/Gm. The mean concentration of ATP fell from 5.59 $\mu$M/Gm. to 4.27 $\mu$M/Gm., a decrease of 24 per cent. There was no corresponding increase in ADP or AMP levels, so that the total adenine nucleotide content was decreased to the same extent. Another significant finding was the mean loss of 34 per cent of the heart's total creatine stores. The loss in six individual cases was so severe that the total creatine content of these ventricles was equal to or less than the normal PC content, thereby imposing a significant limitation upon the ability of the tissue to synthesize PC. Three sham-operated animals were found to have normal levels of PC and ATP.

**CORRELATION OF CHANGES IN HIGH-ENERGY PHOSPHATE COMPOUNDS AND CREATINE WITH PHYSIOLOGICAL AND PATHOLOGICAL MANIFESTATIONS OF HEART FAILURE**

The results described above refer to the mean values for the entire group of 20 experimental animals, without regard to the severity of the signs of congestive heart failure. If the results are arbitrarily ranked according to whether the PC content of the heart was greater than 5 $\mu$M/Gm., between 4 and 5, or less than 4 $\mu$M/Gm., 11 animals fall into the last group. This group also exhibited the lowest mean concentrations of ATP and total creatine. The incidence of abnormal intraventricular pressures (i.e., high right ventricular systolic and high left ventricular diastolic pressures), tachycardia, hydrothorax, pulmonary edema, and "nut-meg" liver was greatest in this group (for further details of the pathological physiology of these animals, see Schwartz and Lee). Seven of these 11 animals had left ventricular diastolic pressures of from 7 to 35 mm. Hg (mean 13 mm. Hg). Normal left ventricular diastolic pressure ranged from 0 to 2 mm. Hg in controls. Right ventricular systolic pressure, in controls, was observed to range from 15 to 20 mm. Hg, whereas the range in the group of experimental guinea pigs, with PC concentrations less than 4 $\mu$M/Gm., was from 24 to 100 mm. Hg (mean 43 mm. Hg). Of the nine guinea pigs with PC concentrations greater than 4 $\mu$M/Gm., only two had elevated right ventricular systolic pressure, while one animal exhibited a higher left ventricular diastolic pressure.

Cardiac hypertrophy was evident in the
HEART FAILURE AND OUABAIN

group of 20 animals with aortic coarctation. The heart weight to body weight ratios are 26 per cent higher than in the controls: 0.0034 ± 0.0001 (standard error) versus 0.0027 ± 0.00007. The gross evidence for cardiac hypertrophy was confirmed by microscopic examination of hemotoxylin-eosin-stained sections of myocardium. Histological sections also reveal signs of passive venous congestion of the lungs and liver. Pulmonary congestion was observed in every one of 12 animals, the tissues of which were examined microscopically.

RESPONSE OF THE HEART TO OUABAIN

In view of the fact that cardiac failure induced by aortic constriction is associated with a loss of energy-rich compounds which are presumed to supply, either directly or indirectly, the energy for contraction, it was of considerable interest to investigate the response to a digitalis glycoside. Digitalis has been shown to produce a positive inotropic response in situations where spontaneous failure of the heart is not associated with a loss of energy-rich compounds, as well as in cases where a loss of ATP, or ATP and PC, has been reported to accompany failure.

The latter observations are most interesting because a positive inotropic response to digitalis glycoside was presumably associated with an increase in energy-rich phosphate stores. The therapeutic action of digitalis was, therefore, attributed to a restoration of energy-rich phosphate compounds. Table 3 shows the results obtained in five animals in which aortic coarctation had been experimentally produced. Each animal was anesthetized with 15 to 20 mg./Kg. of pentobarbital sodium (these animals were usually more sensitive than controls to pentobarbital, so that lower doses were used to produce anesthesia) and maintained under artificial respiration. After the chest was opened, left and right ventricular pressures were recorded, after which 15 μg./Kg. of ouabain was injected slowly into the right ventricular chamber. In every animal, a significant reduction of heart rate occurred. The mean decrease for the group was 16 per cent. Intravenous pressure measurements indicated that ouabain increased left ventricular systolic pressure (mean increase 75 per cent), usually lowered the diastolic pressure, when it had previously been elevated, and also decreased right ventricular systolic and diastolic pressures when they were initially high.

In other words, the characteristic hemodynamic responses to digitalis, which have been observed in congestive failure in humans are noted in experimental cardiac failure in the guinea pig. The raising of left ventricular pressure considerably above normal values, which occurred in some cases after ouabain, can probably be attributed to the continued high resistance to outflow from the ventricle due to the aortic constriction. In eight normal open-chest guinea pigs, ouabain produced a positive inotropic response (mean systolic pressure increase 49 per cent), as determined by left ventricular pressures. Variable changes in rate were produced. In five of the eight animals, the rate was increased by about 10 per cent. One animal developed ventricular tachycardia, with a rate increase of nearly 50 per cent. This positive chronotropic effect is in marked contrast to the consistent slowing of the heart in the cases of experimental congestive failure.

Samples of the left ventricular apex were frozen in liquid nitrogen at a point in time at which a peak in the response to ouabain appeared to have been reached. This was usually from 6 to 12 minutes after the drug had been injected. Analysis of the tissue samples (table 3) revealed that the mean ATP content of digitalized hearts from animals with congestive failure was 4.46 μM/Gm. This is almost identical to the ATP content of the 20 animals with congestive failure which did not receive ouabain. The PC concentration (2.17 μM/Gm.), on the other hand, was significantly lower. The total creatine content was within 10 per cent of the value obtained in the nondigitalized animals and is, therefore, not considered significantly different.

These results indicate that a positive inotropic response to ouabain can occur in
TABLE 3

**Effect of Acute Digitalization* upon the Concentrations of High-Energy Phosphates, Inorganic Phosphate, and Creatine in the Guinea Pig Left Ventricle.**

<table>
<thead>
<tr>
<th>Controls</th>
<th>IP</th>
<th>PC</th>
<th>Total creatine</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.82</td>
<td>7.09</td>
<td>17.54</td>
<td>5.07</td>
<td>0.55</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>4.39</td>
<td>6.37</td>
<td>15.71</td>
<td>5.57</td>
<td>0.56</td>
<td>0.17</td>
</tr>
<tr>
<td>3</td>
<td>5.42</td>
<td>5.82</td>
<td>15.40</td>
<td>4.82</td>
<td>0.76</td>
<td>0.46</td>
</tr>
<tr>
<td>4</td>
<td>5.87</td>
<td>6.45</td>
<td>16.65</td>
<td>5.34</td>
<td>0.84</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>5.72</td>
<td>5.70</td>
<td>16.30</td>
<td>5.47</td>
<td>0.38</td>
<td>0.32</td>
</tr>
<tr>
<td>6</td>
<td>7.76</td>
<td>5.40</td>
<td>18.66</td>
<td>5.62</td>
<td>0.77</td>
<td>0.65</td>
</tr>
<tr>
<td>7</td>
<td>8.04</td>
<td>5.37</td>
<td>19.02</td>
<td>5.04</td>
<td>0.05</td>
<td>0.38</td>
</tr>
<tr>
<td>Mean</td>
<td>6.14</td>
<td>6.03</td>
<td>17.05</td>
<td>5.25</td>
<td>0.56</td>
<td>0.50</td>
</tr>
<tr>
<td>S.E.</td>
<td>±0.48</td>
<td>±0.39</td>
<td>±0.44</td>
<td>±0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10.19</td>
<td>2.09</td>
<td>17.94</td>
<td>4.55</td>
<td>0.84</td>
<td>0.36</td>
</tr>
<tr>
<td>9</td>
<td>7.81</td>
<td>2.87</td>
<td>16.82</td>
<td>5.14</td>
<td>0.46</td>
<td>0.71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experimental congestive heart failure</th>
<th>IP</th>
<th>PC</th>
<th>Total creatine</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (5)</td>
<td>5.37</td>
<td>3.19</td>
<td>10.38</td>
<td>3.96</td>
<td>0.18</td>
<td>0.25</td>
</tr>
<tr>
<td>11 (7)</td>
<td>7.92</td>
<td>2.02</td>
<td>13.82</td>
<td>4.64</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>12 (4)</td>
<td>8.51</td>
<td>1.66</td>
<td>12.39</td>
<td>4.48</td>
<td>0.15</td>
<td>0.41</td>
</tr>
<tr>
<td>13 (3)</td>
<td>6.86</td>
<td>2.56</td>
<td>10.60</td>
<td>4.66</td>
<td>0.15</td>
<td>0.21</td>
</tr>
<tr>
<td>14 (11)</td>
<td>9.75</td>
<td>1.44</td>
<td>14.49</td>
<td>4.58</td>
<td>0.09</td>
<td>0.34</td>
</tr>
<tr>
<td>Mean</td>
<td>7.58</td>
<td>2.17</td>
<td>12.38</td>
<td>4.46</td>
<td>0.18</td>
<td>0.34</td>
</tr>
<tr>
<td>S.E.</td>
<td>±0.56</td>
<td>±0.37</td>
<td>±0.60</td>
<td>±0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*In experiments 1 to 7, 15 μg./Kg. ouabain was injected into the right ventricle. The same dose was given in experiments 10 to 14. In experiments 8 and 9, the dose of ouabain was 30 and 22 μg./Kg., respectively.

†For explanation of abbreviations, see footnote to table 1.

‡Days after aortic coarctation noted in parentheses.

In cardiac failure, characterized by a loss of high-energy phosphate stores. This response is not accompanied by a return of such stores to normal. In fact, it appears as though an additional decrease in PC occurs. Seven control animals receiving ouabain exhibited a mean decrease of 27 per cent in the PC content of the heart, an equivalent increase in IP, but no significant alteration of ATP. Thus, ouabain appears to decrease PC in both control and experimental congestive failure animals in conjunction with its positive inotropic effects.

**EFFECT OF ACUTE ASPHYXIA ON THE FORCE OF CONTRACTION AND THE HIGH-ENERGY PHOSPHATE CONTENT OF THE HEART**

Normal guinea pigs of 650 to 800 Gm. were anesthetized with sodium pentobarbital (35 mg./Kg.) and artificially respired for 10 minutes. The chest was then opened, as previously described, and left and right ventricular pressures were recorded. After suitable control records were obtained, the tracheal cannula was removed and the trachea was occluded with a hemostat. Left ventricular pressure was continuously monitored via a 20-gauge hypodermic needle which was kept in place within the ventricular chamber. The left ventricular apex was removed and frozen in liquid nitrogen at a suitable time after the initiation of the asphyxial period. In this manner, hearts were removed from individual animals at periods from 30 seconds to 15 minutes after tracheal occlusion for analysis of myocardial high-energy phosphate compounds. The mechanical capacity of the hearts was evaluated by observing its ability to increase left ventricular systolic pressure during temporary complete aortic occlusion. In normal hearts a two- to threefold rise in intraventricular pressure can occur, within 5 to 10 seconds, under these conditions, with a maximum pressure development of about 200 mm. Hg.
At the beginning of the asphyxial period, a slight rise or depression of the intraventricular systolic pressure was observed in all experiments, followed by a progressive fall in pressure. This was accompanied by a considerable and progressive lessening in the peak isometric pressure (PIP) obtained by complete occlusion of the aorta. After about one to three minutes of anoxia, very little, if any, increase in PIP occurred, indicating that the ventricle has lost its reserve ability to develop tension. This is good evidence for mechanical failure of the ventricle.

PIP, in several instances, fell even before intraventricular pressures (without aortic occlusion) were significantly depressed. The most striking initial alteration in cardiac function, aside from pressure changes, was the development of atrioventricular (A-V) block which occurred in every experiment between one and two minutes after tracheal occlusion. A-V block was not due to reflex vagal stimulation as prior bilateral vagotomy did not prevent it. It may reflect a greater sensitivity of A-V nodal tissue, than other conducting tissue of the heart, to anoxia. The end-diastolic pressure was increased above normal during the anoxic period.

The recovery from acute asphyxia was studied by resuming artificial respiration at a particular point in time after tracheal occlusion. An eight-minute period of asphyxia was chosen as a suitable time, as this was near the point at which mechanical activity ceased in most animals. In fact, several animals could not be resuscitated at this point. Samples of left ventricular apex were frozen in liquid nitrogen at periods ranging from 1 to 17 minutes after restoration of pulmonary ventilation, and analyzed for high-energy phosphate compounds.

Results of the analyses (table 4) reveal a precipitous fall in PC after tracheal occlusion. Eighty per cent of the PC is lost in
Effects of Acute Asphyxia, and Subsequent Recovery by Rebreathing on Intraventricular Pressures (Left Ventricle) and Rate of the Guinea Pig Heart

<table>
<thead>
<tr>
<th>No. of experiments</th>
<th>Mean intraventricular pressures</th>
<th>Mean heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 8 minutes asphyxia</td>
<td>13 29 7 120 0.2 257</td>
<td></td>
</tr>
<tr>
<td>After 30 seconds to 1 minute rebreathing</td>
<td>7 120 0.2 265</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 5

Due to diffusion out of the cells into the blood. The rate at which ATP decreases is considerably less than that for PC, so that by the time PC has fallen 80 per cent, only 20 to 30 per cent of the ATP has been lost. Loss of ATP is not accompanied by any comparable increase in ADP or AMP, so that a loss of total adenine nucleotide is incurred. The ultraviolet absorption spectrum of the tissue extracts provided no evidence for an accumulation of either inosinic acid or hypoxanthine.

The gross appearance of the heart at the end of the eight-minute anoxic period is that of a markedly dilated, cyanotic, flabby organ. Upon reventilation of the lungs, the heart, in each case that recovered, reverted to a reddish, firm, rapidly and vigorously contracting organ within 15 to 60 seconds after rebreathing was commenced. Intraventricular pressures (systolic as well as PIP) and heart rate returned to preanoxic values within this time period (table 5, fig. 3).

The speed and magnitude of the recovery was surprising, and the primary point of interest became the determination of the rate at which ATP and PC levels were restored in relation to the return of normal mechanical function. The data (table 4) indicate that there is a rapid increase in PC (with equivalent decreases in IP and free creatine) so that it is at 50 per cent of normal in 1 1/2 minutes and reaches the normal level within 10 minutes. However, the rate of recovery of PC is not as rapid as its initial loss. The
recovery of ATP, on the other hand, is very slow. The highest single concentration attained (after 10 minutes rebreathing) was only about 10 per cent greater than at the end of an eight-minute period of anoxia. The most striking physiological change in the recovery experiments was the return of normal sinus rhythm and a marked increase in contractile force (as determined by left ventricular pressure pulses both before and after complete temporary aortic occlusion) within 20 seconds to 1½ minutes after pulmonary ventilation was resumed. Recovery of normal mechanical and conducting properties of the muscle occurred, in most cases, at a time when the concentration of PC had probably not increased to more than 20 or 30 per cent of its control value, and there was no increase at all in ATP. For example, in one animal in which normal rate and pressure were re-established in 20 seconds, a sample of left ventricle was removed after one minute of rebreathing and was found to contain only 3.09 μM/Gm. PC. This is about 37 per cent of the mean control concentration of PC. The actual concentration at the 20-second point was undoubtedly considerably less. In another animal, the PC concentration was 4.30 μM/Gm. (51 per cent of control level) after 90 seconds. However, rate and intraventricular pressure had returned to normal after 25 seconds. In both instances, the ATP levels were in the same range as found in animals after five to nine minutes of anoxia. It appears that the increased mechanical output is not related to a return of energy-rich phosphate compounds to normal steady-state levels. Certainly a rise in ATP concentration is ruled out as a possible factor responsible for the increase in force of contraction.

Discussion
An important difference between the method of inducing myocardial failure used in these experiments and most others reported in the literature is that in the present case a mechanical overloading of the left ventricle is produced. In other procedures (with the exception of Szekeres and Schein who, in acute experiments, obtained similar results to those reported in this paper) either the increased load is imposed upon the right ventricle or failure is spontaneous, or drug induced, in a heart isolated from its normal innervation and the other major organs of the body. Congestive cardiac failure in humans is most commonly associated with chronic mechanical overloading of the left ventricle, due to hypertension, valvular lesions, or other mechanical abnormalities, such as subvalvular aortic stenosis and aortic coarctation. These conditions all have in common a requirement for increased tension development within the myocardium, in order to maintain adequate minute output.

The evidence presented in this paper indicates that mechanical overloading of the left ventricle may lead to a depression of the high-energy phosphate and creatine stores of the heart. The loss of high-energy phosphate compounds may be an early manifestation of failure, for it was observed to some extent even in those animals which exhibit little gross pathology or abnormal intracardiac pressure pulses. While no definitive statement can be made as to any cause and effect relation, the lowered reserves of energy-rich phosphates must have a bearing on many aspects of the metabolism of the heart, which can influence contractility (e.g., active transport of ions).

The loss of total creatine stores observed in the hearts of guinea pigs with experimental congestive failure is in agreement with the results of Cowan and Herrmann, Decherd and Oliver in cases of human congestive heart failure. The loss of total creatine from the guinea pig heart, in all but the seven most severe cases, is primarily a reflection of the fall in PC. That is, the free creatine concentrations are normal, but the total creatine is decreased to the extent that PC is lost. In the more severely affected animals, the concentration of free creatine is also lowered. The loss of creatine may be due to a diffusion out of the cell as the PC level falls and that of creatine rises.
and Eggleton have observed diffusion of creatine out of skeletal muscle due to fatigue or anoxia. Depression of creatine synthesis, which is known to require ATP, is another possible explanation for the low creatine levels in failing hearts. No direct evidence bearing upon these alternative explanations has been obtained. However, it is apparent that the lower the ATP concentration, which was observed in failing hearts, the lower was the total creatine content as well (see table 2). No such relationship between the ATP and total creatine content of the heart was observed in the case of acute anoxia (table 4), where high total creatine concentrations were observed in the presence of very low levels of ATP.

The data presented in this paper do not support any of the previously described work on the effects of the cardiac glycosides in experimental cardiac failure in which these drugs appeared to increase energy-rich phosphate levels. In control animals, ouabain (15 μg./Kg.) was found to produce a significant (25 per cent) fall in PC with no change in ATP. In experimental heart failure in the guinea pig, the same dose of ouabain produced, at the height of the positive inotropic response, a fall in PC without any apparent change in adenine nucleotides. This result is in accord with that of Schwartz and Lee, who found that ouabain did not restore the efficiency of oxidative phosphorylation in heart mitochondria from guinea pigs with aortic coarctation.

The reasons for the discrepancy between the results reported in this paper and those of the other investigators referred to is not readily apparent, but as the control values for ATP and PC in most of the latter were usually quite low and variable, it would seem that extensive breakdown of these compounds occurred which was not a consequence of the action of a cardiac glycoside or the method of inducing cardiac failure. Until more definitive experiments are conducted to make clear the mechanism of action of digitalis glycosides, no final conclusion can be reached concerning the action of these agents on energy metabolism. Lee, Yu, and Burststein have recently been able to demonstrate a positive inotropic action of ouabain in cat papillary muscle under conditions where no effect on oxygen consumption or energy-rich phosphates was noted. At the height of the positive inotropic action of strophanthin on guinea pig auricles, in vitro, Furchgott and deGubareff also have reported that no change in the concentrations of PC or adenine nucleotides occurred. It would appear, therefore, that the action of cardiac glycosides on energy-rich phosphates and respiration are secondary effects of the drugs and not directly responsible for the improved mechanical performance which they induce. A possible explanation for the observed falls in PC concentration in the guinea pig ventricle may be found in the work of Hochrein and Doring, who observed an inverse relationship between the aortic pressure and the PC content of the guinea pig heart. A similar observation was made by Wollenberger on the dog heart.

The return of normal mechanical function of the anoxic heart appears to be more closely related to the restoration of the PC content than to that of ATP. However, an alternate explanation is that recovery from anoxia requires the restoration of some altered state of the cell, other than the energy-rich phosphate content, such as the ionic composition, pH, or concentration of intermediary metabolites. It must be kept in mind that the values of PC and ATP which were determined represent the steady-state values and are in no way indicative of changes in the rates of synthesis and utilization which are probably of greater significance than the actual concentrations. Normal mechanical output of the heart appears to be possible at low steady-state levels of energy-rich phosphate compounds if conditions are such that the rates of synthesis of ATP and PC are sufficient to meet the necessary energy requirements of the muscle. Similarly, the lowered ATP and PC content of cardiac muscle in experimental failure would probably be of
HEART FAILURE AND OUABAIN

less consequence than a depression of the rates of synthesis of these compounds. Schwartz and Lee have demonstrated that mitochondria isolated from the hearts of animals with experimental congestive failure exhibit a depressed respiratory rate and an uncoupling of oxidative phosphorylation. A number of these animals were assayed for ATP and PC and have been reported in this paper. There was excellent agreement between the efficiency of mitochondrial oxidative phosphorylation and the levels of ATP and PC found in the same hearts. This altered state of the mitochondria, if present in the intact living heart, would undoubtedly be of more importance than the lowered steady-state levels of ATP and PC. The loss of energy-rich phosphates would, in fact, be a reflection of a depression in their rates of synthesis.

Summary

The left ventricles of guinea pigs have been assayed for adenosine triphosphate, adenosine diphosphate, adenylic acid, phosphorylcreatine, free creatine, and inorganic phosphate. These determinations have been made under various experimental conditions which affect the performance of the heart in vivo, such as experimental congestive heart failure, acute asphyxia, and the influence of the cardiac glycoside, ouabain. These determinations have been made under various experimental conditions which affect the performance of the heart in vivo, such as experimental congestive heart failure, acute asphyxia, and the influence of the cardiac glycoside, ouabain. In 20 guinea pigs with experimental congestive heart failure, sacrificed 1 to 18 days after surgically producing a coarctation of the ascending aorta, a significant fall in PC (54 per cent), ATP (24 per cent), and total creatine (34 per cent) was observed. The extent of the loss of high-energy phosphate compounds and total creatine paralleled the severity of cardiac failure as determined by abnormal intraventricular pressure pulses, ventricular hypertrophy, and gross and microscopic pathology. In animals with experimental congestive heart failure, the cardiac glycoside ouabain produced significant slowing of the heart, a positive inotropic effect, and a lowering of abnormally high right ventricular systolic and left ventricular diastolic pressures. At the height of the response to ouabain, there was a further fall in PC to 25 per cent of normal without any change in ATP concentration. The positive inotropic action of ouabain is, therefore, not due to an ability of the drug to increase high-energy phosphate levels in failing heart muscle. Acute asphyxia produces a very rapid fall in the PC concentration (85 per cent lost in two minutes) and a much slower fall in ATP (33 per cent lost in eight minutes). Recovery of normal mechanical activity, after a period of asphyxia, by rebreathing occurs with little, if any, restoration of ATP and at a time when the PC concentration is only about 25 per cent of normal. These experiments indicate that steady-state levels of high-energy phosphate compounds are not as important in determining the mechanical capacity of the heart as are their rates of synthesis.

Acknowledgment

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Circulation Research, Volume X, March 1962
Effects of Experimental Congestive Heart Failure, Ouabain, and Asphyxia on the
High-Energy Phosphate and Creatine Content of the Guinea Pig Heart
MAURICE B. FEINSTEIN

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