Energy cost of work in aerobic and anaerobic turtle heart muscle


Aerobic rates of oxygen consumption and anaerobic rates of lactate production, each as a function of work rate at 23-25 C, were compared in 75 isolated perfused hearts from turtles (Pseudemys scripta). Pressure-volume work at constant heart rate was varied over the range of 2-14 × 10⁵ ergs/min. Control experiments established that turtle hearts are capable of sustained (greater than 15 hr) anaerobic work provided some plasma is present in the perfusion medium. Mechanical performance was unaffected by anoxia (pO₂ < 2 mm Hg) except for some decrease in heart rate. Adenosine triphosphate (ATP) demand was the same at any given work rate whether the energy was supplied by oxidative phosphorylation or exclusively by anaerobic glycolysis. The efficiency of the heart, expressed as mechanical work divided by free energy consumed, was therefore independent of the metabolic pathways leading to production of ATP. If the ΔF° of ATP hydrolysis is taken to be 7.0 kcal/mole, the efficiency of the working turtle heart is 59 ± 3.4%.

METHODS

General

Experiments were carried out on 75 isolated turtle hearts, perfused with Ringer-plasma solution equilibrated with oxygen or nitrogen. Cardiac work was set by level of venous return reservoir and by ventricular outflow pressure. Oxygen consumption was calculated from cardiac output and the difference between oxygen partial pressures of inflow and outflow. Glucose utilization and lactic acid production were calculated from changes in the composition of the perfusion reservoir with time. For this latter purpose it was necessary to develop an isolated heart preparation in which fluid losses were reduced to a minimum.

Operative Procedures

Turtles of the species Pseudemys scripta (0.9-1.5 kg), obtained from Lemberger & Co., Oshkosh, Wisconsin during winter months, were anesthetized with pentobarbital sodium (30 mg/kg by stomach tube). Five percent CO₂ in oxygen was administered by tracheal intubation throughout the initial operative procedures in order to prevent hypoxia. Prior to isolation of the heart the animals were placed on their backs, partially submerged in a bath of 0.8% NaCl. A g-in. circle was cut in the plastron over the heart and the bony plug removed under saline to prevent formation of air emboli in the well-developed coronary circulation. The right atrium and the left radix artery were cannulated with polyethylene tubing (PE 330). All other blood vessels entering or leaving the heart were tied, care being taken not to occlude the coronary circulation. Venous drainage from the liver just dorsal to the heart was occluded by several small ligatures passed through liver parenchyma. The heart, together with a small plaque of liver, could then be freed from the carcases and placed in the perfusion system. Fluid loss from the system was seldom more than 2%/hr of the initial perfusion volume (50-70 ml).
Comparison of turtle Ringer’s solution with turtle serum (6)

TABLE 1. Comparison of turtle Ringer’s solution with turtle serum (6)

<table>
<thead>
<tr>
<th></th>
<th>Ringer’s</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>131.3</td>
<td>132.8</td>
</tr>
<tr>
<td>K</td>
<td>3.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Ca</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>Mg</td>
<td>3.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Cl</td>
<td>99</td>
<td>80</td>
</tr>
<tr>
<td>SO₄</td>
<td>3.5</td>
<td>0.5</td>
</tr>
<tr>
<td>PO₄</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>HCO₃</td>
<td>45.0</td>
<td>43.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.77</td>
<td>7.80</td>
</tr>
</tbody>
</table>

Concentrations are in millimoles/liter water.

Perfusion System

Perfusion fluid from the reservoir was led through silicone-treated glass and rubber tubing by gravity flow directly to the atrial cannula. Working conditions of the heart were controlled by selecting the desired inflow and fixing the output pressure against which the heart emptied. The latter was varied with a Starling resistance; interposed between the heart and Starling resistance was a low resistance valve. Arterial pressure was monitored by means of a Statham pressure transducer; heart rate was measured from the arterial pressure record. Minute output of the heart was recorded with an accuracy of ±0.5 ml/min using a Gaddum flowmeter (5). Atrial pressure was computed from reservoir pressure by subtracting the pressure drop in the reservoir-to-cannula tubing (obtained from the pressure flow curve and the measured output of the heart). Experiments were carried out at 23–25 °C (room temperature).

In some experiments changes in the volume of the heart were measured continuously by weighing the isolated heart on a recording balance employing a linear variable differential transformer (Schaevitz Engineering Corp., Camden, N. J.). When the heart weight was measured anaerobically, the balance pan was tightly covered by a plastic chamber through which a large flow of water-saturated N₂:CO₂ (95:5) was directed.

Addition of plasma to a Ringer-perfused heart brings about significant mechanical and efficiency changes (7, 8). At fixed atrial and output pressures, the addition of small amounts of plasma increases the output concomitantly with a decrease in heart volume and oxygen consumption (Fig. 2). Consequently, animals were given heparin (2 mg/kg), bled just before cannulation of the heart, and the separated plasma added to the perfusion fluid. Final protein concentration was not uniform throughout all experiments; in six experiments the concentration, estimated from dry weight, was 0.38 ± 0.02 g/100 ml. Separate control experiments established that heparin alone was without effect.

To show that edema did not occur as a consequence of perfusion (> 2 hr) with this medium, wet and dry weights were measured and per cent water was calculated. The water content of five unperfused and eight perfused hearts was 84.5 ± 1.6% (SE) and 85.2 ± 2.2%, respectively. All hearts together contained 85.0 ± 0.3% water.

Oxygen Measurements

Continuous polarographic monitoring of the oxygen pressure in the perfusion fluid served three purposes: a) determination of inflow and outflow oxygen tensions...
FIG. 2. Effect of small amount of plasma (final concn. 0.4 g/100 ml) on volume of Ringer-perfused aerobic turtle heart (tissue wt. 2.6 g). Weight trace rises during diastolic filling and falls during systole. Addition of plasma decreases end-diastolic volume and increases stroke volume and cardiac output. A third effect of plasma is a large decrease (up to 30%) in the oxygen consumption associated with a given work rate.

TABLE 2. Effect of aerobic-anaerobic transition on heart rate in working turtle hearts

<table>
<thead>
<tr>
<th>Sequence</th>
<th>No. of Animals</th>
<th>Anoxic Rate, min⁻¹</th>
<th>Aerobic Rate, min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ to N₂</td>
<td>22</td>
<td>29.4±1.63</td>
<td>36.1±1.24</td>
</tr>
<tr>
<td>N₂ to O₂*</td>
<td>5</td>
<td>28.2±0.87</td>
<td>36.1±3.10</td>
</tr>
</tbody>
</table>

Values are given ±S.E. * Duration of anoxia was greater than 2 hr in all experiments.

Chemical Determinations

The uptake of glucose and output of lactate were calculated from the product of concentration and perfusion volume as a function of time. Samples (0.5 ml) were removed every 20 min from the perfusion reservoir which was kept well mixed by the gas-exchange pump. Lactate was estimated colorimetrically in triplicate by the method of Barker and Summerson (10). Glucose was determined in duplicate on Somogyi (11) protein-free supernatant in two ways: first, as total reducing substance by the method of Nelson (12), and second, by the glucose oxidase method (13) for which enzyme was purchased from Takamine Lab., Clifton, N. J. (batch no. 140). The presence of nonglucose-reducing substances could not be detected. Glycogen was isolated from heart muscle immediately on terminating an experiment by the method of Good, Kramer, and Somogyi (14) and was hydrolyzed in 5 N sulfuric acid in a boiling water bath for 40 min; glucose was measured on the neutralized hydrolysate by the Nelson procedure. All glycogen figures are presented in terms of glucose equivalents. pH measurements were made with a glass electrode on a model pHM-22 Radiometer instrument (Radiometer, Copenhagen, Denmark).

Replicate chemical analyses agreed within 5% and the chief source of error was associated with volume changes brought about by extravasation from the heart preparation. The perfusion volume was measured at the
TABLE 3. Comparison of work and O₂ consumption in isolated turtle hearts before and after a period of sustained anoxic work

<table>
<thead>
<tr>
<th>No.</th>
<th>Duration of Anoxia, min</th>
<th>Preanoxic</th>
<th>Postanoxic</th>
<th>Ratio ( q_{\text{O}_2} ) pre/post</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>7.7 ( \times 10^5 )</td>
<td>156</td>
<td>1.55</td>
</tr>
<tr>
<td>2</td>
<td>95</td>
<td>8.1</td>
<td>1.68</td>
<td>6.9</td>
</tr>
<tr>
<td>3</td>
<td>85</td>
<td>9.2</td>
<td>1.88</td>
<td>9.3</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>9.2</td>
<td>2.07</td>
<td>9.1</td>
</tr>
</tbody>
</table>

*Workload varied in anoxia and not returned to initial level before readmitting oxygen; postanoxia \( q_{\text{O}_2} \) is corrected to initial work level using Fig. 6.

FIG. 5. Steady-state rates of lactate production (\( \mu \)moles/min heart) as a function of work rate in the isolated anaerobic working turtle heart. Line indicated is least-squares fit to data.

lower than the aerobic rate; the time course of the rate change paralleled the decrease in oxygen tension of the perfusion fluid. Once complete anoxia was achieved, the anoxic rate was well maintained throughout the remainder of the anoxic period, however long. The effect was fully reversible, as indicated in Table 2.

Mechanical performance. Figure 3 compares the size of the heart and the output achieved at a given atrial pressure in a typical heart operating under aerobic and anoxic conditions. Anoxia is not accompanied by dilatation nor by diminution of work output. Some plasma-Ringer's perfused preparations were deprived of oxygen in the presence of glucose as long as 15 hr without becoming hypodynamic.

Oxygen consumption. Table 3 compares the work output and \( q_{\text{O}_2} \) at fixed atrial and arterial pressures before and after an extended period of anoxic work. Neither impairment of workload at specified pressures nor change in oxygen consumption associated with that workload occurs in the turtle heart preparation. The postanoxic rate of oxygen consumption was not significantly greater than the control rate at comparable workload, there was no indication of an oxygen debt.

Energy Metabolism of Anaerobic Heart

Figure 4 summarizes a typical anaerobic experiment in which output pressure and atrial pressure were set prior to anoxia and were not altered subsequently in the course of the experiment. When the heart was made anoxic, no decrease in work rate was observed. Lactate production and glucose uptake were zero in the aerobic period, but once complete anoxia had been achieved a constant lactate production and glucose uptake were observed. Results of 38 similar experiments at widely

beginning and at the end of each experiment. An additional estimate of the perfusion volume was usually made at the midpoint of each experiment from the dilution of added glucose. Leakage from the system was often negligible; whenever leakage was present, however, the apparent rates of glucose uptake and lactate production were corrected to take account of the quantities lost in the leak. Oxygen consumption was computed from arteriovenous oxygen pressure difference and cardiac output (assuming a Bunsen coefficient of oxygen in turtle Ringer's solution of 0.030 ml \( \text{O}_2 \) STP/ml atm). With the indicated error in estimation of oxygen tension and the small error in minute output, the maximum expected error in the \( q_{\text{O}_2} \) figures is less than 10%.

Heart work rate was computed as pressure-volume work per minute (15); in all cases calculated velocity work was negligible. Pressure was obtained by taking the graphic integral of the pressure-time curve of ejection and dividing by the ejection duration.

RESULTS

Effects of Anoxia on Physiological Performance

Heart rate. Table 2 shows effects of anoxia on endogenous pacemaker activity of the perfused working heart. In all cases anoxia slowed the heart to a rate about 25%
ranging workloads are plotted in Fig. 5, which shows that lactate production was linearly dependent on work rate. These data derive principally from experiments in which volume work was varied while arterial pressure was maintained in the range of 25-30 cm H2O; despite the desirability of carrying out comparable measurements at higher arterial pressures, the requirement for a leak-proof preparation made reliable data extremely difficult to obtain. Each point represents steady-state rate of lactate accumulation in the medium estimated from at least four samples collected at 20-min intervals. This precaution was exercised to insure that changes in intracellular lactate concentration would not be mistaken for actual changes in rate of production. The data are plotted in terms of micromoles of lactate per minute per heart; inasmuch as the heart weights used varied from 1.5-4 g, some of the variability observed must be attributed to energy-utilizing processes not directly associated with the accomplishment of external work, i.e., a basal or resting metabolism component. This is indicated also by the intercept at zero workload.

Although the rate of glucose uptake in the experiment depicted in Fig. 4 is almost sufficient to account for the observed rate of lactate production (i.e., 2 moles lactate produced for each mole of glucose utilized), it must be pointed out that glucose uptake is not always stoichiometrically related to lactate production. Factors which determine rate of glucose uptake are discussed in an accompanying paper (16).

Figure 6 shows steady-state rates of oxygen consumption as a function of work rate. The rate of approach to the steady state following a step change of workload was extremely rapid, as indicated by constant arteriovenous oxygen difference within five or six beats. Oxygen consumption, like lactate production, is linearly related to workload and has a positive intercept at zero workload.

The results shown in Figs. 5 and 6 make possible a comparison of the energy cost of anaerobic work with aerobic work in terms of ATP demand. Under aerobic conditions the relation between \( q_{\text{O}_2} \) and rate of synthesis of ATP is relatively unaffected by the nature of the substrate utilized, i.e., whether carbohydrate or fat reserves; this scale factor may be approximated by a P:O ratio of 3.0 (17). A simple scale factor for conversion of lactate production into ATP units is not, however, so readily applied. The stoichiometry of ATP production per mole of lactate formed is a function of the identity of the hexose pool utilized; at lower workloads where glycogen is the principal substrate (16), the molar ratio of ATP: lactate is 1.5. At greater workloads where glucose is utilized from the medium in addition to endogenous glycogen, the molar ratio of ATP synthesized to lactate produced lies somewhere between 1.5 and 1.0. Thus no simple scaling of the lactate curve is adequate to represent these data in terms of ATP production.

This difficulty can be resolved if it is assumed that all the observed glucose uptake (\( q_{\text{GLU}} \) \( \mu \)moles/min heart) is quantitatively converted into lactate, and that the lactate production (\( q_{\text{LAC}} \)) which cannot be accounted for as glucose taken from the medium (\( q_{\text{LAC(GLU)}} \)), derives from breakdown of tissue glycogen stores (\( q_{\text{LAC(GLY)}} \)). This assumption is shown to be valid in the accompany-
...ing paper (16). Rate of production of ATP under anaerobic conditions, \( q_{\text{ATP}}^n \), is then given by

\[
q_{\text{ATP}}^n = 1.5 (q_{\text{LAC(GLY)}} + q_{\text{LAC(GLU)}}) = 1.5 (q_{\text{LAC}} - 2q_{\text{GLU}}) + 2q_{\text{GLU}} \quad (i)
\]

Figure 7 presents the comparison of aerobic and anaerobic work costs in terms of calculated ATP production rates. Comparison by \( t \) test of the least-squares lines computed for these two sets of data indicates no difference at the 0.05 confidence level. Some of the scatter in the data may be attributed to variation in heart weight which would be expected to alter the intercept, though not the slope, of the correlation.

**DISCUSSION**

**Equivalence of Contractile ATP Demand in Aerobic and Anaerobic ATP Synthesis**

It is now widely held that “the energy of metabolic reactions becomes available for work processes after transit through an ATP molecule” (18). Numerous instances of the participation of this molecule in mechanochemical, light emission, and synthetic reactions attest the extensive range of experimental evidence for this generalization. As a consequence, not only is the accumulation of free energy as ATP by cells independent of the redox potentials of the energy-conserving reactions, but the time and place of ATP synthesis may be distinct from the moment and region of ATP utilization within a cell. Provided that ATP produced by one energy pathway, i.e., mitochondrial oxidative phosphorylation, cannot be distinguished from that produced by another pathway, i.e., cytoplasmic glycolysis, ATP demand created by the performance of cell work should be the same when measured exclusively by either pathway. The results summarized in Fig. 7 indicate that, at least for turtle heart muscle, contractile ATP demand does provide equivalent stimulation to either the aerobic or anaerobic energy pathway.

This simple extension of the concept of ATP as free energy currency has not been shown previously for muscle or for any other tissue. One reason for this appears to be that few cells or tissues possess both glycolytic and oxidative pathways having capacity sufficient to sustain prolonged and measurable workloads. Precisely this feature renders the isolated turtle heart preparation suitable for investigation of energy costs or work using different synthetic pathways for ATP. Its glycolytic capacity is able to provide for workloads nearly equivalent to its best performance aerobically.

**Mechanical Efficiency of Working Heart Muscle**

Figure 7 has shown that ATP demand is a function of work rate and does not differ significantly under aerobic and anaerobic conditions. This information permits a computation of the efficiency of the working turtle myocardium. Efficiency is here defined as the ratio of external work performed to the free energy consumed in the process (19). The computation assumes that no portion of the ATP used at zero workload is available for mechanical work. With this assumption the efficiency is defined by the reciprocal of the slope of the line shown in Fig. 7. After conversion to common units the efficiency is:

\[
F = \frac{10^6 \text{ ergs}}{0.68 \text{ umole ATP}} = \frac{10^6 \text{ ergs} \times 2.39 \times 10^{-3} \text{ cal/erg}}{0.68 \text{ umole ATP}} = 3,520 \text{ cal/mole ATP} \quad (2)
\]

This figure may be compared to the work performed by frog rectis abdominis per mole of phosphocreatine as reported by Cain et al. (4) of 2,600 cal/mole. If the \( \Delta F^\circ \) of ATP hydrolysis is taken as 7.0 kcal/mole (18) then an efficiency of 50 + 3.4% is obtained for the turtle heart compared to a figure of 37% for the frog rectis abdominis.

**REFERENCES**