Effect of pressure development on oxygen consumption by isolated rat heart

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Effect of pressure development on oxygen consumption by isolated rat heart. Am. J. Physiol. 212(4):804-814, 1967.—The isolated perfused rat heart has been modified to allow metabolic studies over a range of heart work and pressure development. When the left atrial filling pressure was increased from 0 to 20 cm H2O, cardiac output increased fivefold and systolic aortic pressure rose from 60 to 120 mm Hg. The mechanical performance and oxygen consumption were stable for over 3 hr when the heart was perfused with Krebs-Henseleit bicarbonate buffer containing only glucose. Variation in mechanical performance and oxygen consumption with hearts of various sizes has been noted, indicating that meaningful comparisons of performance must utilize hearts of similar size. Pressure development, as estimated from pressure-time integrals, correlated well with the portion of oxygen consumption involved in generation of mechanical energy. Oxygen supply to the tissue appeared to be adequate. Oxygen consumption increased from an average of 1.44 to 2.85 mmoles/g per hr as atrial pressure was increased from 0 to 20 cm H2O when hearts were perfused with buffer containing either no nutrients, 5 mm glucose, 5 mm acetacetate, or 5 mm acetate.

The relationships between mechanical activity, oxygen consumption, and substrate utilization by the heart can be studied best using a heart preparation that does not include lungs or other tissues in the circuit. The isolated rat heart perfused by the Langendorff technique has been used extensively to study oxygen consumption and substrate utilization and their regulation by hormonal and nonhormonal factors. The relationship between mechanical activity, oxygen consumption, and substrate utilization has only recently been investigated by Opie using a modification of this technique (27). However, Opie’s preparation does not pump fluid or do external work, preventing studies of the effects of these variables on oxygen consumption and substrate utilization.

The relationships between mechanical activity and oxygen consumption have been intensively studied in heart preparations. Factors including heart (2, 18) and contractile element work (6), end diastolic volume or fiber length (9, 19, 33, 35), heart rate (3, 8, 11, 13, 24, 34, 35), developed myocardial tension (13, 29), coronary flow (1, 17, 20, 27), and velocity of contraction (30) have been considered to be of importance in regulating oxygen consumption.

In the present work, a completely isolated rat heart preparation capable of in vitro mechanical work has been devised. Perfusion was introduced into the left atrium at various filling pressures and pumped by the left ventricle against a hydrostatic pressure head. Cardiac work and oxygen consumption increased as left atrial filling pressure was raised. In addition, the rat heart perfused by the Langendorff technique (23) has been found to develop left ventricular pressure up to the level of the aortic pressure with each systole. Oxygen consumption varied with changes in aortic pressure, a parameter that has not been strictly controlled in many earlier studies of substrate utilization by the rat heart (5, 26, 28, 36). These preparations offered the opportunity to vary ventricular pressure development and external work over a wide range.

EXPERIMENTAL PROCEDURES

Preparation of the heart. Rats of the Sprague-Dawley strain weighing 150-350 g were fasted overnight before use. Heparin sodium (2.5 mg) was injected intraperitoneally 1 hr before the rats were killed. The rat was anesthetized with Nembutal (50 mg/rat, ip) and the abdominal cavity was opened by making a transverse incision with the scissors. The diaphragm was transected and lateral incisions were made along both sides of the rib cage. The anterior chest wall was folded back. The pericardium and

NOTE ADDED IN PROOF: More recently, it has been shown that the isolated perfused rat heart is capable of generating mechanical work and oxygen consumption for periods exceeding 30 min (29). These studies have been carried out under conditions that are not completely comparable to those described in the present work, but they lend support to the view that the isolated perfused rat heart is capable of substantial mechanical and metabolic activity for periods exceeding 30 min.
other filamentous tissues of the mediastinum were pulled away from the heart with the fingers. The heart was picked up and the lungs and other chest contents pushed toward the back. Prior to excising the heart, it was important to identify the point at which the pulmonary veins join the left atrium. The heart was then cut free by making a single cut with the scissors through this point and on through the other vessels arising from the heart. The heart was dropped into a beaker containing 0.9% sodium chloride chilled in ice water. Contractions stopped within a few seconds.

Perfusion of working rat hearts. Using a fine tipped forcep, the heart was picked up by the aorta and any connective tissue, thymus, or lung that may have been removed with it was pulled away. When the heart had cooled for 15-20 sec, the aorta was slipped about 3 mm onto a grooved perfusion cannula (Fig. 1) and held in place with a hemostat. Retrograde perfusion down the aorta was begun from a reservoir 70 cm above the heart as soon as the heart was positioned with the hemostat. After the aorta was secured with a ligature, the heart was rotated on the cannula, if necessary, to position the opening into the left atrium to receive the second perfusion cannula. The left atrium was slipped onto this cannula and tied. The accuracy and competence of the cannulation were tested by unclamping the tube leading from the atrial bubble trap and observing the filling of the left auricle. Retrograde perfusion was continued for 10 min (60 mm Hg pressure) with medium containing heparin (10 mg/liter) and the additions that were to be present in the subsequent perfusion. This washing served to remove all blood, to equilibrate the substrate concentrations in the medium with those in the interstitial fluid, and to allow the heart to recover from the period of anoxia associated with excision and initiation of perfusion.

Heart work was begun by clamping the tube from the washout reservoir and unclamping the tubes supplying perfusate to the atrium from an overflow type bubble trap and carrying perfusate to the aortic bubble trap. This bubble trap design eliminated pressure fluctuations due to the pump and insured a constant hydrostatic pressure impinging on the heart. A peristaltic pump delivered perfusate from the oxygenating chamber to the atrial bubble trap. Buffer entering the atrium passed into the pressure chamber attached to the aortic cannula. This chamber was one-third filled with air (approximately 1 ml) to provide some elasticity to an otherwise rigid system. The volume of air was important in determining the size and shape of the aortic pressure curve. When the ventricle contracted, pressure development in this chamber forced fluid out through a Tygon tube into an aortic bubble trap 70 cm above the heart. Overflow from the aortic bubble trap was returned inside the long central oxygenating chamber. Aortic output was measured by collecting the overflow from this bubble trap. After the atrium was cannulated and ventricular filling begun, cardiac output was more than sufficient for coronary perfusion. Coronary flow was returned from the heart chamber through an opening in the central portion of the oxygenating chamber and reservoir. 1) Heart chamber and cannula assembly, and pressure chamber is shown on left. Apparatus consisted of the following parts: 2) Heart chamber and cannula assembly. The male portion of a 35 ball joint was made of Teflon and held two stainless steel cannulas (0.134 inch o.d.) grooved to accommodate ligatures. A tip of 0.109-inch o.d. tubing was soldered into the atrial cannula. The female portion of a 35/25 ball joint was adapted for use as a heart chamber and held in place with a pinch clamp. 2) Aortic and atrial bubble traps and pressure chamber. These were made from female portions of 14/35 standard taper-joints. Male plugs were made of Teflon and fitted with neoprene "O" rings to facilitate sealing and removal of stoppers. A side arm was sealed onto the atrial bubble trap and extended with Tygon tubing and connected to the central portion of the apparatus by an 18/9 ball joint. A side arm for the aortic bubble trap was made from the male portion of a 15 ball joint. This was connected to an adapter (20 ball, 29/42 standard taper) which in turn fitted into the top condenser of the central portion of the apparatus. 3) Oxygenating chamber. Three condensers with 29/42 standard taper connections made up the central portion of the apparatus. Both the top condenser (60 cm long) and the bottom one (20 cm long) were "ful-jak" allihn condensers. A special condenser (manufactured by H. S. Martin and Co., Evanston, Ill.) was used as the middle portion. It was constructed with side arms for receiving overflow from the atrial bubble trap and coronary effluent from the heart chamber. A coarse porosity, sintered glass filter was fitted into the bottom of the oxygenating chamber. 4) Peristaltic pump was a model 505, Harvard Apparatus Co. Water jackets surrounding the glass portions of the apparatus are indicated by shading. Fluid in bubble traps and pressure chamber is indicated by stippling.
artery cannula by the right ventricle. When high coronary flow of hearts perfused in the working apparatus was pumped through the pulmonary artery. The artery was tied onto the cannula after the aortic and atrial cannulae were in place.

Acetoacetate was prepared by the method of Krebs and Eggleston since the openings in the right atrium were usually closed (Langendorff preparation) for cannulation of the pulmonary artery. The artery was tied onto the cannula after the aortic and/or atrial cannulae were in place.

Perfusion medium. Modified Krebs-Henseleit bicarbonate buffer (22), pH 7.40, equilibrated with O₂:CO₂ mixture 95:5 at 37°C was used in all experiments. Final concentrations of the salts in this buffer (in mM) were: NaCl, 118; KCl, 4.7; CaCl₂, 2.5; MgSO₄, 1.2; KH₂PO₄, 1.2; Ca EDTA, 0.5; and NaHCO₃, 25. The addition of Ca-EDTA improved the stability of the preparation, presumably by chelating trace quantities of heavy metals that were present in the reagents. Nutrients were added to the buffer at the concentration indicated in the tables and figures at this time.

Perfusion apparatus for the Langendorff preparation. The apparatus described earlier (26) was modified as follows. The heart was suspended in a 2.5 x 60-cm Allihn condenser with a coarse sintered glass filter inserted into the lower end. Perfusion pressure was changed by varying the degree of compression of the plastic tubing by the peristaltic pump. Following the initial perfusion of the heart, flow of washout buffer was clamped and recirculation of a measured volume (minimum, 15 ml) was begun. The perfusion pressure was adjusted to the value listed in the tables and figures at this time.

Pressure measurements. In some experiments left atrial, intraventricular, and aortic pressures were measured using Statham pressure transducers (model no. P23Gb) connected to a Sanborn recorder (model no. 964). When aortic pressure was to be measured the transducer was connected to the side arm on the aortic cannula. Left atrial pressure was measured with a transducer connected to a Y tube on the atrial cannula. Intraventricular pressures were measured by inserting a 20-gauge needle through the apex of the heart into the left ventricle. A special heart chamber was used with an opening at the bottom for a rubber stopper through which the ventricular needle was inserted and for a tube carrying the coronary flow. Ventricular or aortic pressure curves were integrated by cutting the area under the pressure curves out of the paper and weighing them on an analytical balance. Lengths of paper representing 0.5 sec at several known pressures were used for reference.

Calculations. The rate of external work was calculated by the following formulae:

\[
\text{Pressure work (kg-m/min per g)} = \frac{\text{aortic + coronary flow (ml/min)}}{\text{dry weight of heart (g)}} \times \frac{\text{avg systolic pressure (g/cm²) - 10}^3}{1}
\]

\[
\text{kinetic work (kg-m/min per g)} = \frac{\text{aortic + coronary flow (ml/min)}}{\text{dry weight of heart (g)}} \times \frac{\text{specific gravity of perfusate (g/cm³) - V}^{1.0} - 10^{-9}}{\text{gravitational acceleration (cm/sec²)}}
\]
OXYGEN CONSUMPTION OF ISOLATED RAT HEART

where

\[ V \text{ (cm/sec)} = \frac{\text{aortic + coronary flow (ml/min)}}{\text{cross-sectional area of aorta (cm}^2\text{)}} \times \frac{\text{cycle time (sec)}}{\text{ejection time (sec)}} \times \frac{1}{60} \]

Other indices of mechanical function were calculated as follows: pressure-time integral of working hearts (mm Hg × sec/min) = average aortic systolic pressure (mm Hg) × ejection time (sec) × heart rate (beats/min).

In nonworking hearts, pressure-time integral was calculated by substituting average ventricular systolic pressure for aortic pressure and total duration of systole for ejection time.

**Expression of results.** All heart weights are in terms of grams of dry tissue. Dry heart weight was determined as described earlier (26).

**RESULTS**

**Effect of perfusion pressure on the mechanical function and oxygen consumption of the Langendorff preparation.** Hearts were perfused by the Langendorff technique at varying perfusion pressures as measured by a mercury manometer attached to the top of the bubble trap. When perfusion pressure was increased from 40 to 120 mm Hg, coronary flow increased linearly from 35 to 144 ml/min per g (Table 1). Heart rate also increased as perfusion pressure was raised. The explanation of this change in heart rate is unknown but may be due in part to an increase in the temperature of the perfusate entering the aortic cannula. When a stainless steel cannula with several holes around the intraventricular end was inserted through the mitral valve, increasing amounts of fluid (1-6 ml/min) were pumped from the ventricle as perfusion pressure was raised. Fluid filling the ventricle may have arisen either from Thebesian veins or by leakage through the aortic valves. Distortion of the aortic valve by the cannula a few millimeters away and the use of low viscosity perfusate could predispose this system to valvular incompetence.

Oxygen consumption increased threefold when perfusion pressure was raised from 40 to 120 mm Hg. Since coronary flow increased proportionately more than oxygen consumption, oxygen tension of the coronary effluent rose as perfusion pressure was increased. Correlation coefficients of 0.70, 0.84, and 0.85 were found between oxygen consumption and coronary flow, peak systolic pressure, and pressure-time integral, respectively. These data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Perfusion Pressure, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>187±12</td>
</tr>
<tr>
<td>Coronary flow, ml/min per g</td>
<td>35±2</td>
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<tr>
<td>Aortic pressure, mm Hg</td>
<td>51±4</td>
</tr>
<tr>
<td>Peak systolic Diastolic</td>
<td>43±4</td>
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<tr>
<td>Effluent O2 tension, mm Hg</td>
<td>263±18</td>
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<tr>
<td>O2 consumption, mmol/hr per g</td>
<td>0.80±.09</td>
</tr>
<tr>
<td>Pressure-time integral mm Hg sec/min 103</td>
<td>1.13±.11</td>
</tr>
</tbody>
</table>

Values are means ± se. Hearts were perfused with buffer containing 5.5 mm glucose at various perfusion pressures. The buffer passed through the heart a single time and was collected from the pulmonary artery cannula for estimation of oxygen consumption. Each perfusion pressure was maintained for 3 min prior to making the measurements described in the table. Each heart was perfused at each pressure at random. About 1 hr was required to complete the measurements on each heart. Oxygen tension of buffer entering the heart averaged 514 mm Hg. Five hearts were included in each group. The average dry heart weight was 0.201 g.
indicated that hearts perfused by this modification of the Langendorff method developed intraventricular pressure in amounts that depended on the afterload. Oxygen consumption increased as the pressure was elevated.

**Effect of left atrial filling pressure on mechanical function and oxygen consumption of the working rat heart.** Hearts were perfused in the working heart apparatus at varying left atrial filling pressures. As atrial pressure was elevated,

![FIG. 2. Effects of perfusion pressure on aortic and ventricular pressure of the Langendorff preparation. Hearts were perfused as described in Experimental procedures and Table 1. Simultaneous recordings of aortic and ventricular pressures were made at a paper speed of 100 mm/sec. Aortic pressures were 50/40, 75/85, 115/85, and 135/100 at 40, 60, 80, and 110 mm Hg perfusion pressure, respectively. Peak ventricular pressures at these perfusion pressures were 50, 84, 100, and 120 mm Hg. Diastolic pressures were 2, 4, 8, and 12 mm Hg, respectively.](image)

**TABLE 2. Effect of left atrial filling pressure on cardiac output, aortic pressure, external work, pressure time integral, O₂ consumption, and efficiency of working rat heart**

<table>
<thead>
<tr>
<th>Left Atrial Pressure, mm Hg</th>
<th>Exogenous Substrate, mM</th>
<th>Heart Rate, beats/min</th>
<th>Cardiac Output, ml/min per g</th>
<th>Coronary Flow, ml/min per g</th>
<th>Aortic Pressure, mm Hg</th>
<th>Aortic Work, kg·m/min per g</th>
<th>Effluent O₂ Tension, mm Hg</th>
<th>Efficiency, %</th>
<th>Pressure-Time Integral, mm Hg sec/min kg</th>
<th>O₂ Consump., mmole/hr per g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None (12)</td>
<td>24 ± 13</td>
<td>63 ± 3</td>
<td>49 ± 4</td>
<td>63 ± 0</td>
<td>49 ± 1</td>
<td>0.2 ± 0</td>
<td>121 ± 10</td>
<td>0.21 ± 0.02</td>
<td>22 ± 10</td>
</tr>
<tr>
<td></td>
<td>Glucose (12)</td>
<td>250 ± 13</td>
<td>60 ± 4</td>
<td>60 ± 4</td>
<td>58 ± 4</td>
<td>48 ± 3</td>
<td>0.3 ± 0</td>
<td>113 ± 12</td>
<td>0.20 ± 0.02</td>
<td>20 ± 12</td>
</tr>
<tr>
<td></td>
<td>Acetate (6)</td>
<td>207 ± 25</td>
<td>51 ± 4</td>
<td>51 ± 4</td>
<td>66 ± 4</td>
<td>44 ± 3</td>
<td>0.4 ± 0</td>
<td>115 ± 15</td>
<td>0.21 ± 0.02</td>
<td>24 ± 15</td>
</tr>
<tr>
<td>5</td>
<td>None (6)</td>
<td>242 ± 13</td>
<td>105 ± 8</td>
<td>53 ± 2</td>
<td>75 ± 8</td>
<td>44 ± 3</td>
<td>0.8 ± 0</td>
<td>127 ± 17</td>
<td>0.32 ± 0.03</td>
<td>26 ± 17</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>255 ± 17</td>
<td>71 ± 7</td>
<td>77 ± 3</td>
<td>73 ± 7</td>
<td>47 ± 3</td>
<td>0.9 ± 0</td>
<td>125 ± 15</td>
<td>0.33 ± 0.04</td>
<td>26 ± 15</td>
</tr>
<tr>
<td></td>
<td>Acetoacetate (5)</td>
<td>240 ± 22</td>
<td>95 ± 15</td>
<td>69 ± 4</td>
<td>72 ± 4</td>
<td>47 ± 3</td>
<td>0.7 ± 0</td>
<td>122 ± 17</td>
<td>0.32 ± 0.03</td>
<td>25 ± 17</td>
</tr>
<tr>
<td></td>
<td>Acetate</td>
<td>214 ± 21</td>
<td>100 ± 9</td>
<td>64 ± 3</td>
<td>90 ± 5</td>
<td>37 ± 3</td>
<td>0.9 ± 0</td>
<td>129 ± 25</td>
<td>0.39 ± 0.04</td>
<td>27 ± 18</td>
</tr>
<tr>
<td>10</td>
<td>None (12)</td>
<td>234 ± 14</td>
<td>191 ± 12</td>
<td>69 ± 4</td>
<td>95 ± 13</td>
<td>43 ± 4</td>
<td>0.8 ± 0</td>
<td>122 ± 17</td>
<td>0.29 ± 0.03</td>
<td>24 ± 17</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>244 ± 18</td>
<td>134 ± 14</td>
<td>78 ± 4</td>
<td>94 ± 14</td>
<td>46 ± 5</td>
<td>0.7 ± 0</td>
<td>125 ± 15</td>
<td>0.34 ± 0.03</td>
<td>26 ± 17</td>
</tr>
<tr>
<td></td>
<td>Acetoacetate (5)</td>
<td>260 ± 22</td>
<td>195 ± 15</td>
<td>75 ± 5</td>
<td>110 ± 6</td>
<td>39 ± 6</td>
<td>0.7 ± 0</td>
<td>124 ± 17</td>
<td>0.29 ± 0.03</td>
<td>23 ± 17</td>
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<tr>
<td></td>
<td>Acetate</td>
<td>200 ± 22</td>
<td>195 ± 15</td>
<td>73 ± 4</td>
<td>110 ± 2</td>
<td>35 ± 4</td>
<td>0.6 ± 0</td>
<td>123 ± 17</td>
<td>0.28 ± 0.03</td>
<td>22 ± 17</td>
</tr>
<tr>
<td>20</td>
<td>None (12)</td>
<td>233 ± 15</td>
<td>260 ± 21</td>
<td>79 ± 4</td>
<td>110 ± 16</td>
<td>37 ± 4</td>
<td>0.9 ± 0</td>
<td>124 ± 10</td>
<td>0.26 ± 0.03</td>
<td>28 ± 10</td>
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<tr>
<td></td>
<td>Glucose</td>
<td>260 ± 18</td>
<td>318 ± 22</td>
<td>106 ± 5</td>
<td>116 ± 10</td>
<td>35 ± 5</td>
<td>0.9 ± 0</td>
<td>124 ± 10</td>
<td>0.26 ± 0.03</td>
<td>28 ± 10</td>
</tr>
<tr>
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<td>1.0 ± 0</td>
<td>125 ± 16</td>
<td>0.30 ± 0.03</td>
<td>29 ± 12</td>
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<tr>
<td></td>
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<td>299 ± 20</td>
<td>77 ± 4</td>
<td>126 ± 8</td>
<td>34 ± 6</td>
<td>0.8 ± 0</td>
<td>124 ± 15</td>
<td>0.28 ± 0.03</td>
<td>27 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± se. Hearts were perfused with buffer containing substrates listed in the table. The buffer passed through the coronary vessels a single time and was collected from the pulmonary artery cannula for estimation of oxygen tension. Each atrial filling pressure was maintained for 3 min prior to making the measurements described in the table. Each heart was perfused at each atrial pressure at random. Oxygen tension of buffer entering the heart averaged 614, 531, 660, and 534 mm Hg for medium containing no substrate, glucose, acetoacetate, and acetate, respectively. The dry heart weights averaged 0.226, 0.199, 0.218, and 0.232 g for hearts perfused with buffer containing no substrate, glucose, acetoacetate, and acetate, respectively. The number of hearts in each group is indicated in parentheses.
cardiac output rose progressively regardless of the substrate present in the buffer (Table 2). At 0 cm, nearly all the cardiac output passed through the coronary circulation; at 20 cm, 30% of cardiac output was accounted for by coronary flow. As the quantity of fluid that was pumped increased, peak systolic aortic pressure rose, and diastolic aortic pressure fell from an average of 48 mm Hg at 0 cm to 36 mm Hg at 20 cm H$_2$O atrial pressure. The conformations of the aortic and ventricular pressure curves were similar to those observed in vivo (Fig. 3). Peak systolic ventricular pressure was 2-4 mm Hg higher than the comparable aortic pressure. Left ventricular end diastolic pressure was approximately equal to left atrial pressure.

Heart work, calculated from measurements of cardiac output and aortic pressure, increased linearly as atrial pressure was raised. The pressure-time integral approximately doubled as atrial filling pressure was increased from 0 to 20 cm H$_2$O. Oxygen consumption doubled as atrial pressure was varied over this range. Oxygen consumption was linearly related to coronary flow, pressure-time integral, and peak systolic pressure with correlation coefficients of 0.80, 0.84, and 0.76, respectively. Effluent oxygen tension fell from an average of 252 mm Hg at 0 cm H$_2$O left atrial pressure.

Heart work, calculated from measurements of cardiac output and aortic pressure, increased linearly as atrial pressure was raised. The pressure-time integral approximately doubled as atrial filling pressure was increased from 0 to 20 cm H$_2$O. Oxygen consumption doubled as atrial pressure was varied over this range. Oxygen consumption was linearly related to coronary flow, pressure-time integral, and peak systolic pressure with correlation coefficients of 0.80, 0.84, and 0.76, respectively. Effluent oxygen tension fell from an average of 252 mm Hg at 0 cm H$_2$O left atrial pressure.

Heart work, calculated from measurements of cardiac output and aortic pressure, increased linearly as atrial pressure was raised. The pressure-time integral approximately doubled as atrial filling pressure was increased from 0 to 20 cm H$_2$O. Oxygen consumption doubled as atrial pressure was varied over this range. Oxygen consumption was linearly related to coronary flow, pressure-time integral, and peak systolic pressure with correlation coefficients of 0.80, 0.84, and 0.76, respectively. Effluent oxygen tension fell from an average of 252 mm Hg at 0 cm H$_2$O left atrial pressure.
cm H₂O to 189 mm Hg at 20 cmH₂O. External efficiency, calculated from measurements of work and oxygen consumption, increased from 4 to 15% over the range of atrial pressure. The mechanical performance and oxygen consumption were similar whether the buffer contained no substrate, glucose, acetate, or acetoacetate. These observations indicated that the perfused rat heart could be induced to do controlled quantities of external work when perfused in vitro. The quantity of work performed at 20 cm H₂O atrial pressure was approximately equal to that reported for the rat heart in vivo by Beznak (4).

Stability of performance of heart preparations. Hearts were perfused for 3 hr at 10 cm H₂O left atrial filling pressure (Table 3). Heart rate varied about 10% during the experiment. Cardiac output was 158 ml g⁻¹ initially and 147 ml after 3 hr. Aortic pressure development was well maintained. Total heart work decreased from 18 to 16 kg-m g⁻¹ min⁻¹ and oxygen consumption fell from 2.22 to 1.73 mmol g⁻¹ hr⁻¹ after 3 hr. Efficiency of heart work increased from 11 to 12%. These observations indicated that performance of hearts working at 10 cm H₂O left atrial pressure was stable for 3 hr. Progressive deterioration of mechanical function was observed during the 4th and 5th hr of perfusion. When left atrial pressure was increased to 20 cm H₂O, performance was stable for 1-1.5 hr. The Langendorff preparation perfused at a pressure of 60 mm Hg maintained a constant heart rate and coronary flow for periods in excess of 4 hr.

Effect of heart size on mechanical performance and oxygen consumption of the working heart. Since ventricular volume is not a linear function of heart weight, stroke volume did not change in proportion to weight. As seen in Table 4, an increase in dry heart weight from 0.13 to 0.24 g resulted in a 60% increment in stroke volume. Since the perfusion apparatus had a constant outflow resistance, peak systolic aortic pressure increased as stroke volume rose. Heart rate was slower, however, in the larger hearts. The combination of these changes resulted in a 25% increase in cardiac output and work as heart weight increased from 0.13 to 0.24 g when expressed per heart. When expressed per gram of dry tissue, both cardiac output and work fell about 40% over this weight range. The pressure-time integral decreased as size increased because the decrease in heart rate was proportionately more than the increase in peak systolic pressure. No relationship between pressure-time integral and oxygen consumption was observed when the results were expressed per heart. When expressed per gram of dry tissue, oxygen consumption correlated well with the pressure-time integral.

These observations demonstrate the importance of using hearts of comparable size for studying the metabolic effects of heart work. This conclusion also applied to the Langendorff preparation. When ventricular pressure was measured in hearts of this range of size, peak systolic pressure rose to a value slightly above aortic pressure regardless of size. Since small hearts beat at a faster rate, the pressure-time integral of these hearts was greater than that of larger hearts. The oxygen consumption of the small heart was less when expressed per heart.

Correlation of mechanical function and oxygen consumption. Attempts to correlate mechanical activity and oxygen consumption of the heart have resulted in several empirical relationships. Inability to measure, in physical terms, the energy consumed in development of tension accounts for the use of other parameters of mechanical function. External work, calculated from pressure development and cardiac output, correlated poorly with oxygen consumption in dog hearts (11, 29). As seen in Table 5, a similar lack of correlation could be demonstrated in the rat heart. In Experiment A, heart work was increased by simultaneously raising left atrial pressure from 0 to 20 cm H₂O and compressing the tube leading to the aortic bubble trap. As a result of these manipulations, the hearts were not allowed to eject a greater volume of fluid, but did generate greater aortic pressure. A doubling of external work, under these conditions, increased oxygen consumption 65%. When the pressure-time integral was related to oxygen consumption, a correlation co-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Avg Heart Wt, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>Stroke vol, μl</td>
<td>123±12</td>
</tr>
<tr>
<td>Heart rate, beats/ min</td>
<td>38±7 ±18</td>
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<td>Aortic pressure, mm Hg</td>
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<tr>
<td>Peak systolic</td>
<td>38 ±1</td>
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<tr>
<td>Diastolic</td>
<td>13 ±1.5 (100)</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td>36 ±2 (273)</td>
</tr>
<tr>
<td>Total cardiac output, ml/min</td>
<td>0.32 ±0.004 (24)</td>
</tr>
<tr>
<td>Work, kg-m/min</td>
<td>1.53 ±0.05</td>
</tr>
<tr>
<td>Pressure time integral, mm Hg</td>
<td>0.39 ±0.02 (5.9)</td>
</tr>
<tr>
<td>O₂ uptake, mmol/ hr</td>
<td></td>
</tr>
</tbody>
</table>

Hearts were perfused with buffer containing 5.5 mM glucose at 10 cm H₂O atrial pressure. The buffer passed through the heart a single time and was collected from the pulmonary artery cannula for estimation of oxygen tension. Numbers in parentheses represent the value for the parameter expressed per gram dry tissue. Three hearts were perfused.
The perfused rat heart preparation has been modified to allow for metabolic studies over a range of ventricular pressure development. Pressure development was increased either by raising the perfusion pressure of the modified Langendorff preparation or left atrial filling pressure of a preparation doing external work. The pressure-time integral increased approximately 2.5-fold. Significant improvements in the stability of the preparation were also achieved. Lorber (25) described a characteristic fall in efficiency of isolated cat hearts as failure developed. By this criterion, rat hearts perfused by the present method were not in cardiac failure after 3 hr. In fact, efficiency had increased slightly at the termination of perfusion.

Several changes in perfusion technique appear to have been responsible for the improved stability. 1) Ca EDTA was included in all buffers to chelate trace quantities of heavy metals in reagents and water. 2) Heparin was added to the buffer used for the initial perfusion of the heart to prevent the formation of thrombi within the vascular bed. 3) Perfusion pressure was increased to 60 mm Hg to improve coronary flow. 4) Preliminary perfusion of the heart was lengthened to 10 min and a larger volume of buffer was recirculated. These procedures reduced the quantity and final concentration of protein in the perfusate arising from the extracellular fluid of the heart. Oxygenation of the buffer by bubbling gas through it denatures protein and leads to formation of emboli. When bubbling was omitted, oxygen tensions above 500 mm Hg were found in arterial perfusates of the Langendorff preparation at all perfusion pressures and in arterial perfusates of the working heart at 0 and 5 cm Hg atrial pressure. Bubbling was especially deleterious when hormones or other proteins were added to the buffer. Relative importance of these changes or other subtle improvements in technique cannot be evaluated, but the over all improvement in the preparation has been dramatic.

Energy requirements of the heart can be considered to consist of 1) that portion of chemical energy utilized in maintenance of the completely quiescent tissue and 2) that portion of chemical energy converted to mechanical preparation did not change as perfusion pressure was varied. As seen in Fig. 4, oxygen consumption correlated well with peak systolic pressure and pressure-time integral of either working or Langendorff preparations. Since the slopes of regression lines relating peak systolic pressure and pressure-time integral to oxygen consumptions of working and Langendorff preparations were not significantly different (P > 0.5), a heart consumed the same amount of oxygen whether or not it was pumping fluid. From the regression line, a heart developing zero pressure was estimated to require from 0.4 to 1.6 mmoles O_2/hr per g for tissue maintenance. These observations indicated that pressure development correlated well with oxygen consumption in either preparation.

**DISCUSSION**

**TABLE 5. Effects of cardiac output and pressure development on O_2 consumption of working hearts**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment A (changes in cardiac output minimized)</th>
<th>Experiment B (changes in aortic pressure minimized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac output, ml/min per g</td>
<td>124 ± 20</td>
<td>142 ± 13</td>
</tr>
<tr>
<td>Aortic pressure, mm Hg</td>
<td>116 ± 5</td>
<td>128 ± 6</td>
</tr>
<tr>
<td>Peak systolic</td>
<td>87 ± 3</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>Diastolic</td>
<td>160 ± 6</td>
<td>131 ± 7</td>
</tr>
<tr>
<td>Work, kg-m/min per g</td>
<td>1.7 ± 6</td>
<td>2.9 ± 6</td>
</tr>
<tr>
<td>O_2 consumption, mmoles/hr per g</td>
<td>1.60 ± 20</td>
<td>2.36 ± 20</td>
</tr>
</tbody>
</table>

Experiment is described in text. Four hearts were perfused and used for both experiments A and B. Average heart weight was 0.236 g.

Table 5: Effects of cardiac output and pressure development on O_2 consumption of working hearts.
energy. Extrapolation of the relationships between oxygen consumption and pressure development to zero pressure development indicated that the quiescent heart consumed about 0.70 mmoles O₂/g per hr (Fig. 4).

There have been many attempts to determine the physical parameter which best represents the rate of ATP splitting concerned with development of mechanical energy by the heart and which would, therefore, best correlate with oxygen consumption. The first question that must be answered when choosing a parameter to correlate with oxygen consumption is whether oxygen consumption is limited by delivery of oxygen to the cells or by contractile activity of the muscle. High efficient oxygen tensions found in the present study support the conclusion that oxygen supply was adequate. In earlier experiments, Fisher and Williamson found that oxygen consumption remained constant when coronary flow was increased in rat hearts by perfusing with erythritol tetranitrate (14). In addition, these workers have shown that the Langendorff preparation perfused in the presence of 10⁻³ M dinitrophenol could reduce the effluent oxygen tension to very low values without a change in coronary flow. The observation that working hearts extract more oxygen at the same coronary flow than the Langendorff preparation also indicated that coronary flow per se was not determining oxygen consumption.

Correlation of several parameters of mechanical energy output with oxygen consumption was investigated. This correlation was sought with two purposes in mind. First, a parameter of mechanical function that could be directly related to oxygen consumption would be convenient for later studies of substrate utilization. Second, the present experiments may demonstrate that some of the relationshipships between mechanical function and oxygen consumption that have been described for hearts of other species apply to the completely isolated working rat heart.

It has long been recognized that the heart is more efficient if pumping fluid against a low resistance than if pumping against a high resistance (12). An example of this was described in the present study by showing that a twofold increase in cardiac output and external work, associated with a decrease in mean aortic pressure, was performed with little or no increase in oxygen utilization. On the other hand, elevated aortic pressure in association with a constant cardiac output resulted in a doubling of oxygen consumption. Also, results summarized in Fig. 4 show that for a given developed pressure a working heart consumed the same amount of oxygen as the Langendorff preparation, even though the working heart pumped more fluid and did external work. These findings confirm that cardiac output and work are poor indicators of the rate of energy consumption.

Parameters related to pressure development by the heart correlated well with oxygen consumption. Correlation coefficients relating oxygen utilization to peak systolic pressure and pressure time integral of working and Langendorff preparations varied from 0.76 to 0.86. Since these parameters are easily measured, they appear to be convenient indicators of the energy utilized for contraction.

If end-diastolic ventricular pressure is used as an index of end-diastolic volume, increased development of pressure in hearts perfused with either increasing perfusion pressure or left atrial pressure appeared to be explained by increased fiber length. Diastolic ventricular pressure increased from 2 to 12 mm Hg in the Langendorff preparation as perfusion pressure was raised from 40 to 120 mm Hg (Table 6). End-diastolic ventricular pressure in working hearts was approximately equal to left atrial filling pressure over the range from 0 to 20 cm H₂O. Correlation coefficients relating oxygen consumption to end-diastolic pressure were 0.74 for working hearts and 0.73 for Langendorff preparations. The relationship between fiber length and contractile force was originally described by Frank (16) and Starling (32) and has been recently reinterpreted as a relationship between sarcomere length and contractile force (31).

Fisher and Williamson demonstrated that oxygen consumption of the nonworking heart was only slightly altered whether hearts were perfused with buffer containing no substrate, glucose, succinate, acetoacetate, or β-hydroxy butyrate (15). Recently, Challoner and Steinberg have presented evidence that oxygen consumption was increased when the perfusate contained palmitate (7). In the present study, oxygen consumption and cardiac work were generally unaltered over the range of atrial pressures that were imposed when either 5 mM glucose, acetoacetate, acetate, or no exogenous substrate were provided. An exception to this generalization were hearts perfused with 5 mM glucose at high atrial pressure where oxygen uptake was slightly lower, apparently as a
result of less tension development. Hearts perfused with no exogenous substrate have been shown to utilize tri-
strates or a variety of exogenous substrates can be readily
oxidized to support contractile activity.

These findings indicate that either endogenous sub-
strates or a variety of exogenous substrates can be readily
oxidized to support contractile activity.

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