Oxygen Consumption and Tension of Isolated Heart Muscle During Rest and Activity Using a New Technic

By William J. Whalen, Ph.D.

A new method is described by which the relationship between length, tension, rate of contraction and oxygen consumption can be studied in masses of cardiac tissues weighing as little as 2 mg. (dry weight). The results presented suggest that satisfactory physiologic conditions prevail.

In our studies on cardiac efficiency, it was essential to record simultaneously the length, tension, rate of contraction and oxygen consumption of isolated cardiac muscle. Available technics1,2 were inappropriate and a new method was devised. Emphasis in this paper is on the description and testing of the technic and the differences in QO2 during rest and activity with and without added epinephrine.

Methods

Figure 1 presents a labeled diagram of the apparatus in the operating position with one of the 3 chambers submerged almost to the incite floor (Q) of the water bath. The chamber is shown cut-away to reveal inner structures. The basic material used for the chamber was molded epoxy resin (Epocast)* which is an inert, hard but machinable plastic. The total volume of the system is about 13 ml. including the manometer and connecting line. When 8 ml. of media are used and with 2,2,4-trimethylpentane (specific gravity 0.7) as the manometer fluid, the 3 chamber constants are 0.35, 0.37 and 0.41.

For the preliminary testing of the chambers the excised, spontaneously beating rat atria3 and the rat diaphragm were used. Later experiments utilized the cat papillary4 and the rat ventricle strip.5 The media used are listed in Table 1. With the bicarbonate-buffered media, diethanolamine solution,6 stabilized with 0.1 per cent thiourea,7 was the CO2 absorber. Potassium hydroxide KOH (20 per cent) was used with the phosphate-buffered media.4

Before the tissues were prepared, three uncovered chambers were partly submerged in the lucite-walled, constant temperature bath maintained at 37.3 ± 0.02 C. The medium was pipetted into the chamber and two strips of fluted filter paper (0.3 x 7 cm. before folding) were soaked in the absorber solution and placed in the absorber cup (I). A temporary cover (not shown) was placed over the chamber and the aerator valve (N) opened, permitting gas flow through the sintered glass disc (O). A ridge of grease was applied at the outer edge of the flange (H).

The excised strip of muscle (M) was tied to one end to the pin at the bottom of the muscle holder (F), and at the other end to monofilament nylon thread (C) which had been previously inserted through the mercury seal (G) in the cover. The temporary cover was removed and the regular cover fitted on the chamber, which brought the muscle into place over the aerator as shown in figure 1. Spring clips held the top in position and insured a perfect seal. The thread (C) was tied to the strain gage (B) which is mounted on a micrometer stage. Stimulation of the muscle is accomplished by means of paired platinum electrodes (L) sealed into the wall of the vessel. The electrodes in all three chambers were connected in series to assure the same current flow in each. Two chambers contained tissue, and the third served as the thermobarometer and as a control for absorption of oxygen by the diethanolamine in the Fardel's solution.6

The chambers were submerged almost to the bottom of the bath directly over the Mag-Mix under the bath. Tension on the muscle was adjusted to 0.5 Gm. and the contractions were recorded on an Offner oscillograph until a steady state was attained. The aerator valve (N) was then closed, the Mag-Mix (R) turned on, activating the Alnico magnetic stirrer (K) in the chamber, and the manometer stopcock closed. After an equilibration period of 30 min., manometer readings were begun.

When a drug was to be added, an oiled 1 ml. tuberculin syringe was attached to the B-D stopcock on the external end of the drain tube (not shown). The drain valve was opened while observing the manometer column, and about 0.2 ml. of fluid was drawn into the syringe. Injections of 0.005 to 0.05 ml. of the drug were made into the rubber tube section of the drain line by means of a microsyringe (Aloe). Sufficient fluid from the tuberculin syringe was then reinjected into the chamber to return the manometer column to its original position and the

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* Furano Plastics Inc., Los Angeles, Calif.
TABLE 1.—Media Used in Current Experiments in mM/L.

<table>
<thead>
<tr>
<th>Solution</th>
<th>In equilibrium with</th>
<th>For tissues</th>
<th>Membrane</th>
<th>For tissues</th>
<th>Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>98% O₂- 2% CO₂</td>
<td>rat papillary, rat atria</td>
<td>cat papillary</td>
<td>rat ventricle</td>
<td>rat diaphragm</td>
</tr>
<tr>
<td>Na⁺</td>
<td>122</td>
<td>154</td>
<td>161</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>1.8</td>
<td>5.6</td>
<td>5.6</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Ca ++</td>
<td>1.8</td>
<td>1.08</td>
<td>5.6</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Mg ++</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>145</td>
<td>161</td>
<td>170</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>HPO₄⁻ and H₂PO₄</td>
<td>0.36</td>
<td>0.50</td>
<td>0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>SO₄</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>7.1</td>
<td>0</td>
<td>7.1</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Preliminary Tests of Chambers. As a means of crudely estimating the “circulation time” of the chambers, they were filled with 8 ml. of fluid, placed in the operating position over the Mag-Mix, and a drop of dye introduced into the uncovered vessel. The complete dispersion of the dye was almost instantaneous indicating that circulation was rapid and left no “pockets” of unstirred media.

Eight experiments were conducted to determine adequacy of the CO₂ absorption of the Pardee’s solution. The chambers were readied as outlined except that instead of the physiologic media, a solution of bicarbonate was used which was calculated to yield 110 to 120 mm. of CO₂ when acidified. About half of the released gas was absorbed before any readings could be taken, and equilibrium was practically complete in 5 to 6 min. From 5 to 10 per cent of the CO₂ is not absorbed even after 15 min.; which agrees with the calculations of Krebs.

In 4 experiments using KOH as the CO₂ absorber, all of the CO₂ was taken up in 5 min., otherwise the curves were similar. These results essentially confirm the original observations of Pardee. The agreement of the results using the 2 absorbers was sufficiently close in our experiments to warrant use of the diethanolamine, particularly because of the importance of the bicarbonate ion in muscular contraction.

Another type of test of the absorption characteristics of the chambers using diethanolamine absorber is shown in figure 2. It is apparent that, for the rat diaphragm, readings taken at 3 min. intervals can be used to obtain a fairly reliable estimate of the QO₂. Also the lag in the system must be less than 6 min. since the control level was approximated during the second 3 min. interval after stimulation. The first period after stimulation was significantly above the control level but interpretation is confounded by the possibility that the
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Fig. 2. Oxygen consumption of 4 rat diaphragm strips during rest (open blocks) and during stimulation at 120/min. (solid blocks). Cross-hatched areas represent ±1 S.E. The 100 per cent level is the QO₂ of these strips during the previous hour which was 6.7 ± 0.72.

difference may be due in part to an "oxygen debt."

To provide evidence on whether the oxygenation and stirring in our system was such that it would not be a limiting factor in performance, experiments were carried out in which the isolated, spontaneously beating rat atria was alternately gassed then stirred. In every instance both the rate and amplitude of the contractions were greater during the period of stirring alone, than during the periods when the tissue was maximally gassed in the conventional manner (fig. 3). It was concluded that, at least temporarily, stirring created a more favorable environment for the rat atria than gassing.

Information concerning the long term effects of stirring as opposed to gassing in unchanged media was obtained by comparing rate of decrease in activity with stirring as seen in figure 3 with rate of decline observed when 10 rat atrial preparations were continually gassed. The slope of the curves for rate and amplitude was similar for the 2 series of experiments but with stirring alone the absolute values were always significantly higher. As for the duration of activity, many of the preparations were active for 12 hours or more in the unchanged solution without additionally gassing.

Further confirmation of the validity of the method was sought in the comparison of our values for QO₂ with those of others. Previous investigators using the Warburg technic have reported a rather wide range of mean values for in vitro oxygen consumption of rat atrial muscle. Muus et al.⁹ and Olson et al.¹⁰ found QO₂'s of 15.7 and 14.77 respectively. Presumably the atria were beating spontaneously. Webb et al.¹¹ using rat atria, which may not have been spontaneously contracting, found much lower values of 4 to 5. Ransom and Loomis¹² reported that the active rat atria had a QO₂ of about 5.9. Thus, the oxygen consumption of atrial tissue as measured by us (mean of 13.2 for the first hour—fig. 3) is in the upper range of the reported values. This finding is somewhat surprising in view of the fact that Pearson and associates¹³ showed that the QO₂ of rat ventricle slices fell from 12.0 to 5.8 when the phosphate concentration was reduced from 20 mM/L to 2.5 mM/L. In our studies none of the solutions contained more than 1.2 mM/L of phosphate.

For a more direct comparison with the results of Pearson et al. the rat ventricle slice was studied.⁶ Both the solutions of Gardner et al.² and Feigen et al.⁵ were employed in this series and, since no significant differences in QO₂ were found, the data were pooled. The mean resting QO₂ of 14 strips was 7.6 ± 0.69, which is also higher than expected in view of the phosphate concentration.

Obviously, there are several possible explanations for the high rate of oxygen uptake with low phosphate, but they were not of immediate
Table 2.—Mean and Standard Error of $Q_O_2$'s Obtained During Rest and Activity

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Media</th>
<th>Rest*</th>
<th>Stimulus (60/min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat papillary muscle</td>
<td>CG</td>
<td>(15)</td>
<td>3.4±.44</td>
</tr>
<tr>
<td>Cat papillary muscle</td>
<td>GWF</td>
<td>(11)</td>
<td>2.6±.27</td>
</tr>
<tr>
<td>Left rat atria</td>
<td>GWF</td>
<td>(7)</td>
<td>7.7±.95</td>
</tr>
</tbody>
</table>

* Numbers in parentheses equal the number of experiments.

Concern. The feature of special interest is the relatively high and steady state of the respiratory activity. The muscle in this series of experiments was alternately stimulated and rested at hourly intervals for a total of 4 hours. The periods were criss-crossed on alternate days. Table 2 presents the results of the series using cat papillary muscle and the isolated left atria of the rat. These unpaired data do not show a significant difference between rest and activity. With paired values, however, a significant difference was found.

The comparatively low $Q_O_2$ for the cat papillary muscle was an unexpected finding. Lee obtained a mean of 3.3 during rest and 5.5 during activity using the same media and the same stimulation rate. Thus, the cat papillary data are at variance with the data for the other tissues studied, all of which gave high values with reference to reports in the literature. The discrepancy is, at present, unexplained. It may be that differences in the tension placed on the muscle are partly responsible. Experiments are now in progress which will test this suggestion.

The low values cannot be explained on the basis of differences in muscle size since, mindful of Lee's calculations of maximal thickness, only those muscles were used which had a maximum diameter not greater than 1.2 mm. Therefore, oxygen diffusion should have been ample. Two muscles which approached the limiting thickness were eliminated from consideration because they showed a decrease in contraction strength greater than 50 per cent.

Table 3.—Effects of Epinephrine on $Q_O_2$ and Isometric Contractions of Cat Papillary in Per Cent of Control Level, Muscle not Stimulated During Periods in Italics.

<table>
<thead>
<tr>
<th>Exp.*</th>
<th>QO$_2$ (min. after injection)</th>
<th>Contractions (min. after injection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-10</td>
<td>10-20</td>
</tr>
<tr>
<td>2 a</td>
<td>1.0</td>
<td>817</td>
</tr>
<tr>
<td>27 a</td>
<td>.2</td>
<td>315</td>
</tr>
<tr>
<td>20 a</td>
<td>.1</td>
<td>813</td>
</tr>
<tr>
<td>b</td>
<td>.1</td>
<td>600</td>
</tr>
<tr>
<td>31 a</td>
<td>.1</td>
<td>199</td>
</tr>
<tr>
<td>b</td>
<td>.1</td>
<td>199</td>
</tr>
<tr>
<td>38 a</td>
<td>.1</td>
<td>270</td>
</tr>
<tr>
<td>30 a</td>
<td>.1</td>
<td>180</td>
</tr>
<tr>
<td>b</td>
<td>.1</td>
<td>201</td>
</tr>
<tr>
<td>47 a</td>
<td>.1</td>
<td>161</td>
</tr>
<tr>
<td>b</td>
<td>.1</td>
<td>180</td>
</tr>
</tbody>
</table>

* a = first injection, b = second injection.

Otherwise the muscles responded normally as judged by contraction height and stimulation threshold.

Effects of Epinephrine. That epinephrine increases oxidative metabolism is well known, but the mechanism has not been clarified. Nor is it known how the extra energy produced is utilized to increase the rate and strength of contraction. The following experiments were initiated as a preliminary to our studies on the mechanism of action of epinephrine, to provide further indirect evidence that oxygen diffusion was not a limiting factor in the technic and to test the method of injection. Epinephrine was injected into the chambers during rest and during stimulation at 60/min. in criss-cross fashion using a "crossover" experimental design. At least 30 min. elapsed between injections on the same strip. The same quantity of epinephrine was also injected into the control chamber in most of the experiments.

Most conspicuous among the data in table 3 on the effects of epinephrine is the high oxygen consumption during the first 10 min. interval following addition of epinephrine regardless of the concentration used. The outcome of the larger doses appeared to be largely a matter of the persistence of the high oxygen uptake and increased contractions throughout the 30 min. test period. In a few experiments readings were
taken 5 min. after the injection. These readings showed even higher oxygen uptakes, exceeding a $Q_O^2$ of 24 in one experiment. By way of comparison, calculation of Lorber’s data from the blood-perfused cat heart performing light work gives an approximate $Q_O^2$ of 15 based on 4.6 to 1 wet weight, dry weight ratio.

Lee’s studies on the cat papillary found that increases in $Q_O^2$ during the first 5 min. in 1 to 10 million epinephrine* averaged about 170 per cent of the control level. This increase is less than that found in the present investigation for reasons not obvious at present. The present series of experiments do demonstrate, however, that oxygen is available to the tissues in excess of that required for normal function.

With regard to the relation between oxygen consumption and contractions, after epinephrine, there are hints in this preliminary series which suggest a decreased efficiency with epinephrine. For example, during rest, $Q_O^2$ is approximately doubled, and often the increase in oxygen consumption during stimulation was much larger than the increase in contractile force.

**SUMMARY**

A technic has been described which makes possible the simultaneous recording of oxygen consumption and contractions in isolated cardiac muscle in bicarbonate media. Oxygenation is accomplished by violent stirring of the medium using a magnetic stirrer. Rat atria, rat ventricle, and cat papillary muscle, as well as rat diaphragm were studied. The contractions were well maintained and, with the exception of the cat papillary, the $Q_O^2$ obtained were in the high range of reported values. It is concluded that oxygenation is at least as adequate as provided by gassing or by shaking. Epinephrine increased the $Q_O^2$ of active cat papillary as much as 8 fold which suggests that oxygen was available in amounts approaching the in vivo situation. Epinephrine also markedly increased resting oxygen consumption. This new technic should be useful in revealing interrelations of the chemical and mechanical events in all types of muscle.

**SUMMARIO IN INTERLINGUA**

Es describite un technica que rende possibile le registration simultanee del consumption de oxygeno e del contractiones in isolate musculos cardiac mantenite in medios a bicarbonato. Le oxygenation es effectuate per violenta agitacion del medio con un agitator magnetic. Esseva studiate atrios de ratto, ventriculos de ratto, e musculos papillar de catto e etiam diaphragmas de ratto. Le contractiones esseva ben mantenite, e—con le exception del valores pro musculos papillar de catto—le $Q_O^2$ obtenite esseva in omne casos in le area superior del valores reportate. Es conclutite que le oxygenation es al minus tanto adequate como illo effectuate per gasar o succucer le medio. Epinephrina augmentava le $Q_O^2$ del musculo papillar de catto per usque a 700 pro cento, lo que pare indicar que oxygeno esseva disponibile in quantitates proxime al nivello del situation in vivo. Epinephrina etiam augmentava marcatemente le consumption de oxygeno in stato de reposo. Il es a previdere que iste technica va provar se utile in clarificar le interrelaciones del eventos chimic e mechanic in omne typos de musculo.

**ACKNOWLEDGMENT**

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