Observations on the Heart-Lung Preparation in the Rat

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THE RAT heart-lung preparation has, in the past, been very little used by physiologists, largely because the small physical dimensions have presented great difficulties in the way of accurate pressure and flow measurements. Cruickshank and Kosterlitz1 and Cruickshank2 employed the rat heart-lung preparation for the investigation of substrate metabolism in the heart and the effects of positive and negative pulmonary ventilation, but no close study of cardiac dynamics was made. Begovic and Stern3 produced the preparation under hypothermia. Bubnoff and associates,4 utilizing the technique of Begovic and Stern to produce the heart-lung preparation, widened the observations upon it by direct cannulation of the right atrium and by measurement of flow through the use of a bubble flowmeter. Kukovetz5 also employed the technique of Begovic and Stern, and measured output with a device resembling a water mill. Malinow and associates6 described a technique of preparing the rat heart-lung, but reported no observations upon it. Pietra and Minelli7 also described a technique, and measured flow with a mechanical drop counter. Solomon and associates8,9 developed a technique for preparing a heart-lung preparation in which the lungs were allowed to collapse and oxygenation was carried out by an external oxygenator; this modification was considered to extend survival of the preparation through the avoidance of pulmonary edema.

It would not appear that a close study has been made, in the papers referred to above, of the influence of certain variables on the relationship between right-atrial pressure and cardiac output. This paper reports a study which was undertaken as a preliminary to experiments on cardiac dynamics in nephrectomized animals,10 in order to define more clearly the influence of such variables on the heart-lung preparation. Aising out of the study of cardiac dynamics in nephrectomized animals, a more detailed investigation of the effects of sodium and potassium on the heart-lung preparation was carried out and is also reported in the present paper.

Methods

Animals

Female rats of an inbred Wistar strain were employed when they were in the weight range of 180 to 300 Gm. In later work the weight range was narrowed to 180 to 220 Gm.

Apparatus (Figure 1)

A perspex tank was constructed to carry a brass operating tray below which water constantly circulated. Included in the tank was a small reservoir of perspex containing blood (4 to 8 ml.) which was an integral part of the extracorporeal circulation. The water in the tank was maintained at 37 C. and vigorously circulated. When the extracorporeal circulation was established, a lid was placed over the tank which thus enclosed the preparation and all connecting tubing in an atmosphere saturated with water vapor.

All tubing was of either silicone rubber or perspex. The variable resistance (R) on the arterial side was of the standard Starling type; in many experiments, a length of very thin-walled, silicone-rubber tubing (D) was placed proximal to the resistance to damp the pulse wave. Blood passed from the variable resistance directly to the perspex reservoir (VR) and thence through an electromagnetic flowmeter (F) back to the heart. A bypass for the flowmeter was incorporated so that the zero could be checked from time to time. The air space above the blood in the reservoir was in communication with a pressure bottle (PB) and a large volume syringe, the latter permitting an extremely sensitive control over air pressure in the reservoir.

Artificial respiration was carried out with a specially constructed pump with a perspex valve which, together with the tracheal cannula, had a dead space of 0.2 ml. During the preparation
Figure 1

Diagram of heart-lung and the extracorporeal circulation. From the aortic cannula, blood passes through a thin-walled, silicone-rubber damping tube (D), thence through a resistance (R), and into a perspex venous reservoir (VR). The air in the reservoir is in communication with a pressure bottle (PB) where the air pressure is finely controlled with the aid of a syringe. Leaving the reservoir, blood returns to the right atrium through an electromagnetic flowmeter (F) and a cannula placed in the inferior vena cava (IVC). A bypass for the flowmeter is provided for checking zero. Arterial and right-atrial pressures are measured through fine cannulas introduced through the innominate artery (IA) and the right superior vena cava (RSVC), respectively.

Recording Techniques

Arterial and right-atrial pressures were measured with cannulas placed in the innominate artery (IA) (or right common carotid artery) and the right atrium (via the right superior vena cava—RSVC), respectively, using photoelectric manometers. In the case of the right-atrial pressure, the reference point was taken to be the middle of the right superior vena cava as it entered the right atrium. A pointer on a swinging arm was screwed down into this position and then swung around to allow the height of the water in the reference bottle to be adjusted accordingly. By this means, it was considered that this reference point could be defined to within ±0.5 mm. Attempts to find the most suitable reference point from which to measure right-atrial and ventricular pressure have been the subject of some controversy. There is no doubt that changes in the positioning of the heart in the chest, which is partly determined by the degree of inflation of the lungs, must account for some of the differences between preparations. Probably, the theoretical reference point is the gravitational center of the blood in the right ventricle at the end of diastole, but no attempt was made to define this. The heart rate was directly measured by timing with a stopwatch in conjunction with a high-speed counter actuated by a flip-flop circuit triggered from the arterial-pressure manometer. Serial electrocardiographic recordings were made using conventional limb leads.

Operative Technique

Rats were anesthetized with urethane (0.5 ml. of 50 per cent wt./vol., administered partly intraperitoneally, partly subcutaneously) and received an injection of heparin (60 units) at the start of the experiment. In some cases, an injection of pentolinium tartrate (1 mg.) was also given at the start, in order to avoid the rise in blood pressure which was sometimes found to be considerable in the early stages of the operation. The technique was similar to that originally employed by Knowlton and Starling. The order of the steps in the operation was carefully planned, with the objective of maintaining the arterial pressure throughout as near 100 mm. Hg as possible, and of avoiding transient changes beyond the extreme range of 50 to 150 mm. Hg. Large, thin-walled, nylon cannulas were placed in the aorta and in the inferior vena cava (IVC), and the left superior vena cava and vena azygos were ligated. The pericardium was removed. The operation lasted from 55 to 90 minutes. Heparinized donor blood (8 units per ml.) from two or more rats was used to fill the extracorporeal circulation, the volume required being from 10 to 15 ml.

Observations on the Isolated Heart-Lung

General

Once established, the isolated rat heart-lung continued to beat for four to six hours at a rate varying between 250 and 400 per minute. The rate tended to rise gradually at the start and fall abruptly preterminally due to a rise in the plasma-potassium concentration. The potassium must have come mainly, if not entirely, from the red cells in the circulation or the lung, since measurement of myocardial potassium at the end of a long experiment revealed no significant fall (vide infra). Although hemolysis occurred slowly and progressively, this was not the only cause of the
rise in the plasma potassium, for the amount of potassium present was out of proportion to the degree of hemolysis. When the heart rate fell abruptly toward the end of a long run, exchange transfusion with fresh blood restored the rate to its original high level, but did not improve the deteriorating mechanical function of the heart (vide infra). The oxygen content of the reservoir blood was measured serially in five experiments. It remained constant at around 20 volumes per cent over three hours, but beyond this time, or earlier if pulmonary edema was accelerated by heart failure, the oxygen content fell.

Standard Observations

Serial observations were made on the relationship between the mean right-atrial pressure (RAP) and the cardiac output (CO) (which does not include the coronary blood flow) over a fairly wide range of CO. In such a series, the reservoir pressure was raised in a stepwise manner and, concurrently, the peripheral resistance was lowered to keep the mean arterial pressure constant. Observations were made on the RAP and the CO at 5, 15, and 35 seconds after each rise in reservoir pressure. With the heart working in an external circuit of this kind, the delay in equilibrium was manifested by a falling RAP accompanied by little alteration in the new level of CO. Such changes following a rise in reservoir pressure are shown in the recording (fig. 2). This overshoot of the RAP and delay in attaining equilibrium after a rise in reservoir pressure were only of slight degree when the heart-lung was fresh, but became progressively more pronounced as the preparation fatigued. In the fatigued state, partial to almost complete abolition of this overshoot and delay could be brought about by the infusion of epinephrine (0.2 μg./min.) into the right atrium. After the CO had been raised to a certain prearranged level, the reservoir pressure was lowered in a stepwise manner to complete the cycle of observations, which were then plotted as shown in figure 3.

The relationship between RAP and CO is practically linear in a fresh preparation over a range of CO of 5 to 30 ml./min., and has been shown to be linear up to 40 or even 50 ml./min., although observations were usually restricted to a CO below 35 ml./min. in order to avoid overstraining the heart. At high levels of RAP, the curve of RAP versus CO bends over to the pressure axis, and this tendency occurs at a lower level of RAP as the heart fatigues. Many experiments were performed at a mean arterial pressure of 150 mm. Hg. In comparison with the observations made at a mean arterial pressure of 100 mm.
Hg, the curve relating RAP and CO is unaltered at lower levels of output, but tends to bend over to the pressure axis at a lower level of RAP. Since the heart fatigued even more rapidly under these circumstances, all subsequent observations reported here were made at a constant mean arterial pressure of 100 mm Hg.

**Effect of Altering Pulmonary Ventilation on Relationship Between Cardiac Output and Mean Right-Atrial Pressure**

The artificial respirator was normally set at a stroke output of 6.5 ml. The possibility that slight alterations in the extent of pulmonary ventilation might alter the relationship between the CO and the RAP was investigated. The results are shown in figure 4. As a consequence of dead space in the pump, the extent of pulmonary ventilation was only directionally, and not proportionately, related to the respirator stroke output. When the respirator stroke output was raised to 11 ml., the lungs were greatly distended at each insufflation and the heart was lifted forward slightly. This caused the heart rate to fall and the curve relating CO to RAP to shift to the right. Reducing the pulmonary ventilation again brought about quickening of the heart and return of the curve to approximately the initial position. Reducing the respiratory stroke output to 3.8 ml. resulted in barely appreciable movement of the lungs, yet there was no significant alteration in the curve. Thus, it seemed reasonable to assume that providing the lungs were not grossly overdistended, alterations in the pulmonary ventilation were unlikely to disturb the relationship between CO and RAP.

**Effect of Altering Arterial Pulse Pressure on Relationship Between Cardiac Output and Mean Right-Atrial Pressure**

The nylon cannula which had been used in the aorta constituted a considerable resistance to the blood flow and led to large pulse pressures at high flow rates. The effect that this might have on the relationship between CO and RAP was investigated in two experiments by introducing a larger cannula into the aorta and then allowing the blood to flow into an air-damped system before entering a narrow resistance segment. The air damping could be switched on or off at will. The results of one of these experiments are shown in table 1. At low flow rates and relatively small pulse pressures, the presence or absence of damping had no effect on the relationship between CO and RAP. At larger flow rates, the absence of damping led to an increase of RAP for a given CO, and eventually to acute left-ventricular failure. The effect of this phenomenon on the standard curve relating CO to RAP was to lessen the slope of the curve and shift it slightly to the right. The effect was negligible when the preparation was fresh, but became pronounced when it was fatigued. In consequence of this finding, damping was provided in subsequent experiments by using as large an aortic cannula as possible, followed by a damping system of very thin-walled, silicone-rubber tubing.

**Effect of Raising the Heart Rate on Relationship Between Cardiac Output and Mean Right-Atrial Pressure**

This investigation was undertaken because it had been found that certain changes in the composition of the circulating blood might alter both the heart rate and the relationship between CO and RAP; therefore,
Table 1
Effect of Altering the Pulse Pressure on the Relationship Between Cardiac Output (CO) and Mean Right-Atrial Pressure (RAP)

<table>
<thead>
<tr>
<th>Blood pressure* (mm. Hg)</th>
<th>Ratio CO/RAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Damped</td>
</tr>
<tr>
<td>CO (ml./min.)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>110/90 (20)</td>
</tr>
<tr>
<td>20</td>
<td>114/84 (30)</td>
</tr>
<tr>
<td>30</td>
<td>124/82 (42)</td>
</tr>
<tr>
<td>40</td>
<td>132/82 (50)</td>
</tr>
<tr>
<td>50</td>
<td>136/81 (55)</td>
</tr>
</tbody>
</table>

*Pulse pressure in parentheses.

RAT HEART-LUNG PREPARATION

considered necessary to discover whether the former could be directly responsible for the latter.

The heart was electrically stimulated with a square wave pulse (20-msec. pulse width) applied to the inferior vena cava as it entered the right atrium. Fourteen satisfactory experiments were made. The heart rate was increased artificially to rates up to double the natural frequency. When the frequency of electrical stimulation approached the natural frequency, the heart rate tended to become irregular; there was, however, no significant directional change in the relationship between the CO and RAP when the heart was electro-stimulated at approximately the same rate as the natural frequency. In some instances, the CO relative to the RAP rose slightly, and in others it fell. Observations were made, in the previously described manner, of the relationship between the CO and RAP when the heart rate was electro-stimulated at approximately the same rate as the natural frequency. In some instances, the CO relative to the RAP rose slightly, and in others it fell. Observations were made, in the previously described manner, of the relationship between the CO and RAP at heart rates up to 25 per cent over the natural frequency. The result from one such experiment is shown in figure 5. No significant shift of the curve relating CO to RAP took place. In other experiments, the CO was kept as nearly constant as possible as the heart rate was raised progressively by electrical stimulation, and observations were made of changes in RAP. The ratio of CO to RAP was then plotted against the heart rate as shown in figure 6. When the heart rate was thus raised in steps, there was a delay before the heart settled down to the new conditions, as shown in the figure, where observations made at 5 and 35 seconds after the change in heart rate have been entered. This effect resulted in the ratio of CO to RAP being higher for a given heart rate when the heart rate was being raised than when it was being lowered. In the majority of experiments, the ratio of CO to RAP remained approximately constant (within 10 per cent, and usually within 5 per cent) as the heart rate was raised from the natural frequency (of 250 to 330 per minute) to about 400 per minute, and then fell more and more steeply. In one experiment, the ratio increased markedly up to a rate of 400, particularly at the higher levels of CO; in only one experiment did the ratio fall progressively as the rate increased from the natural frequency. In three experiments performed during constant infusions of epinephrine1 or norepinephrine,2 the ratio of CO to RAP appeared to remain constant up to a higher heart rate than normally.

Changes in Cardiac Dynamics During a Prolonged Experiment

The curve relating CO to RAP altered little in slope during the first 60 to 90 minutes from the start of the extracorporeal circulation (fig. 7). The position of the curve, however, tended to move along the pressure axis, to a small and sometimes quite negligible extent in the first hour, but later progressively. After approximately 90 minutes, the slope of the curve commenced to fall and continued to do so until the end of the experiment. At first,
Constancy of the relationship between cardiac output (CO) and right-atrial pressure (RAP) as the rate is raised by electrical stimulation to over 400 beats per minute. During the experiment, the CO was kept approximately constant at 20 ml. per minute.

Change in the relationship between cardiac output and right-atrial pressure during a prolonged experiment with the heart-lung preparation. It was thought that the steadily mounting potassium concentration might account for this deterioration, but the addition of small quantities of potassium neither shifted the curve to the right along the pressure axis nor decreased its slope, until the potassium concentration rose to such an extent that the heart was greatly slowed. Furthermore, repeated exchange transfusions with fresh blood (estimated to replace about 75 per cent of the blood in circulation) prevented the potassium concentration from rising, yet made no difference whatsoever in the rate of deterioration of function of the isolated heart (fig. 8). The curve in a fatiguing heart was noted to be restored, although usually incompletely, to its initial position during an infusion of epinephrine of 0.2 μg./min.

Water, Sodium, and Potassium Transport in the Isolated Beating Heart

The finding of a progressively increasing potassium concentration in the circulating plasma, indicating transfer of the ion out of cells, suggested the possibility that the myocardium might take part in this process. To investigate this possibility, the distribution of water, sodium, and potassium in the ventricular myocardium was studied in a few experiments after different times of extracorporeal circulation. The technique consisted of the injection of inulin into the circulation one hour before termination of the experiment. At the end of this time, the heart was quickly removed, the atria cut off, and the ventricles sliced,blotted, divided into three portions, and weighed. The portions were then treated, as described previously, 13 for the estimation of dry weight, sodium, potassium, and inulin. The results of five such experiments are shown in table 2 where, in addition, the figures for the normal heart in situ are given as a baseline. 14 It will be seen that in the two short-lasting experiments (nos. 1 and 2), the distribution of water and electrolytes was approximately the same as in the normal heart in situ, with the exception that the plasma-
potassium concentration had already started to rise. In the two long-lasting experiments, the volume of extracellular fluid had increased and there appeared to be less intracellular water in relation to the dry weight of the tissue. The concentration of sodium in intracellular water had fallen, whereas that of potassium had risen. When the electrolyte content of the intracellular fluid was expressed in terms of the dry weight of the tissue, the sodium content was seen to have fallen, and the potassium remained constant. This would suggest that sodium had left the cells, and that no transfer of potassium had taken place, the rise in the concentration of this ion in intracellular fluid being due to the passage of water out of the cell. Hence, there was no evidence of potassium's leaving the cells of the ventricular myocardium. The significance of the loss of intracellular sodium was not apparent. The slope of the curve relating CO to RAP was very much lower in the long-lasting experiments, as indicated in an earlier section. It is not possible at this stage to say whether this was in any way related to the alteration in myocardial electrolytes. The results in experiment no. 5 are tabulated separately. In this experiment, sodium chloride was added to the circulating blood to raise the concentration of sodium in the plasma. This produced cellular dehydration with increased intracellular concentration of sodium and potassium, but there was no evidence of passage of sodium into or of potassium out of the cells. This procedure did not materially alter the slope of the curve relating CO to RAP.

Effect of Raising Concentrations of Sodium and Potassium in the Plasma

Earlier work had indicated that artificial elevation of the level of potassium in the circulating blood shifted the curve relating cardiac output and right-atrial pressure to the left, signifying a greater cardiac output for the same right-atrial pressure. A number of experiments were undertaken to explore this phenomenon further. After preliminary observations on the heart-lung, solutions of sodium or potassium chloride (of volume not exceeding 0.3 ml.) were added to the circulating blood. The effects which these additions had on the heart rate and on the relationship between cardiac output and right-atrial pressure are shown in table 3. The addition of isotonic saline and of small quantities of hypertonic saline had no effect on the above characteristics of the heart-lung preparation. When the concentration of sodium was raised to a greater extent, there was a highly sig-

Table 2

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Duration of extracorporeal circulation (min.)</th>
<th>Slope of curve CO vs. RAP (ml/min/mm Hg)</th>
<th>Plasma concentration (mEq./L.)</th>
<th>Volume of extracellular fluid</th>
<th>Index of intracellular hydration</th>
<th>ICF concentrations (mEq./L.)</th>
<th>Electrolyte content of ICF per Kg solids (mEq./Kg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>2.2</td>
<td>148</td>
<td>6.2</td>
<td>13.1</td>
<td>2.35</td>
<td>20.5</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>1.4</td>
<td>148</td>
<td>5.9</td>
<td>12.6</td>
<td>2.45</td>
<td>25.0</td>
</tr>
<tr>
<td>3</td>
<td>203</td>
<td>0.2</td>
<td>151</td>
<td>10.9</td>
<td>17.1</td>
<td>2.21</td>
<td>13.7</td>
</tr>
<tr>
<td>4</td>
<td>239</td>
<td>0.3</td>
<td>151</td>
<td>8.6</td>
<td>16.7</td>
<td>2.29</td>
<td>13.3</td>
</tr>
<tr>
<td>5</td>
<td>97</td>
<td>2.1</td>
<td>212</td>
<td>6.0</td>
<td>16.9</td>
<td>1.83</td>
<td>33.7</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>145.8</td>
<td>4.2</td>
<td>12.3</td>
<td>2.31</td>
<td>22.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Na K</th>
<th>Index of intracellular hydration</th>
<th>Na K</th>
<th>Na K</th>
<th>Na K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>±0.5 ±0.2</td>
<td>1.8</td>
<td>±0.9 ±2.2</td>
<td>±2.9 ±4.6</td>
<td>±2.9 ±4.6</td>
</tr>
</tbody>
</table>

*ECFV refers to the volume of extracellular fluid in the myocardium.

The index of intracellular hydration is the volume of intracellular fluid per Kg. wet tissue expressed as a fraction of the dry weight per Kg. of wet tissue.
Table 3
Mean Changes in Position and Slope of the Curve Relating Cardiac Output (CO) to Right-Atrial Pressure (RAP) After Addition of Various Solutions to the Circulating Blood.

<table>
<thead>
<tr>
<th>Solution added</th>
<th>Number of experiments</th>
<th>Change in RAP at CO of:</th>
<th>Ratio of slope of curve CO vs. RAP before and after addition of solution</th>
<th>Change in heart rate per min.</th>
<th>Change in plasma Na concentration (mEq./L.)</th>
<th>Change in K concentration (mEq./L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotonic NaCl</td>
<td>5</td>
<td>-0.08 ± 0.10 mm. H2O</td>
<td>0.987 ± 0.015</td>
<td>+ 8</td>
<td>-0.6</td>
<td>+0.43</td>
</tr>
<tr>
<td>Hypertonic NaCl (1)</td>
<td>5</td>
<td>+0.19 ± 0.10 mm. H2O</td>
<td>1.050 ± 0.027</td>
<td>+13</td>
<td>+7</td>
<td>+0.14 (range 5-11)</td>
</tr>
<tr>
<td>Hypertonic NaCl (2)</td>
<td>5</td>
<td>+1.73 ± 0.49 mm. H2O</td>
<td>0.940 ± 0.036</td>
<td>+13</td>
<td>-29</td>
<td>+0.26 (range 19-40)</td>
</tr>
<tr>
<td>Hypertonic KCl</td>
<td>5</td>
<td>-0.98 ± 0.14 mm. H2O</td>
<td>0.962 ± 0.048</td>
<td>+ 6</td>
<td>-1</td>
<td>+3.16 (range 2.5-4.3)</td>
</tr>
</tbody>
</table>

The sign ± denotes standard error of the mean.
P refers to the significance of difference from the results after addition of isotonic NaCl.

Figure 9
Change in the relationship between cardiac output and right-atrial pressure, following consecutive elevation of the concentration of sodium and potassium in the circulating blood.

Significant shift of the CO versus RAP curve to the right, without any alteration in slope. Conversely, elevation of the level of potassium shifted the curve to the left. When consecutive additions of sodium and potassium were made in the same preparation, the expected shifts of the curve took place. An example of this is shown in figure 9, which illustrates an experiment in which, first, the concentration of sodium was raised, with consequent shift of the curve to the right, and secondly, the concentration of potassium was raised with resulting shift of the curve to the left. This latter shift was of such a degree in this particular experiment that the final curve lay to the left of the initial curve. No significant change in heart rate took place following the addition of sodium and potassium, except in one instance when the addition of potassium produced a transient slowing of the heart rate from 293 to 163 per minute. The aforementioned experiments were extended by making further additions of potassium: it was found that when the concentration of potassium was raised to between 8 and 11 mEq./L. the heart rate fell abruptly from about 250 to 300 to 100 to 160. At this stage, the addition of hypertonic sodium chloride, raising the sodium concentration to 160 to 180 mEq./L., restored the initial rapid heart rate. By raising the sodium concentration further, up to a maximum of 210 mEq./L., the heart rate was maintained, despite elevation of the potassium concentration to as high as 16 mEq./L.

Serial electrocardiographic recordings were made during these imposed changes in electrolyte concentration in the circulating blood. The results in two such experiments are shown in figure 10. All recordings were made at the same cardiac output and with the respirator temporarily switched off. Raising the level of sodium concentration caused slight, but consistent, elevation in the R and T waves, whereas potassium caused lowering and broadening.
of the T wave, with prolongation of the Q-T interval. These two effects opposed one another when potassium and sodium were administered consecutively, as is well shown in figure 10 (B).

Discussion

The rat heart-lung would appear to be a very satisfactory and convenient preparation for the study of cardiac dynamics. The disadvantages of its small size have been largely overcome, but studies involving measurement of coronary flow and of ventricular and left-atrial pressures, which are possible on larger hearts, would prove most difficult in the rat heart-lung preparation at this stage in technical development. The observations recorded in the present paper have been mainly concerned with net systemic cardiac output in relation to right-heart filling pressure. The various factors which might influence this relationship have been explored, and it has been shown that over the first hour or two of extracorporeal circulation, the relationship remained fairly constant. Thus, it would seem reasonable to assume that, over this period, the observed relationship represented the basic behavior of the heart when in the body. In the latter situation, of course, various humoral and nervous influences would be modifying this basic relationship. Through the use of the direct Fick principle, a CO of the order of 40 to 50 ml./min. has been observed in intact, resting rats of similar weight to those used in the present experiments. Thus,
in the experiments reported here, cardiac outputs approaching those in the intact rat have been employed.

The effects that certain variables, inherent in the heart-lung preparation, could have on the relationship between output and filling pressure have been investigated. The depth of artificial pulmonary ventilation was not critical, and only with extreme overventilation did the heart rate slow and the cardiac output fall. The influence of the degree of damping of the arterial pulse was inappreciable, except when it was greatly restricted in a fatiguing heart. Coronary blood flow is believed to take place exclusively during diastole.17, 18 It is possible that the low diastolic pressure occurring in the absence of damping is insufficient to perfuse the coronary vessels.

The effect of altering the heart rate upon the relationship between CO and RAP has been investigated previously by Markwalder and Starling19 and Patterson and Starling.20 Changes in heart rate were achieved by cooling and warming the blood perfusing the heart, and the latter authors reported that if the venous pressure were kept constant, the CO rose with increasing heart rate. From a study of their data, it would appear that the hearts were already in some degree of failure when the experiments were carried out, since the rate of adaptation to imposed changes was extremely sluggish. This makes interpretation of their observations rather difficult. Furthermore, it does not seem justifiable to assume that the observed changes were the result of the lowering of heart rate per se, rather than some effect on myocardial activity brought about by cold. In the present experiments, the relationship between CO and RAP has been found to be approximately constant as the heart rate was raised to a figure of 25 to 30 per cent above the spontaneous rate. At this point, the CO began to decrease relative to RAP. During epinephrine or norepinephrine infusions, this change took place at a higher rate of stimulation. No attempts were made to study the effect of lowering the heart rate below the spontaneous rate, but on a few occasions, during experiments in which potassium was added to the blood, the heart rate was observed to slow very greatly and then revert to its previous level. In such cases, the increased stroke output was inadequate for matching the reduction in rate, and the CO fell relative to the RAP. It would thus appear that in the physiological range of heart rate, the heart is operating in such a way as to give maximum CO for a given filling pressure.

In the early stages of all experiments, the spontaneous heart rate was observed to increase as the RAP and CO were raised, and to fall again as the RAP was lowered. Similar observations have been made by Binks.21 Later in the course of the experiment, this phenomenon became less marked and might disappear entirely.

The reason for the progressive deterioration of the function of the heart-lung with time is unknown. The observation that repeated exchange transfusions with fresh blood failed to make any difference to the rate of deterioration not only ruled out the increase in plasma potassium as the cause, but also made it most improbable that either the accumulation of other substances or the complete consumption of some essential metabolites in the blood was responsible. No measurements of pulmonary resistance were made, and it is possible that a rise in resistance might have led to altered right-ventricular function. This was thought unlikely to be the complete explanation, for when right-ventricular function had deteriorated, it was found that the left ventricle was incapable of sustaining a raised arterial pressure. In fact, the left ventricle might go into failure with great dilation of the left atrium as the output of the right ventricle was raised in the later stages of the experiment. Thus, it would appear that both ventricles were failing, and some explanation had to be sought which would account for the combined failure. Inadequate oxygenation in the later stages was a possibility, but, as has been stated earlier, there was usually no fall in oxygen content of the blood during the first two or three hours of the experiment and this, therefore, seemed an unlikely explanation. It is possible that some cardiomyotoxic
substance is continuously being formed in the circulating blood and is completely removed by one passage through the lungs. After some hours, the powers of the lungs to remove such a substance may become impaired so that the substance enters the coronary circulation. This would explain the failure of exchange transfusions with fresh blood to delay the onset of deterioration. Newton noted that the toxicity of blood was reduced by passage through the heart-lung preparation and suggested that histamine release might be responsible for the observed changes in myocardial function and pulmonary-vascular resistance. Daly and Thorpe pointed out that in a preparation in which an isolated heart is pumping blood through an artificial oxygenator, cardiac failure occurs early, in contrast with the much later failure in the heart-lung preparation. They found that the development of cardiac failure could be delayed by passing the blood through cambric filters, and suggested that either particulate matter or some unspecified toxic substances were being removed from the blood by the filters.

The results of the preliminary investigation of water, sodium, and potassium transport in the isolated beating heart may be compared with the earlier work of Wood and Moe. These workers reported an increase in the extracellular-fluid content of the heart, and the present results confirm this finding in the two experiments of long duration, but not in the experiments of short duration. Wood and Moe used the chloride space to define the extracellular compartment, whereas inulin was used in the present studies. On this basis, they further reported no change in the intracellular water and electrolyte content, in contrast with the present finding of a reduction in intracellular sodium. This latter observation and the lack of evidence that potassium left the myocardial cells as the preparation gradually deteriorated are the reverse of what might be expected as cell function becomes depressed.25 These conclusions naturally depend on the validity of the inulin technique for defining the extracellular space. The findings could be explained on the assumption that inulin entered the cells of the heart in the later stages of failure of the preparation. No convincing evidence has yet been presented that inulin enters healthy muscle fiber.

A preliminary report of the action of potassium on the heart-lung preparation has already appeared.10 The interpretation of the reported findings in regard to the action of both sodium and potassium is difficult, since changes in cardiac output relative to right-atrial pressure could theoretically be due to alterations in one or more of three variables. These variables are myocardial contractility, pulmonary-vascular resistance, and coronary blood flow. In the isolated frog's heart, increase of the potassium concentration has been reported to diminish the force of contraction.26 However, McDowall and associates demonstrated slight stimulation of isolated strips of the right ventricle from the rat by moderate elevations of potassium, with depression at higher levels. Elevation of the level of sodium decreases the force of contraction in the isolated heart of both the frog28, 29 and the rat.27 Thus, it is possible that changes in myocardial contractility could account for the opposing actions of sodium and potassium on the heart-lung preparation, reported here. Turning to the problem of changes in pulmonary-vascular resistance, there have been many reports that intravenous injection of hypertonic sodium chloride induces a transient rise in pulmonary-artery pressure.30, 31 If a prolonged and stable rise in the pulmonary-vascular resistance were to occur in the heart-lung preparation when the sodium concentration was raised and maintained at a higher level, this would account for the observed changes in the relationship between cardiac output and right-atrial pressure. Dawes found that raising the potassium concentration had no effect on the blood flow through perfused dog's lungs, although in peripheral vessels, small changes produced vasodilatation. Thus, there is little evidence to support the possibility that a fall in pulmonary-vascular resistance could account for the action of potassium on the heart-lung, although such a mechanism cannot be excluded. If the coronary blood flow were...
greatly diminished by potassium and raised by sodium, the change in the relationship between cardiac output and right-atrial pressure could be equally well explained. Katz and Linder, working with the Langendorff preparation of dog's heart, did, in fact, find that sodium caused coronary vasodilatation, whereas potassium dilated the coronaries in concentrations up to 34 to 37 mg. per cent and constricted them above this level. Thus, the action of sodium in the heart-lung preparation might be explained in this manner, but not that of potassium. No data are available on the absolute level of coronary blood flow in the heart-lung preparation of the rat, but it should be noted that in the intact rat with a cardiac output of approximately 45 ml./min., the coronary flow is reported to be less than 1.8 ml./min. These figures cannot, of course, be unreservedly applied to the heart-lung preparation, but they make it less likely that the magnitude of the increase in cardiac output relative to right-atrial pressure could be explained by a reduction in coronary flow.

Whatever is the explanation of the change in this relationship, the significance of it is that elevations in potassium would be expected to increase, and sodium to decrease, systemic cardiac output for a given right-heart filling pressure.

The chronotropic action of high levels of potassium on the heart-lung preparation and the antagonistic action of sodium in this respect are compatible with the observation of the action of sodium in reducing the toxicity of potassium in mice and in dogs.

**Summary**

A technique for producing and studying a heart-lung preparation in the rat is described. The relationship between filling pressure and cardiac output was found to remain constant for 60 to 90 minutes after the start of the extracorporeal circulation. The influence on this relationship of alterations in pulmonary ventilation, arterial pulse pressure, and heart rate has not been found to be critical. The mechanisms underlying deterioration of the preparation have been investigated, but no firm conclusions have been reached. Deterioration was not prevented by the introduction of fresh blood into the circulation. During prolonged experiments, the extracellular-fluid content of the heart was found to increase, the intracellular potassium was unaltered, and the intracellular sodium was reduced. Acute elevation of the sodium concentration in the circulating blood lowered, whereas potassium raised, the cardiac output for a given filling pressure. These opposing actions were found to summate. Slowing of the heart brought about by high concentrations of potassium was antagonized by sodium.

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**References**


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