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# THE OXYGEN UPTAKE OF THE PERFUSED RAT HEART

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Since the publication of the papers of Laitinen & Kolthoff (1941*a*, *b*) and Davies & Brink (1942) stationary, vibrating or rotating platinum electrodes have been extensively used for the estimation of the oxygen consumption of surviving tissues and cell suspensions. This paper describes the development of a method of this kind for the determination of the oxygen consumption of the rat heart perfused through the coronary arteries with a saline-bicarbonate medium.

The results show that although the oxygen uptake of the isolated rat heart is three to four times as great as that of rat heart slices, it is feasible to supply considerably more oxygen than the basal oxygen requirement of the heart. The isolated rat heart preparation is shown to be resistant to short periods of complete anoxia, or longer periods of partial anoxia, and it is suggested that survival time after anoxia is affected by the initial glycogen content of the heart. Some characteristics of the oxygen uptake of hearts perfused without added nutrient are described.

## METHODS

### *Principle of the polarographic method*

The rat heart is supplied with a bicarbonate-saline medium which is kept in equilibrium with a gas mixture containing a known partial pressure of $O_2$ with $CO_2$. The oxygen concentration in the medium issuing from the heart is measured polarographically, and the rate of flow of medium through the heart is measured with a bubble flow meter. The product of the arteriovenous ($A-V$) oxygen-concentration difference and the flow rate gives the oxygen consumption of the heart.

Determination of the oxygen concentration of the effluent from the heart depends on the measurement of the current flowing between a platinum electrode maintained at a suitable negative potential, and a standard non-polarizable anode. The current is produced by the reduction of oxygen molecules to water at the platinum surface, and the magnitude of the current depends on the area of the exposed platinum, the concentration of oxygen in solution, and the prevailing hydrodynamic conditions which determine the rate of diffusion and convection of oxygen molecules to the platinum surface. The chief disadvantages of the method are that the current produced by a constant concentration of oxygen tends to change with time, and that the oxygen current is influenced by the immediate past history of the electrode.

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The experience of many investigators (reviewed by Connelly, 1957) has shown that the most reproducible results are obtained by careful control of the hydrodynamic conditions in the electrode chamber, either by preventing direct access of the exposed platinum to the solution by allowing the oxygen molecules to diffuse through a thin membrane covering the platinum surface, or by allowing the electrode to recover between short periods of polarization (intermittent polarization). As the flow rate of fluid passing through the electrode chamber does not remain constant, a stationary membrane electrode cannot be used for our experiments. Consequently a rotating platinum electrode of the exposed type, similar to the gold electrode used by Longmuir (1954), is used, together with the intermittent-polarization technique. This method has proved reliable, and is suitable for any system in which blood-free solutions flow through an electrode chamber.

*Perfusion method*

The perfusion fluid is a modified Krebs-bicarbonate medium containing half the calcium concentration recommended by Umbreit, Burris & Stauffer (1949). The perfusion apparatus was derived from that used by Bleehen & Fisher (1954), modified by introducing a more efficient method of continuous filtration of the perfusion medium than the Whatman Soxhlet thimbles originally used. In the modified apparatus (Fig. 1) fluid passes into the heart under a hydrostatic pressure of 54 cm $H_2O$, from a small water-jacketed chamber ($R_2$), and is returned to a chamber ($R_1$) at the same level as the first by a gas lift ($G_1$) through which 5 % $CO_2$ in oxygen passes. Fluid then passes under a hydrostatic pressure of about 100 cm $H_2O$ to a large sintered-glass filter ($F$) of porosity 4, and the filtered fluid is returned to the chamber ($R_2$) above the heart by a second gas lift ($G_2$). This chamber is sealed and the level of the fluid is kept constant by a siphon ($S$) leading into the chamber ($R_1$) which is open to the atmosphere. A Soxhlet thimble is retained in reservoir $R_1$, as this delays the blocking of the sintered-glass filter. Blocked filters are washed with concentrated sulphuric acid containing a small amount of potassium chlorate and sodium nitrate.

This basic perfusion apparatus was modified for the measurement of the oxygen uptake of the heart by the introduction of two calibration circuits ($C_1$ and $C_2$), and three glass taps ($T_1$, $T_2$ and $T_3$) which allowed fluid from each of the calibration circuits or the heart circuit either to flow straight through, or to pass independently into a water-jacketed electrode chamber ($E$), and then to the appropriate gas lift. A diagram of one of these glass taps in the two positions is shown in Fig. 2, and Fig. 3 shows the three taps connected together and to the electrode chamber. Each calibration circuit is a simplified version of the heart circuit, with the second upper reservoir and the sintered-glass filter omitted. Fluid, the flow of which is restricted by a screw clip, passes from a water-jacketed upper chamber through the glass tap and is returned to the chamber by a gas lift, through which passes a gas mixture containing 5 % $CO_2$ and a known percentage of oxygen.

The electrode chamber is sealed at one end with a rubber bung 2 cm in diameter and 0·8 cm. thick, through which passes the platinum electrode. The bung rests against a collar in the glass of the chamber and acts as the fulcrum for the rotating platinum electrode. The walls of the electrode chamber flare out slightly from the collar to allow for the swept volume of the rotated electrode, and a glass tube sealed into the far end of the chamber serves as the fluid inlet. The fluid outlet is near the collar. This design of the electrode chamber reduces the fluid dead space to a minimum.

The electrode consists of a platinum wire sealed into a glass tube 0·6 cm in diameter and 17 cm long. The centre of the bung through which the electrode passes into the chamber is 5 cm from the platinum tip. A steel sleeve is fitted over the far end of the electrode tube and seated in a self-aligning ball-race, which is set eccentrically 0·7 cm. from the centre of a circular brass disk mounted on the motor shaft. The diameter of the circle described by the platinum tip is 0·3 cm. The water-jacketed electrode chamber and motor are mounted rigidly on a wooden board fixed to the bench, making a vibration-free rotating electrode assembly.

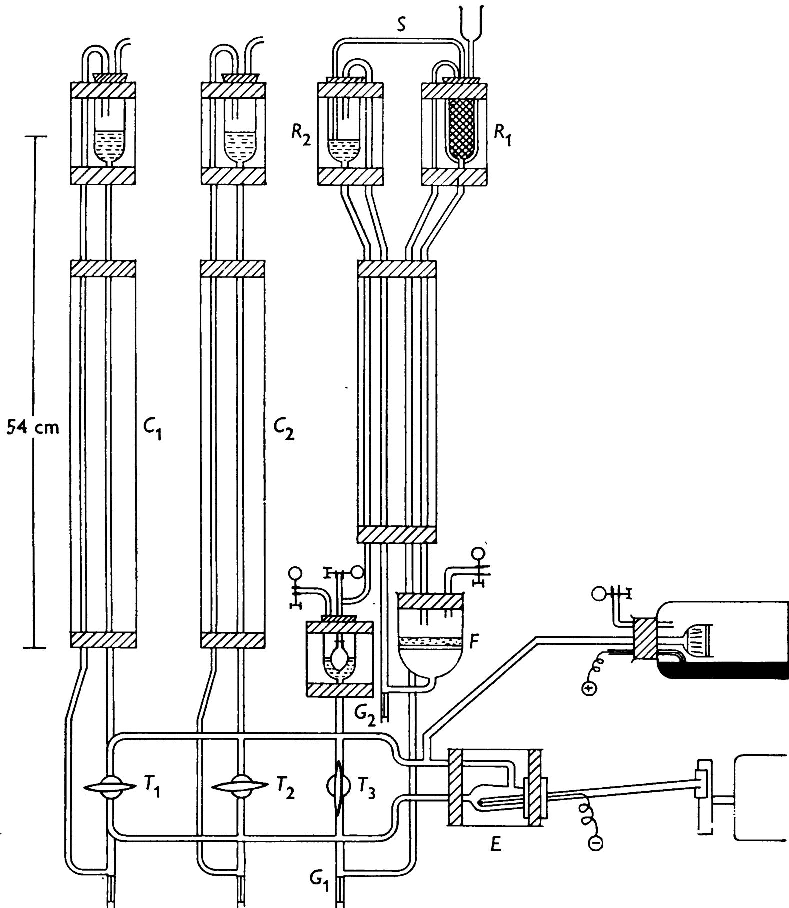


Fig. 1. Arrangement of apparatus for the determination of the oxygen uptake of the perfused rat heart. $C_1$, $C_2$, calibration circuits; $E$, electrode chamber; $F$, filter; $G_1$, $G_2$, gas lifts; $R_1$, $R_2$, water-jacketed chambers; $S$, siphon; $T_1$, $T_2$, $T_3$, glass taps. For further details see text.

*The accuracy and reproducibility of the oxygen electrode*

The chief factors which were found to determine the rate of drift and the reproducibility of the oxygen current were:

*a*, the preparation and care of the electrodes;
*b*, constancy of rotation of the platinum electrode;
*c*, use of an intermittent polarization technique; and
*d*, presence of calcium ions in the solution.

*Preparation and care of the electrodes.* The biggest single factor influencing the stability and reproducibility of the oxygen current undoubtedly resides in the platinum electrode. With the exposed type of platinum electrode used two factors are particularly important. One factor is that careful annealing of the platinum–glass seal prevents the formation of

small cracks in the glass around the seal, visible only under the microscope. The second is freedom from scratches of the platinum surface. The most satisfactory electrodes are made by sealing a short length of 27 s.w.g. Pt wire, which has the end fused into a sphere, into a hard-glass tube of 0·6 cm external diameter, so that just the hemispherical tip is exposed. A length of silver wire is fused to the other end of the platinum wire, and passes out of the glass tube through a small hole 8 cm from the tip. Satisfactory electrodes, not needing the services of a skilled glass-blower, were also made by sealing the platinum–silver wire into

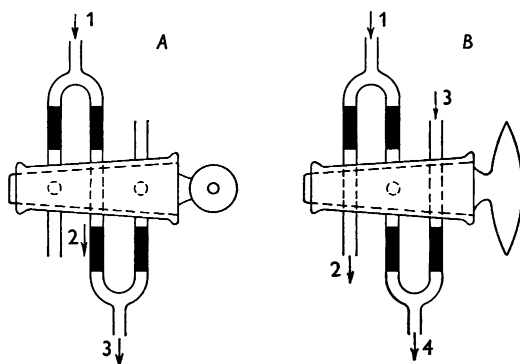


Fig. 2. Special taps for diversion of perfusate to the measuring cell. In *A* perfusate from a calibration circuit or from the heart passes directly to the gas-lift returning it to its reservoir. In *B* it passes to the measuring cell (arrow at bottom left), returns to the tap (arrow at top right) and thence to the gas lift.

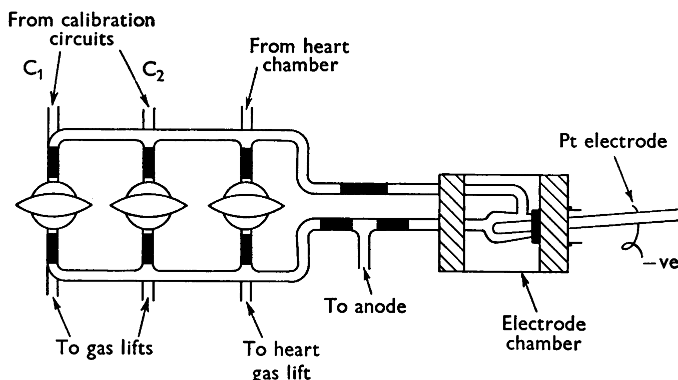


Fig. 3. The connexions of the special taps with the remainder of the apparatus: cf. Fig. 1.

Marco resin SB 26C (Darbre, 1957), using as a mould a glass tube of internal diameter 0·6 cm, coated on the inside with oleic acid as a releasing agent. When not in use electrodes are stored in 10 N nitric acid. Small scratches tend to appear on the platinum surface with use, and these are removed by polishing the surface with jeweller's rouge and then with a fine Perspex polish (No. 3 Perspex polish; I.C.I.).

The type of anode is not critical, provided that it is large enough to eliminate any possibility of anode polarization (Longmuir, 1957), and does not allow diffusion of mercury on to the platinum electrode (Giguère & Lauzier, 1945*a*, *b*). The anode used is a mercury pool of

about 100 cm² surface area, contained in a 500 g bottle resting on its side and filled with saturated KCl solution. A 4 cm coarse sintered-glass filter, cut down to the sintered-glass plate, with the stem partly filled with a 1 cm-thick layer of 4 % agar gel saturated with KCl, serves as a bridge. The bottle is tightly stoppered by a rubber bung, through which projects the stem of the filter, and electrical contact with the mercury is made by a platinum wire sealed into a glass tube, which also passes through the bung.

*Constancy of rotation of the platinum electrode.* Experiments in which the platinum electrode was rotated by a Velodyne motor (Dickinson, 1950) showed that when the fluid was saturated with the gas mixture containing 5 % of $CO_2$ and either 20 % $O_2$ or 95 % $O_2$, the oxygen current increased with increasing speed of rotation of the electrode up to 5000 rev/min (see Fig. 4). This finding is contrary to that of Longmuir (1954), who observed the beginning of a plateau region above 1000 rev/min.

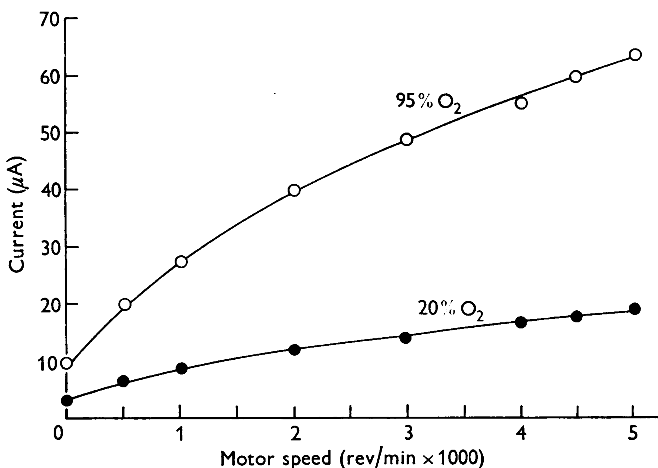


Fig. 4. The relation between speed of rotation of the intermittently polarized electrode and oxygen current.

To ensure a constant speed of rotation, a Fracmo 1/10 h.p. synchronous motor was used to rotate the electrode at 3000 rev/min. At this speed, changes in the flow rate of fluid entering the electrode chamber between 3 and 10 ml./min produce very small changes in the oxygen current.

*Intermittent polarization.* The oxygen current was found to fall in an approximately exponential manner from the time of switching on the polarizing potential, and in some case an hour of pre-polarization at $-1{\cdot}0$ V (Longmuir, 1954) was needed before steady values were obtained. The necessary pre-polarization time is reduced to a few minutes, and the stability of the oxygen current is improved, by reducing the average current density at the electrode surface by applying the intermittent polarization technique of Carlson, Brink & Bronk (1950). A 1-rev/min synchronous motor is used to drive a brass cam, which closes two separate micro-switches each for 10 sec in every minute. One micro-switch controls the polarizing potential to the platinum electrode, while the other operates the chart motor of a Cambridge pen-recording polarograph. During the 10 sec period of polarization the pen reaches its maximum excursion, and a series of vertical lines are recorded on the chart at 1 min intervals. The height of the maximum excursion of the recording pen is a linear function of the percentage of oxygen in the equilibrating gas, as is shown in Fig. 5.

*Effect of calcium ions in the perfusion medium.* Before the intermittent-polarization technique was introduced it was observed that the rate of drift of the oxygen current was

considerably greater when the fluid was Krebs-bicarbonate medium than when it was isotonic mixture containing sodium chloride and the same concentration of sodium bicarbonate as the Krebs medium. The difference between the two fluids was found to depend on the presence of calcium in the medium, and it appeared that prolonged exposure of the platinum electrode to solutions containing calcium, and equilibrated with gas mixtures rich in oxygen, leads to the deposition of a film on the platinum surface reducing its effective area. Reduction of the current density at the platinum surface by the intermittent-polarization technique greatly reduces the fall of sensitivity of the electrode with time which this deposition causes.

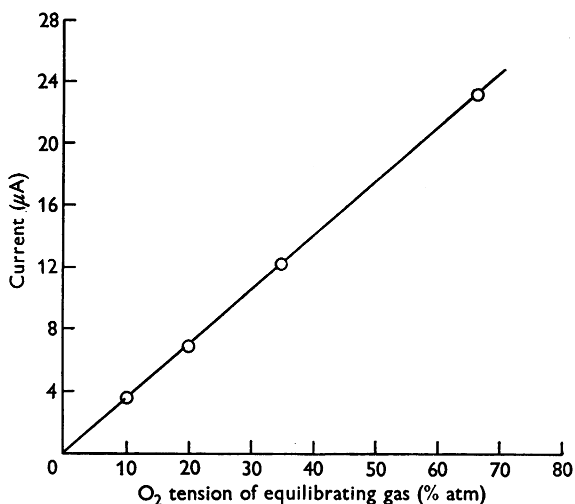


Fig. 5. A typical calibration curve of an electrode rotated at 3000 rev/min, and polarized for 10 sec/min at −0·6 V.

*Current voltage curves*

In common with the other workers using rotating electrodes (Giguère & Lauzier, 1945*b*; Longmuir, 1954) we found no well defined current–voltage plateaux at the speeds of rotation. With a stationary electrode good plateau regions from −0·4 to −1·0 V were obtained, but with the increasing speeds of rotation of the platinum electrode the length of the plateau region decreased, until at 3000 rev/min only a short region of inflexion between 0·6 and −0·8 V remained. However, since the speed of rotation of the electrode is kept constant by the synchronous motor this is not important, and a polarizing potential of −0·6 V is used.

*Application*

Before beginning a perfusion the two calibration circuits and the heart circuit, each containing Krebs-bicarbonate medium, are equilibrated with 5 % $CO_2$ in air, and the flow rates of the calibration circuits are adjusted until the same oxygen current is recorded on each of the three circuits, with the flow rate through the heart circuit set at 6 ml./min. The heart circuit is then equilibrated with 95 % $O_2$ and 5 % $CO_2$, and the two calibration circuits are equilibrated with gas mixtures containing 5 % $CO_2$, and different oxygen contents (usually 20 and 35 %). If the oxygen tension of the effluent from the heart is expected to be greater than 35 % saturation the heart circuit is equilibrated with 5 % $CO_2$ and 70 % $O_2$, so that the oxygen tension of the effluent is within the calibration range. The platinum electrode is calibrated immediately before the start of perfusion, and normally 15 min later and every

subsequent 30 min. The oxygen tension of the effluent is estimated to the nearest ½ % of an atmosphere. The flow rate through the perfused heart is measured at 5 min intervals by timing the fall of a gas bubble between two marks on the glass tube conducting fluid into the heart, and the bubble is removed from the circuit by a bubble trap in the cannula. The oxygen uptake of the heart (in $\mu$l./min) is calculated at 5 min intervals, from the product of the arteriovenous oxygen tension difference, the appropriate value of the Bunsen solubility coefficient for oxygen (Dixon, 1951) and the flow rate. The oxygen uptake is converted to $Q_{O_2}$ values ($\mu$l. $O_2$/mg dry wt./hr) after determination of the dry weight of the heart.

### *Procedure*

*Animals.* Male albino rats of Wistar stock weighing 200–260 g were used. The rats were bred in the Department and allowed unrestricted access to food and water.

*Perfusion fluids.* The basic perfusion fluid was Krebs–bicarbonate medium (KBM) made up according to the instructions of Umbreit *et al.* (1949), except that the calcium content was halved. Different substrates were added as required to this medium, which was always equilibrated with oxygen gas mixtures containing 5 % $CO_2$.

*Insulin.* Crystalline ox insulin (British Drug Houses) containing 23 i.u./mg was used. A stock solution was made up containing 500 m-u./ml., and stored for periods up to 3 months at −18° C as 2 ml. samples in small test tubes.

*Heparin.* Heparin (British Drug Houses) containing 100,000 u./g was used. The dose given was 1 ml. of a solution in NaCl 0·9 g/100 ml., containing 400 u./ml.

*Preparation of the heart for perfusion.* The rat was anaesthetized with ether, and 1 ml. of heparin solution was slowly injected into the femoral vein. After 2 min the thorax was opened and the heart quickly excised and placed in cold KBM. The aorta and branches were cleaned from connective tissue, and the aorta tied on to a glass cannula through which flowed KBM at room temperature. At this stage the heart commenced beating, and blood was quickly washed out of the coronary circulation. The heart, complete with cannula and rubber bung, was then fitted into the heart chamber of the perfusion apparatus, and perfusion commenced, using a pressure head of 54 cm $H_2O$, and a fluid volume of 60–70 ml.

*Estimation of glycogen.* Immediately after perfusion, the heart was taken off the cannula, all tissue above the auriculo-ventricular septum was removed, the ventricles were transected longitudinally and both halves were quickly blotted and weighed on a torsion balance. One half was minced in cold KOH solution, 30 g/100 ml., and the glycogen was separated and hydrolysed by the method of Good, Kramer & Somogyi (1933). The other half was dried overnight in the oven. The glycogen hydrolysate was neutralized with NaOH, 40 g/100 ml., and diluted to 50 ml. The glucose was estimated in this solution by the method of Nelson (1944).

## RESULTS

### *The time course of the oxygen uptake*

Figure 6 shows the change in $Q_{O_2}$ ($\mu$l. $O_2$/mg dry wt./hr) with time of a heart perfused with nutrient-free Krebs-bicarbonate medium (KBM) at 38·5° C and the accompanying changes in the coronary flow and effluent oxygen tension. There is a fall in oxygen consumption over the first 15 min of perfusion and, in parallel with this, a fall in the coronary flow. There is then a long period of steady oxygen consumption during which the coronary flow remains constant. Finally, there is a fall in oxygen consumption. During this time the force of beating of the heart diminishes visibly. This pattern was typical of hearts perfused in the absence of sub-

strate, but the time of onset of the eventual fall in the oxygen uptake varied considerably from heart to heart over the range of 40–90 min from the beginning of perfusion.

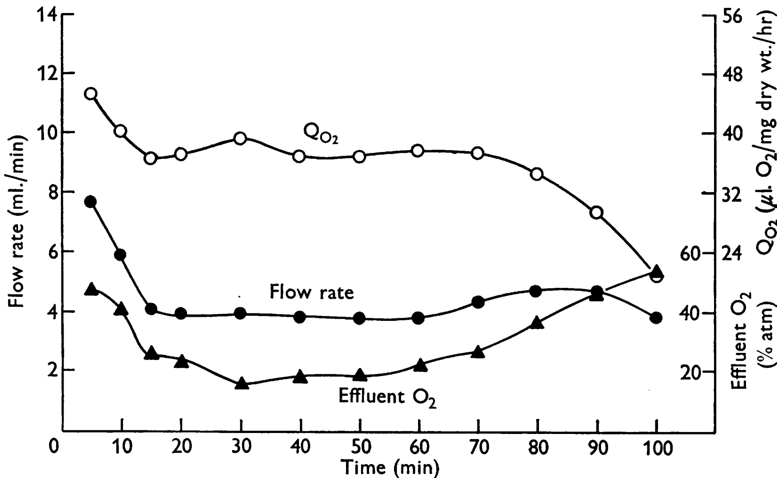


Fig. 6. Measurements at 5 min intervals of oxygen uptake ($Q_{O_2}$ ○), effluent oxygen tension (% atm ▲) and coronary flow rate (ml./min ●).

*Adequacy of oxygen supply*

The fact that there was regularly a measurable oxygen concentration in the coronary effluent (see Fig. 6) is some indication of adequacy of the oxygen supply. But it might be that the heart cannot extract oxygen efficiently below a certain oxygen tension in the fluid supplying it. Experiments of two kinds were made to test this point. In the first set erythritol tetranitrate (final concentration 10 mg/60 ml.) was added after 80 min to a heart perfused at 38·5° C with medium containing 8·3 mM glucose. The erythritol tetranitrate produced a considerable increase in coronary flow, but no systematic increase in the oxygen consumption (see Fig. 7). The effluent oxygen tension also increased considerably, since oxygen supply was greatly increased as the result of the larger coronary flow. In the second set of experiments 2,4-dinitrophenol (DNP) was added to the perfusate 30 min after the start of perfusion, to give a final concentration of $10^{-5}$M. As Fig. 8 shows, it produces a marked rise in the oxygen consumption and a fall in the effluent oxygen tension, without appreciably affecting the coronary flow. The first of these experiments shows that the heart does not extract more oxygen when the supply is increased by erythritol tetranitrate; the second shows that it can extract more from the normal coronary flow when its metabolism is stimulated by DNP. Thus the normal effluent oxygen tension is not so low as to limit oxygen uptake.

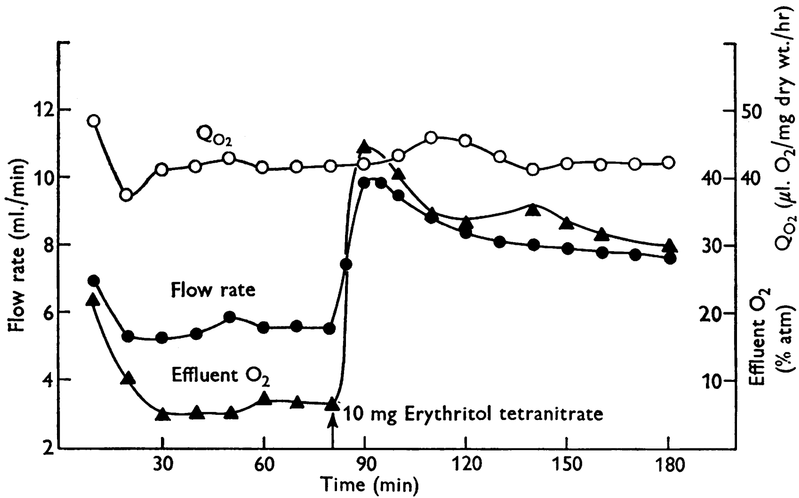


Fig. 7. The effect of erythritol tetranitrate (10 mg in 60 ml.) on $Q_{O_2}$ (○), coronary flow rate (ml./min ●) and on effluent oxygen tension (% atm ▲). The perfusate contained 8·3 mM glucose.

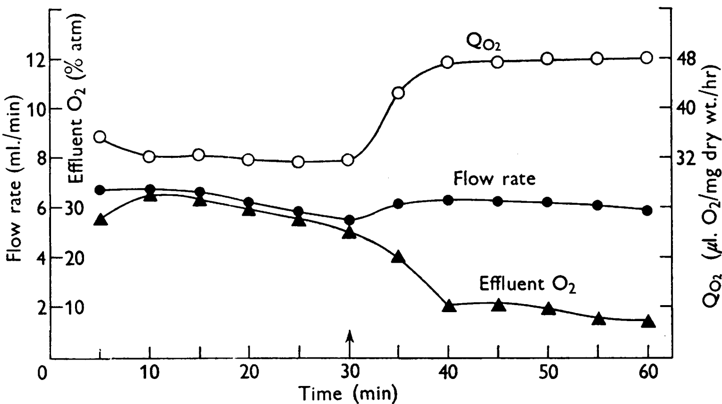


Fig. 8. The effect of 2,4-dinitrophenol (DNP, $10^{-5}$ M, at arrow) on the $Q_{O_2}$ (○), coronary flow rate (ml./min ●) and effluent oxygen tension (% atm ▲) of a heart perfused with 8·3 mM glucose.

*Completeness of equilibration of perfusate with gas mixture*

The two quantities measured are the oxygen concentrations in the coronary effluent and the coronary flow. The A–V oxygen concentration difference is calculated on the assumption that the perfusate returned to the heart reservoir is in equilibrium with the gas mixture used in the gas lifts. If there is an error in this assumption, i.e. if the perfusate is less than saturated, increase in coronary flow will produce a fall in the calculated

oxygen consumption. Also, if equilibration is imperfect, alteration of the oxygen content of the equilibrating gas mixture should change the calculated oxygen uptake. The experiments with erythritol tetranitrate show that the first of these effects does not occur, and several trials with changes of the oxygen tension of the gas mixture have failed to detect the second effect.

A third way of testing this point is to immerse a small sintered-glass gas-bubbler in the perfusate reservoir supplying the heart, and to examine the effect on the apparent oxygen uptake of passing the gas mixture through this as well as through the gas-lifts. No diminution in calculated oxygen uptake was observed when this was done.

*Effect of temperature on oxygen uptake*

The range of temperature studied was small. In view of the initial drop in oxygen consumption, and the decline in the oxygen consumption of some hearts perfused with nutrient-free medium after 40 min, the oxygen uptake in the period 15–40 min is taken as characteristic of the steady state of the heart. The mean $Q_{O_2}$ of eight hearts at 34° C was $32{\cdot}8 \pm 0{\cdot}9$, and the mean $Q_{O_2}$ of thirteen hearts at 38·5° C was $43{\cdot}9 \pm 0{\cdot}9$. In general it is undesirable to perfuse at the higher temperature, since, on occasion, the coronary flow may be too low to give a comfortable margin of oxygen supply. The increase in oxygen consumption with increase in temperature corresponds to a $Q_{10}$ of 2·31, which gives an estimated $Q_{O_2}$ of 38·8 at 37° C.

*The endogenous substrate for oxidation*

Experiments were performed in which hearts were perfused at 37° C for varying periods up to 60 min with nutrient-free KBM in the presence and absence of 2 m-u./ml. insulin. The glycogen contents of these hearts were then determined. The results, shown in Table 1, indicate that the glycogen content falls steadily throughout the first hour of perfusion, and that insulin has no effect on the course of this fall.

In other experiments hearts were made anoxic by perfusing for different periods of time with the perfusion fluid equilibrated with 5 % $CO_2$ in nitrogen before transferring the hearts to fully oxygenated perfusate, and continuing perfusion for a total time of 15 min. The results of glycogen determinations on these hearts are given in Table 2. In one series the hearts were anoxic for a standard time of 10 min, by which time they had all ceased to beat. On transference to the oxygenated medium beating recommenced within 1 min, and at the end of 5 min all the hearts were beating forcibly. In another series the hearts were kept anoxic until they ceased to beat, and were then immediately transferred to the oxygenated medium. Table 2 shows that the apparent amount of glycogen content of the hearts

was not dependent on the period of anoxia, but that in each case the glycogen content was very low, and at much the same level as the glycogen content of the hearts perfused aerobically for 60 min.

The apparent residual glycogen after 15 min anoxic or 60 min aerobic perfusion is not in fact glycogen. Determination of the apparent glucose in final hydrolysates by the glucose oxidase method (Huggett & Nixon, 1957)

TABLE 1. Time course of the glycogen disappearance from rat hearts perfused with nutrient-free medium at 37° C

| Time of perfusion (min) | Apparent glycogen content as glucose (mg/g dry wt.) | |
|---|---|---|
| | Nutrient-free medium | Nutrient-free medium containing insulin 2 m-u./ml. |
| 0 | 16·3 ± 1·1 (16) | 16·3 ± 1·1 (16) |
| 15 | 13·1 ± 1·8 (8) | 12·4 ± 1·7 (12) |
| 30 | 10·5 ± 1·3 (4) | 8·1 ± 1·0 (6) |
| 60 | 3·6 ± 0·9 (4) | 4·1 ± 0·9 (4) |

Standard errors of means are shown; number of experiments given in parentheses.

TABLE 2. Glycogen content of the rat heart after anoxia. The perfusion medium was equilibrated with 95 % $N_2$ + 5 % $CO_2$ during anoxia and the heart was transferred to a second perfusion apparatus equlibrated with 95 % $O_2$ + 5 % $CO_2$ and perfused for a total period (anaerobic + aerobic) of 15 min

| Apparent glycogen content as glucose (mg/g dry wt.) after 15 min perfusion | Duration of anoxia (min) |
|---|---|
| 2·1 | 10 |
| 3·1 | 10 |
| 2·7 | 10 |
| 2·4 | 10 |
| Mean = 2·3 ± 0·4 | |
| 2·0 | 4 |
| 3·5 | 8 |
| 3·5 | 7 |
| 3·5 | 7 |
| Mean = 3·2 ± 0·4 | |

Standard errors of the mean are shown.

has shown that there is consistently less glucose present than is indicated by the Nelson (1944) method. In a large series it has been shown that the mean difference is 2·73 + 0·21 (23) mg/g dry wt., and this difference is independent of total apparent glycogen in the heart. Most, if not all, of the apparent residual glycogen found in the experiments reported in Table 2 is therefore not glycogen.

*Effect of anoxia on survival*

Figure 9 shows the effect on the $Q_{O_2}$, flow rate, and effluent oxygen tension of changing the oxygen tension of the gas equilibrating the perfusion medium from 70 % saturation to 20 % saturation and back again. The $Q_{O_2}$ was reduced to about 40 % of its original value during the 20 min period of partial anoxia, but recovery was complete. The oxygen tension of the effluent fell from 20 % saturation to zero, indicating a complete

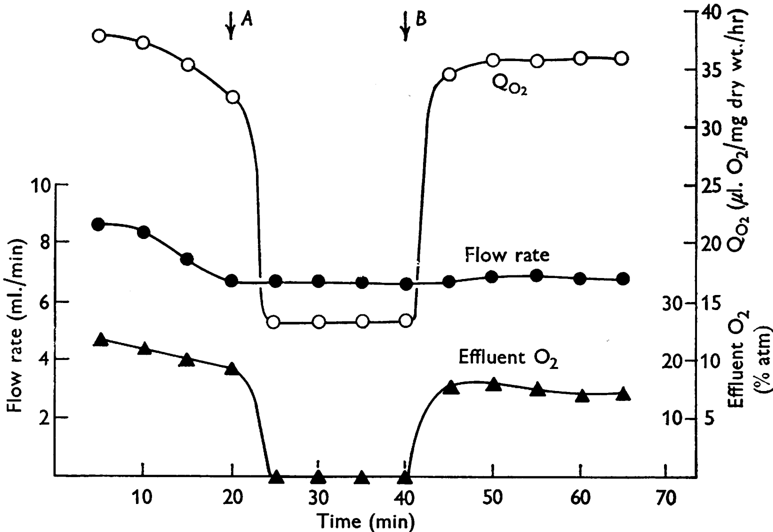


Fig. 9. The effect of altering the oxygen content of the equilibrating gas on $Q_{O_2}$ (○), coronary flow rate (ml./min ●), and effluent oxygen tension (▲). At the beginning and end of the experiment the gas is 5 % $CO_2$ in oxygen. Between arrows *A* and *B* it is 5 % $CO_2$ in air.

extraction of all the available oxygen, but the flow rate did not increase to compensate for the lower oxygen availability. In this experiment the perfusion medium contained 8·3 mM glucose, and the temperature was 37° C, but similar results were obtained with other perfusion media, and when the period of partial anoxia occurred at a later stage during perfusion.

In other experiments hearts were perfused with nutrient-free KBM equilibrated with 5 % $CO_2$ in nitrogen until they stopped beating, and were then transferred to the apparatus for determination of oxygen consumption and perfused for a further 60 min with the nutrient-free KBM equilibrated with 5 % $CO_2$ in oxygen. The time course of the oxygen consumption of these hearts is compared with that of hearts perfused from the beginning with oxygenated medium in Table 3.

All the hearts commenced to beat vigorously within 1 min after instituting aerobic perfusion but the mean $Q_{O_2}$ during the period 0–60 min is only about 70% of that of the controls over the corresponding period, although oxygen consumption in the two series of hearts follows similar time courses. The mean rate of oxygen uptake in both series of the experiments is fairly steady from 10 to 50 min of perfusion, but shows a marked fall after 50 min.

TABLE 3. Effect of anoxia on the oxygen consumption of the isolated rat heart perfused with nutrient-free medium at 37° C

| Time of perfusion (min) | $Q_{O_2}$ of normal hearts (ml./mg dry wt./hr) | $Q_O$ after anoxia (ml./mg dry wt./hr) |
|---|---|---|
| 5 | 45·2 ± 1·8 (22) | 29·8 ± 1·7 (4) |
| 10 | 42·7 ± 1·6 | 27·6 ± 1·6 |
| 15 | 40·0 ± 1·5 | 26·9 ± 1·7 |
| 20 | 39·4 ± 1·3 | 27·3 ± 1·5 |
| 30 | 38·5 ± 1·3 | 29·6 ± 1·5 |
| 40 | 37·5 ± 1·4 | 29·2 ± 1·3 |
| 50 | 36·8 ± 1·6 | 28·4 ± 1·2 |
| 60 | 34·9 ± 1·8 | 25·2 ± 1·0 |
| Mean 0–60 min | 39·4 ± 1·5 | 28·0 ± 1·4 |

Standard errors of the mean are shown; number of experiments in parentheses.

### *Effect of repeated anoxia*

The fact that hearts which had been exposed to anoxia for sufficient time to free them of glycogen would recover apparently completely, and resume a beat of normal rate and force, led us to make some experiments to test the effect of repeated periods of anoxia. The first heart studied ceased to beat after 6 min of anoxia, recovered rapidly when perfused with oxygenated medium, ceased to beat 5 min after switching to anoxic perfusate, recovered again within 5 min and continued to beat strongly for some time. Two further hearts treated similarly ceased to beat 1 min after the beginning of the second period of anoxia, recovered within 1–2 min after reinstituting aerobic perfusion 20–30 min later, but became feeble in approximately 20 min. Each of these hearts was exposed to nitrogen-equilibrated perfusate during the first period of anoxia for long enough to deplete it effectively of glycogen, so that it is difficult to see what source of energy was available to the last two hearts during their second prolonged period of anoxia. It seems probable that, as long as the heart is not performing external work, it can maintain the organization on which a propagated beat depends for some time after it is deprived of a source of energy, even when it is maintained at normal body temperature.

## DISCUSSION

The most important finding in this work is that when an isolated heart of the rat, an organ which might be expected to have a high metabolic rate, is perfused with a saline medium of low oxygen capacity, it is possible to provide a margin of oxygen supply over requirement, provided that the perfusion temperature is not too high. (The upper limit of temperature of adequate oxygenation is probably 38·5–39° C.)

The tests described of the adequacy of oxygenation of the medium show that the equilibration of the gas mixture with the perfusate must be very nearly complete in our conditions and that, in consequence, our estimates of oxygen uptake may be considered reliable.

The level of oxygen uptake of 37° C, 39 $\mu$l./mg dry wt./hr, is much in excess of figures quoted for heart slices (Pearson, Hastings & Bunting, 1949), though the hearts in our experiments are performing no external work.

The reason for the initial fall in oxygen consumption has not been determined. It is suggestive that there are parallel falls in coronary flow and heart rate. All these changes could be accounted for by destruction or elution from the heart of adrenaline.

The maintenance of the oxygen consumption for a considerable period of perfusion with a simple saline perfusate implies that there is available a large supply of endogenous nutrient. The mean glycogen content of hearts before perfusion is 13–14 mg/g dry wt. (data of Table 1 corrected for non-glucose reducing matter measured by the Nelson method). Complete oxidation of this would supply the heart with substrate for 14–19 min at the observed rates of oxygen consumption. As the respiration rarely falls off seriously within an hour, the majority of the substrate must be something other than glycogen and probably other than carbohydrate.

Measurements of glycogen after different times of perfusion show that it falls off linearly with time, disappearing after the first hour. During this time its metabolism would account for 20–30 % of the oxygen consumed. It is of interest, that when hearts are made anoxic initially, the whole of the glycogen disappears and the oxygen uptake when the hearts are subsequently perfused aerobically is in the region of 70 % of that of hearts perfused aerobically throughout the experiment. It looks as though two independent kinds of oxidative metabolism were going on side by side.

A point of some interest arises when one compares the oxygen uptake of these hearts with other estimates of heart oxygen uptake in the literature. No other figures are available for the rat heart, but values are available for blood-perfused cat and dog hearts. Extrapolating the results of Evans & Matsuoka (1915) to conditions of zero external work, one gets a $Q_{O_2}$ of 14

for the dog heart. The data of Laurent, Bolene–Williams, Williams & Katz (1956) for the dog heart beating at 100/min and doing no external work yield a $Q_{O_2}$ of 13. A Langendorff perfusion of the dog heart used by van Citters, Ruth & Reissmann (1957) gives a $Q_{O_2}$ of 9 for the same conditions. Lorber (1953) found a $Q_{O_2}$ of 15 for the perfused cat heart in diastolic arrest. If we take it that the metabolic rate per unit mass of similar tissues is proportional to the third or fourth root of body weight (Brody, 1945), these data are comparable to the present figures for rat hearts. This suggests that neither the nutrients supplied by blood, such as lactate, nor such other major constituents as are missing from our saline perfusate, such as proteins and lipids, have any marked effect on the oxygen uptake.

It appears possible therefore that perfusion of larger—and more slowly metabolizing—hearts with oxygenated saline solution might supply them adequately for considerable periods of time, so long as they were not performing external work.

## SUMMARY

1. A method is described for the determination of the oxygen consumption of the saline-perfused rat heart.

2. The oxygen consumption, coronary flow rate and heart rate are found to fall during the first 20 min of perfusion. Thereafter they stay steady for approximately 40 min.

3. During this period the glycogen content of the heart declines linearly.

4. During the period of steady oxygen consumption the $Q_{O_2}$ at 37° C is 39 and the $Q_{10}$ of oxygen uptake is 2·3.

5. Perfusion for 4–10 min with saline perfusate equilibrated with 5 % $CO_2$ in $N_2$ results in complete disappearance of glycogen from the heart.

6. Hearts so treated show normal rate and force of beat within a few minutes of reinstitution of aerobic perfusion.

7. In a few instances it was shown that such hearts can withstand a second period of anaerobic perfusion, lasting as long as 20–30 min.

8. It is concluded (*a*) that the heart has a large reserve of oxidizable material other than glycogen and (*b*) that the cardiac organization on which the propagated beat is dependent is not readily disintegrated when metabolic energy supplies are withdrawn.

## REFERENCES

Bleehen, N. M. & Fisher, R. B. (1954). The action of insulin in the isolated rat heart. *J. Physiol.* **123**, 260–276.

Brody, S. (1945). *Bioenergetics and growth.* New York: Reinhold.

Carlson, F. D., Brink, F. & Bronk, D. W. (1950). A continuous flow respirometer utilizing the oxygen cathode. *Rev. sci. Instrum.* **21**, 923–932.

Connelly, C. M. (1957). Methods for measuring tissue oxygen tension; theory and evaluation: the oxygen electrode. *Fed. Proc.* **16**, 681–684.

DARBRE, A. (1957). A plastic homogenizer. *J. Physiol.* **136**, 2*P*.

DAVIES, P. W. & BRINK, F. (1942). Microelectrodes for measuring local oxygen tension in animal tissues. *Rev. sci. Instrum.* **13**, 524–533.

DICKINSON, C. J. (1950). *Electrophysiological technique*, p. 105. London: Morgan.

DIXON, M. (1951). *Manometric Methods*, 3rd ed. Cambridge University Press.

EVANS, C. L. & MATSUOKA, Y. (1915). The effect of various mechanical conditions on the gaseous metabolism and efficiency of the mammalian heart. *J. Physiol.* **49**, 378–405.

GIGUÈRE, P. A. & LAUZIER, L. (1945*a*). Le dosage polarographique de l'oxygène dissous dans l'eau de mer. *Canad. J. Res.* B, **23**, 76–83.

GIGUÈRE, P. A. & LAUZIER, L. (1945*b*). Analyse polarometrique de l'oxygène dissous avec une micro-electrode de platine. *Canad. J. Res.* B, **23**, 223–233.

GOOD, C. H., KRAMER, H. & SOMOGYI, M. (1933). The determination of glycogen. *J. biol. Chem.* **100**, 485–491.

HUGGETT, A. St G. & NIXON, D. A. (1957). Use of glucose oxidase, peroxidase and *o*-dianisidine in determination of blood and urinary glucose. *Lancet*, **273**, 368–370.

LAITINEN, H. A. & KOLTHOFF, I. M. (1941*a*). Voltammetry with stationary microelectrodes of platinum wire. *J. phys. Chem.* **45**, 1061–1079.

LAITINEN, H. A. & KOLTHOFF, I. M. (1941*b*). Voltammetric determination and amperometric titrations with a rotating microelectrode of platinum wire. *J. phys. Chem.* **45**, 1079–1093.

LAURENT, D., BOLENE-WILLIAMS, C., WILLIAMS, F. L. & KATZ, L. N. (1956). Effects of heart rate on coronary flow and cardiac oxygen consumption. *Amer. J. Physiol.* **185**, 355–364.

LONGMUIR, I. S. (1954). Respiration rate of bacteria as a function of oxygen concentration. *Biochem. J.* **57**, 81–87.

LONGMUIR, I. S. (1957). Respiration rate of rat-liver cells at low oxygen concentrations. *Biochem. J.* **65**, 378–382.

LORBER, V. (1953). Energy metabolism of the completely isolated mammalian heart in failure. *Circ. Res.* **1**, 298–311.

NELSON, N. J. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *J. biol. Chem.* **153**, 375–380.

PEARSON, O. H., HASTINGS, A. B. & BUNTING, H. (1949). Metabolism of cardiac muscle; utilization of $^{14}C$ labelled pyruvate and acetate by heart slices. *Amer. J. Physiol.* **158**, 251–260.

UMBREIT, W. W., BURRIS, R. H. & STAUFFER, J. F. (1949). *Manometric techniques and tissue metabolism*. Minneapolis: Burgess.

VAN CITTERS, R. L., RUTH, W. E. & REISSMANN, K. R. (1957). Effect of heart rate on oxygen consumption of isolated dog heart performing no external work. *Amer. J. Physiol.* **191**, 443–445.