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A NEW PERFUSION METHOD

BY J. R. VANE

From the Nuffield Institute for Medical Research, University of Oxford

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Recently, Gaddum, Peart & Vogt (1949) described a very simple perfusion system, similar to a method used by Richards & Drinker (1915) and Binet & Burstein (1945). The advantages of this system were its simplicity, the small bulk of the apparatus near to the animal and the *relatively* small quantity of blood in the external circulation. Its main disadvantages were (1) the perfusion pressure was not independent of the mean arterial blood pressure, (2) the system did not give an accurate measure of mean arterial resistance, (3) additional blood from a second animal was sometimes required, and (4) the temperature of the blood was not adequately controlled.

The method of Gaddum *et al.* was used as the basis for the apparatus to be described, originally designed to perfuse the vessels of the stomach. This passed through a number of modifications, and the first type to be described was evolved from many preliminary tests and was used successfully in well over one hundred perfusion experiments.

METHODS

Perfusion arrangement type I

The arrangement of the perfusion circuit is shown in block diagram form in Fig. 1. Blood from an artery of the cat was pumped by the cat's heart through a resistance (*R*) into a reservoir (*Q*) and from there to a measuring chamber. The flow of blood from the reservoir to the measuring chamber (*M*) was controlled by an electromagnetic tap (*T*). The blood in the measuring chamber was pumped by a Dale-Schuster pumping unit (*P*) (Dale & Schuster, 1928) into the artery of the organ to be perfused. The venous return to the heart remained intact.

Pumping and measuring unit. This consisted of three 'Pyrex' glass units, fitted together with ground glass joints (Fig. 2). The measuring chamber (*M*) was surrounded by a water jacket through which hot water was circulated from one unit of a double Dale-Schuster pump to maintain the blood at 37° C.

Blood entered through a side arm and was drawn through the valve V_1 (Guy Ross dental valve) into the valve chamber, to which the pulsations of the other

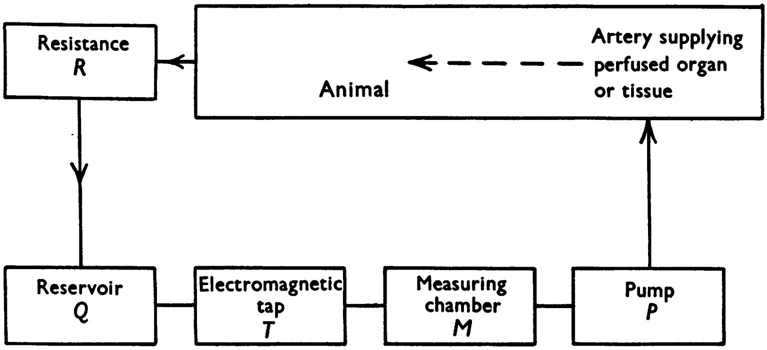

Fig. 1. Block diagram of the perfusion circuit type I. Blood is pumped by the cat's heart through an artificial resistance (R) into a reservoir (Q). The flow of blood from the reservoir to the measuring chamber is controlled by an electro-magnetic tap (T). The blood is pumped by the pump (P) into the artery supplying the perfused organ.

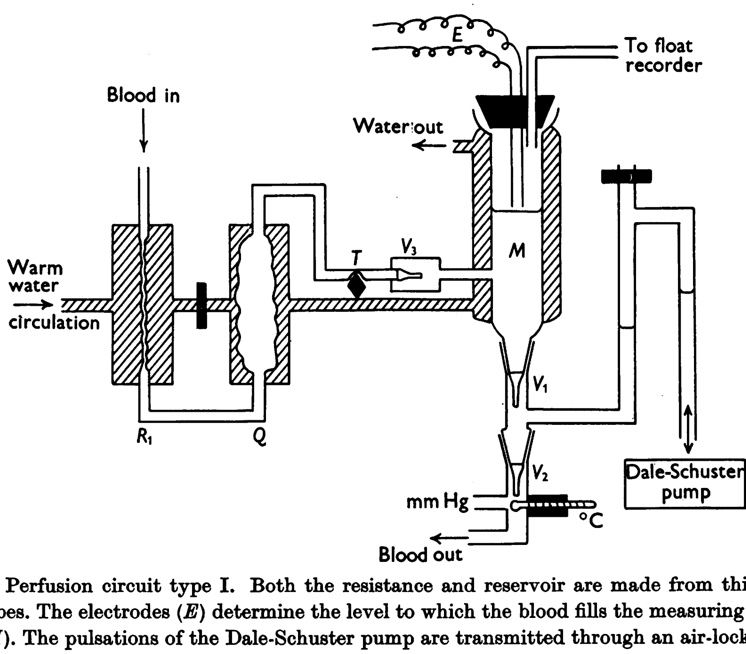

Fig. 2. Perfusion circuit type I. Both the resistance and reservoir are made from thin rubber tubes. The electrodes (E) determine the level to which the blood fills the measuring chamber (M). The pulsations of the Dale-Schuster pump are transmitted through an air-lock in order to conserve blood. The temperature and pressure of the blood are measured as it leaves the apparatus.

Dale-Schuster pump unit were transmitted through a length of 'Portex' tubing containing Ringer's solution. The blood was separated from the Ringer solution by an air lock, and was expelled through a second valve (V_2).

When the blood in the measuring chamber (*M*) reached the level of the electrodes (*E*) the inflow was stopped automatically by the electromagnetic tap *T* and the volume of blood leaving the chamber with each pump-stroke was registered by the float recorder on a smoked drum. The level of blood in the chamber was allowed to fall for 2, 4, 6, 8 or 12 pump-strokes, then the tap *T* opened and the chamber refilled to its initial level, determined by the position of the electrodes *E*. As soon as this level was reached the tap *T* closed again and the float recorder once more measured the outflow of blood from the chamber. The alternate emptying and filling of the chamber was controlled by the circuit shown in Fig. 3. With each pump-stroke a cam on the pump shaft

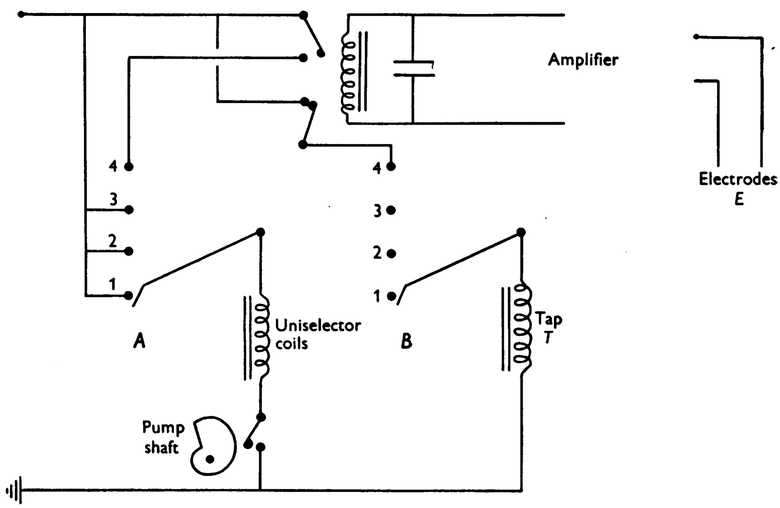

Fig. 3. The circuit used to control the emptying and filling of the measuring chamber (*M*). For details see text.

closed a pair of contacts and energized the coils of a telephone uniselector. Each impulse moved the arms of the uniselector on to the next contact on the two banks of contacts, *A* and *B*. At contact *A* 4, the arms stopped because the uniselector circuit was broken. As contact *B* 4 was made, the electromagnetic tap *T* was opened and blood entered the measuring chamber. The chamber filled until the surface of the blood joined the electrodes *E*; this energized a relay which broke the circuit of the springloaded electromagnetic tap *T*, so that the inflow of blood to the measuring chamber was stopped. At the same time the uniselector circuit through *A* 4 was completed by a second pair of contacts on the relay and the arms of the uniselector moved on to the next cycle. A condenser across the relay prevented 'chatter'.

The circuit was arranged so that the recording phase of the cycle could be altered to 2, 4, 6, 8 or 12 pump-strokes by a rotary switch. There was no interruption of the perfusion during the alternate emptying and filling of the measuring chamber as the Dale-Schuster pump worked continuously.

If the blood were pumped by the heart from the artery directly to the measuring chamber, the apparatus worked, but the resistance against which the heart pumped would suddenly decrease with each filling of the chamber and the amount of blood in the external circuit would vary. A reservoir was therefore needed which filled up with blood during the recording phase of the cycle, ready to discharge its contents into the chamber when the tap T opened; and a resistance was required to control the flow of blood from the cat into the reservoir.

The reservoir (Q, Fig. 2) was made from a tube of thin rubber. The outer jacket was included in the hot water circulation to keep the blood warm.

The resistance (R). The first type of resistance used (R_1) is shown in Fig. 2. Water was pumped round the outside of a thin-walled rubber tube (made from a finger-stall) and maintained at a high pressure by limiting the outflow with a screw clip. The resistance to the flow of blood could be lowered either by opening the screw clip or by decreasing the stroke of the water pump. The following arrangement ensured a free flow of blood from the reservoir to the measuring chamber when the spring-loaded electromagnetic tap was opened. The design of the tap was such that in the open position it partially constricted the hot-water circulation between reservoir and measuring chamber. Consequently, the water pressure round the rubber bag (Q) increased, transferring the blood from the reservoir to the measuring chamber.

The resistance (R_1) was sometimes unreliable as the flow of blood to the perfusion circuit was dependent upon the cat's blood pressure; it needed constant attention whenever a change of blood-flow or general blood pressure occurred. A pressure-reducing valve whose action was independent of changes in blood pressure or rate of blood flow was desirable. A design based on the same principle as a carburettor float chamber ultimately proved to be satisfactory.

The valve (R_2) (Fig. 4) was made from Perspex and consisted of a float chamber and lid. A ping-pong ball acted as a float. If the float rose, the needle valve tended to close and stop the inflow of blood. If the float fell, the valve opened and allowed more blood to enter the chamber. Except at its tip, which was conical, the needle valve had a triangular cross-section. Blood filled the reservoir through the valve (V_3). This system reduced the systemic arterial blood pressure to a (constant) few centimetres of water.

Float recorder. A Palmer float recorder of 5 ml. capacity was modified by using a light lever with a frontal writing point, pivoted from a new fulcrum above the metal float (Fig. 5). The frequency response is thereby improved and the sensitivity increased; the writing point traces a vertical line on the kymograph and the zero is at the bottom, instead of the top of the record. The

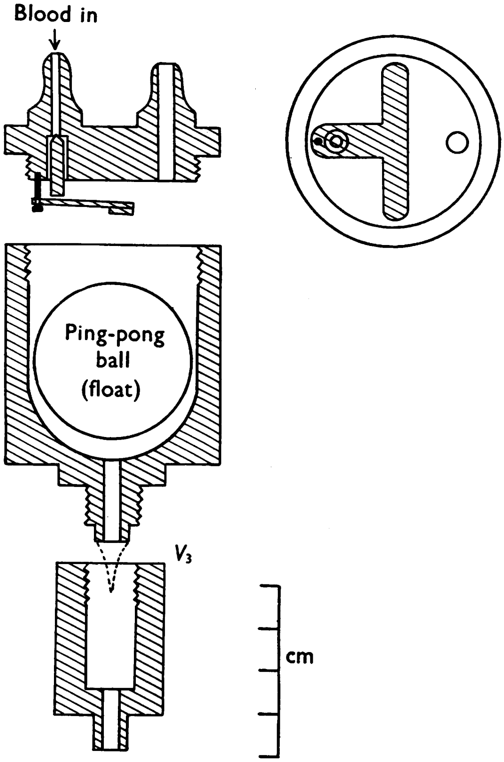

Fig. 4. Pressure-reducing valve (resistance R_2). Except at its tip, which is conical, the needle valve has a triangular cross-section. Top right: underside of lid showing shape of lever. For details, see text.

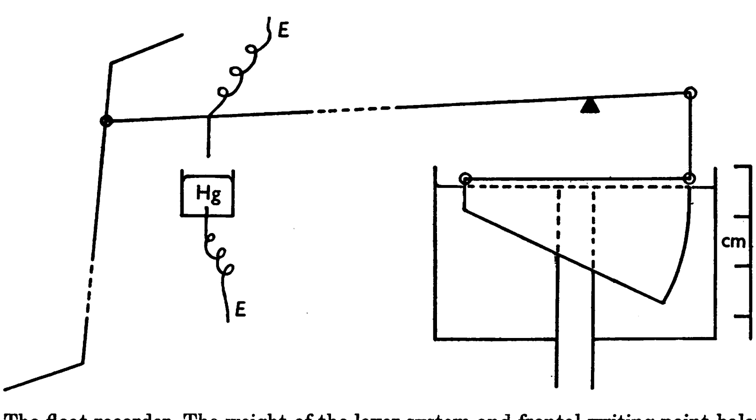

Fig. 5. The float recorder. The weight of the lever system and frontal writing point balances the weight of the float. The electrodes (*E*) which determine the level of blood in the measuring chamber can be transferred to the lever of the float recorder.

electrodes (E) were transferred from the measuring chamber to the lever of the float recorder (Fig. 5). As the blood refilled the chamber, the lever fell until the contact dipped into the mercury cup. The amplifier was then no longer required to activate the relay in Fig. 3.

Procedure. The whole of the perfusion system was mounted on a trolley so that it could be assembled and tested away from the operating table. The pumping and measuring unit (*P.M.*) was mounted on a platform jutting out from the side of the trolley, so that it could be pushed over the operating table. All glass parts of the perfusion apparatus were kept in chromic acid solution overnight. The apparatus was assembled on the trolley and filled with 0·9% (w/v) NaCl. The perfusion cannula was connected to the input side of the resistance (R_1 or R_2) and the saline solution was circulated. The three Pyrex units were held together by rubber bands slipped over small glass pegs, and, by gently easing the ground glass joints apart, any air in the valve chambers was allowed to escape. The apparatus was washed 3–4 times with fresh saline and then filled with Dextran solution. When this had reached a temperature of 37° C, the apparatus was ready for use. The capacity of the external circuit was 20–30 ml., equivalent to 10–15% of the blood volume of a cat weighing 2·5–3·5 kg.

As the apparatus measured directly the volume of blood entering the perfused organ it was necessary to calibrate the float recorder for volume only once. Because of the tendency for the lever of the float recorder to overshoot, any error in the measurement of flow overestimated the flow. Using saline in an artificial perfusion circuit and recording the rate of outflow with a measuring cylinder and stop-watch the estimated volume of flow never exceeded the actual value by more than 2·5% for flow rates between 20 and 90 ml./min and 5% for rates of 10–110 ml./min.

Perfusion arrangement type II

The apparatus described in the previous section was used in many successful experiments (Thompson & Vane, 1951, 1952, 1953) but because it was complicated and difficult to use the apparatus was simplified by omitting the reservoir (Q) and the measuring chamber (M). In the pressure-reducing valve, blood is automatically kept at a constant level in the float chamber and the pump can draw blood directly from the chamber. The modifications made in the apparatus are depicted in Fig. 6.

The glass valve chambers of type I apparatus were replaced by ones turned from Perspex, with screw junctions between each other and the pressure-reducing valve. Small air-release valves were provided in each valve-chamber. The pressure-reducing valve was made with a central needle and by using a smaller ball (2 cm diameter) the size of the chamber was greatly reduced. The capacity of the whole of the apparatus when assembled was only

7 ml., including the blood in the h-piece, which was attached to the valve chamber by Portex tubing. The upper part of the unit could be enclosed within a small Perspex cylinder through which warm water was circulated.

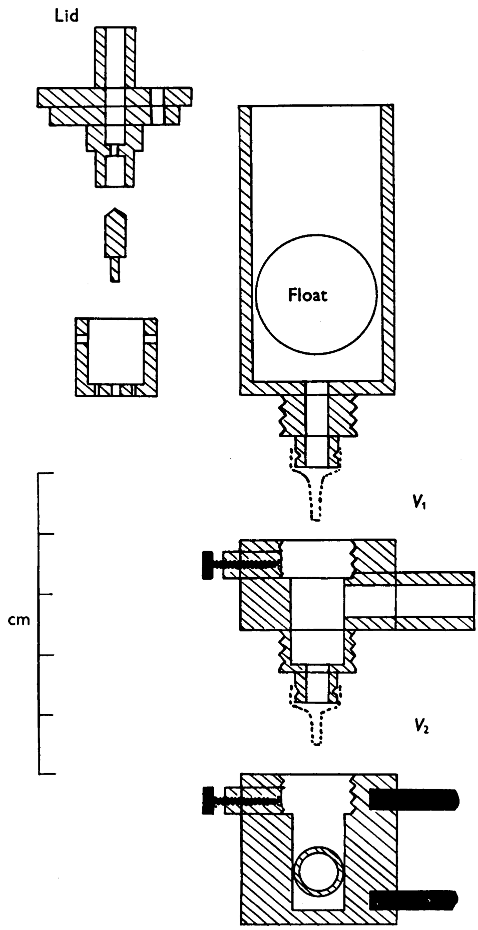

Fig. 6. Perfusion pump type II. The needle valve is in the centre of the lid and is held in place by a small cup. Air can be removed from the valve chambers by opening the small screw-valves. The pulsations of the Dale-Schuster pump are transmitted through an air lock to the side arm of the upper valve chamber. Two brass plugs fixed to the lower valve chamber can be used to mount the pump on a duraluminium panel.

The main problem with this improved perfusion apparatus was the measurement of the rate of flow of blood and at first it seemed impossible to do this by a direct measurement of volume. Consequently, the rate of blood flow was measured indirectly using a venturimeter and a differential manometer. The venturimeter (Wagoner & Livingston, 1925; Daly, 1926) was turned from Perspex, and a differential manometer to write directly on a smoked drum was made by mounting a pair of metal bellows in opposition (Lawson, 1940). The venturimeter and differential bellows recorder were included in the perfusion circuit just before the blood entered the perfused organ. The working capacity of this whole apparatus (type II A) was 10–15 ml., a considerable improvement over type I apparatus. At the end of each experiment blood was pumped through an artificial resistance and the flow recorder was calibrated. When perfused with this apparatus the stomach secreted acid juice (pH $< 1{\cdot}0$) for 3–6 hr in response to an intra-arterial infusion of histamine ($15\,\mu$g/min).

The disadvantages of this type of flow recorder were that it could only be calibrated at the end of the experiment and that changes in viscosity of the blood during the experiment affected the calibration. Moreover, in spite of large doses of heparin it was found that changes in the calibre of the venturi constriction occurred because of deposition of fibrin. It was also difficult to assess the rate of flow during the experiment as the height of the record was approximately related to the square of the flow rate. To overcome these drawbacks without forfeiting the many advantages afforded by the improved perfusion machine presented a difficult problem. A return to the principle of making actual volume measurements was necessary and, in collaboration with Dr G. S. Dawes, a new flow recorder was devised. This is described elsewhere (Dawes, Mott & Vane, 1953). It gives a direct measurement of the rate of flow of blood in terms of volume and time.

This combined pump and flowmeter (apparatus type II B, working capacity 12 ml.) proved very satisfactory and has been used for perfusing the vessels of the stomach, intestines, hind-limbs and lungs of the cat, and the coronary arteries and internal mammary arteries of the dog. It was convenient to use a hot-air box instead of a hot-water circulation to keep the blood at 37° C. The perfusion pump and flowmeter were mounted on a duraluminium panel and totally enclosed by a Perspex cover. The box so formed ($13 \times 13 \times 4$ cm) was heated by a small thermostatically controlled heater.

The pulse pressure produced by these pumping systems was high, sometimes double the 'normal' pulse pressure of an anaesthetized cat. By direct measurement with condenser manometers, it was found that the pulse pressure could be effectively reduced to 'normal' values by the inclusion of a small air buffer (2·0 ml.) in the perfusion circuit.

SUMMARY

1. The development of a new type of perfusion apparatus is described.

2. The first system had a capacity of 20–30 ml. and was used in many successful experiments.

3. The final perfusion arrangement had a much smaller working capacity

(10–15 ml.), was easier to use and could handle flow rates up to 150 ml. blood per min at physiological pressures. The temperature was maintained at 37° C and the rate of blood flow was measured directly.

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