METHODS

DETERMINATION OF FLOW RESISTANCE (VASCULAR TONE) BY A PERFUSION PUMP METHOD

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One of us has previously described the method of recording flow resitance (vascular tone) by stabilizing the flow rate with a special perfusion pump, and the necessary conditions which this apparatus is required to fulfil have been reviewed [1].

In transferring blood from the proximal to the distal end of the cut artery supplying the region being investigated, the pump is required to maintain a constant minute volume which is independent both of the arterial pressure at the input of the pump and of the tone of the perfused vessels. The perfusion pressure recorded at the output of the pump is then a function of flow resistance, and may be taken as an index of vascular tone.

It is important that the pump should be made to reproduce the physical characteristics of normal blood flow without causing any change in physiological composition which might react on the blood vessels. This means that trauma to the blood must be reduced to a minimum, that the flow must be pulsating, and that the initial values of the average and pulse perfusion pressures should approximate the normal values for these quantities in the particular animal investigated.

The PN-2 perfusion pump described below (Figs. 1a and 1b)* has been specially designed to record vascular tone, and consists of three main portions: the drive to the pump A, the working head of the pump B, and the recording mercury manometer C; these are connected hydraulically and electrically.

We will now consider one complete working cycle of the pump.

Blood from the central end of the artery passes along pipe 1 through the input chamber 2 ("auricle") to enter the thin-walled polyethylene pipe 3 ("ventricle") which is placed inside the working head. The chamber 4 of the working head is made of transparent plastic filled with water, and is connected by pipe 5 with piston pump 6 of the input. Valves 7a and 7b are electromagnetic relays operated from pulses generated by a cam through contacts 8a and 8b which in turn are closed by the eccentric wheel 10 turned by pinion 9. The armatures of the relays can close the short rubber tubes completely.

Suppose valve 7a (Fig. 1a) is open, so that blood is free to flow into tube 3, due to the sucking action of the piston in cylinder 11. Valve 7b is closed and prevents blood flowing from tube 3 to the peripheral end of the artery and so to the perfused organ. As soon as piston 6 (operated by cam 12 and lever system 13) reaches the end of its travel, contact 8a closes and valve 7a closes sharply, thus cutting off the perfused artery from the rest of the apparatus. Almost immediately afterwards, contact 8b breaks, valve 7b opens, and as piston 6 moves downwards it pushes water into the chamber of the working head, causing blood to move along tube 14 into the vessels (see

* Engineers N. M. Bardier and I. I. Berger helped in the construction and design of the apparatus.

Fig. 1b). When the piston reaches the lower end of its travel, valve 7b closes sharply, then valve 7a opens, and the piston begins to more upwards, causing a further supply of fresh blood to be sucked in, etc. The cycle now repeats.

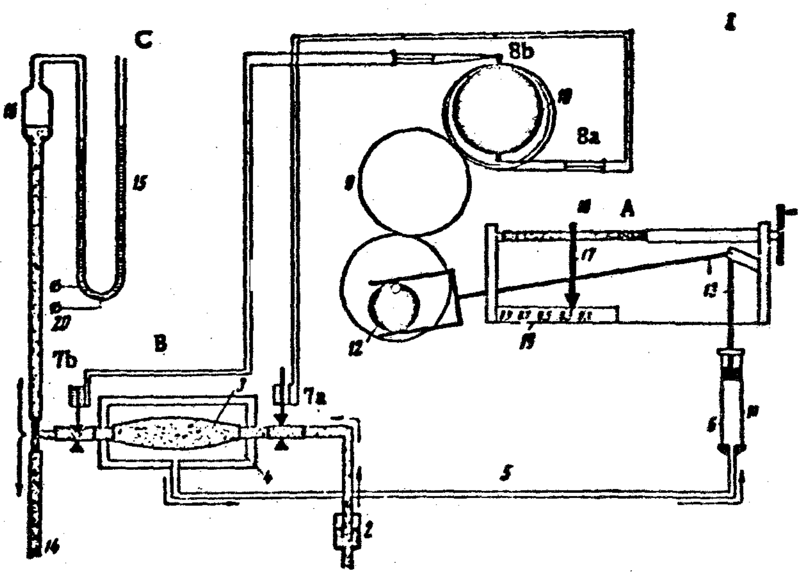

Fig. 1a. Diagram of apparatus PN-2 in the position of "diastole".
A) Drive unit; B) working head; C) recording mercury manometer.

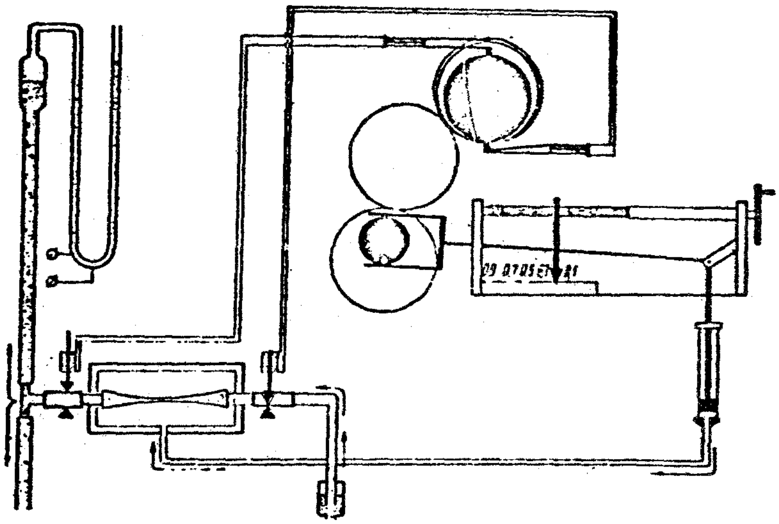

Fig. 1b. Diagram of apparatus PN-2 in the position of "systole".

Correct phasing of the piston and valve action is ensured by a control of the contact mechanism (not shown in Figs. 1a and 1b), which allows the time of opening and shutting of each valve and their operating periods to be adjusted both relative to each other and to the phase of the piston.

Thus during each cycle, a certain volume of blood, corresponding ideally to the difference in volume of tube 3 in the initial and in the compressed condition, passes from the input to the output of the pump, i.e. from the central to the peripheral end of the artery. The resulting perfusion pressure is recorded by the mercury manometer 15, and is determined by the ratio of the conductivity (or resistance) of the vessels to the minute volume. If the vessels contract, some of the blood enters the manometer causing an increase of perfusion pressure so that it is possible to maintain the original minute volume through the contracted vessels. On the other hand, if the vessels dilate blood passes from the manometer so that the same minute volume is maintained under a smaller pressure. Changes in the perfusion pressure are proportional to the change in resistance (tone) of the vessels.

Chamber 16, which corresponds to the aorta, is included to reduce the pulse pressure variations; it has a capacity of about 15-20 ml and is partly filled with air; its volume determines the extent to which the pressure variations are damped out.

The cylinder and piston are those of an ordinary injection syringe. Preliminary coarse adjustments are made for syringes of 1, 2 or 5 ml according to the organ to be perfused. Fine regulation within a range of 1 : 10 is effected by moving the pivot 17 by the screwed rod 18. This gives continuous variation of piston travel with corresponding control over the degree of compression of tube 3 in the working head, and thus gives control of stroke volume which is indicated on scale 19.

The output per minute is equal to the product of the stroke volume and the pulsation frequency. The reduction gear to the crank allows the frequency of the pulsation pressure to be set at 90, 120, 150, 180, 240 or 300 per minute. Either the stroke volume or the pulsation frequency may be changed while the pump is working, without stopping it.

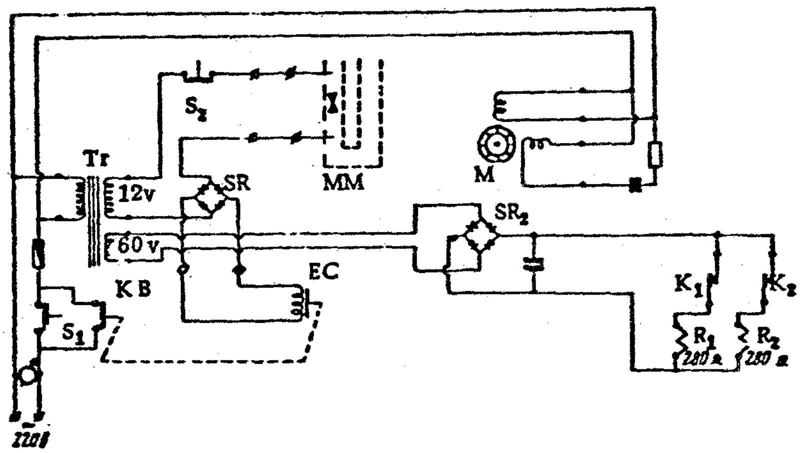

Fig. 2. Electrical circuit of apparatus PN-2.

A particular feature of this instrument is that the output is independent both of changes in the arterial pressure at the input to the pump, and of changes in resistance in the outflow. This is due partly to the use of external valves, and partly to the rigidity of the hydraulic system. The positively operated external valves are free from the defect of the internal self operated type, of allowing leakage of blood through them. The use of electromagnetic relays is particularly effective: the small mass of the armature results in a complete and rapid action at high pulsation frequencies, and independent adjustment eliminates any leakage. The hydraulic system consists of a completely rigid cylinder with its piston and the compartment 4. The wall of the fluid duct is made of semitransparent chlorvinyl tubing, made rigid by treatment with ether, which dissolves out the plasticizing substances. The air contained by the water in the system is removed by boiling, and a layer of vaseline is poured over the piston to prevent its entrance through the cylinder. The transparency of the cylinder, connecting tube and working head affords a complete check on the absence of air bubbles.

The only parts which are not rigid are the rubber connections under the relay armatures. However these are short and for most of their length are covered externally by metal sleeves. It must be noted that the walls of these connections show no wear after several dozen experiments lasting many hours.

To avoid the effect of fluctuation of the line voltage, a *DVA-U4 electric motor* with a large excess of power is used, and this maintains a constant rate of revolution when the line voltage falls. The latter is monitored by a voltmeter mounted on the drive assembly.

Tests showed that the pump produced very nearly the required constant output. A sharp jump in the input pressure of 150-180 mm of mercury caused only a 2-3% change in the output, which was therefore almost independent of variations in the arterial pressure of the animal. If the resistance to the output changed by an amount equivalent to a jump in input pressure from 10 to 250 mm of mercury, the output over the whole of the regulated range did not change by more than 2-3 %.

To avoid the ejection of mercury from the manometer through excessive increase in perfusion pressure, the motor was connected to the reduction gear through an electromagnetic clutch connected via contacts 20 of the recording manometer (see Fig. 1a and 1b). When the perfusion pressure exceeds 300 mm, the mercury column moves, and so opens the contact, thus causing the pump to stop and both valves to open.

The electrical circuit is shown in Fig. 2 and operates as follows. When contacts S_1 ("start") are closed, the mains voltage is supplied to the primary winding of the transformer Tr and to the motor M. The voltage of the secondary winding is applied through the normally closed contacts of the switch S2 and the mercury manometer MM to the selenium rectifier SR, the output from which is applied to the electromagnetic clutch EC. The latter, when it operates, holds itself by closing contacts KB on switch S1.

The clutch EC and motor M continue to operate until contacts KB are again opened. This occurs when the stop switch S2 is opened manually or when the manometer contacts are broken. The pump stops almost instantaneously since the clutch EC makes a mechanical break in the cam drive to the piston from the motor M, which has considerable inertia. At the same time the primary of the transformer is open-circuited, so that current no longer flows from the rectifier through contacts K1 and K2 on the drum to the electrically operated valves R1 and R2, which therefore open.

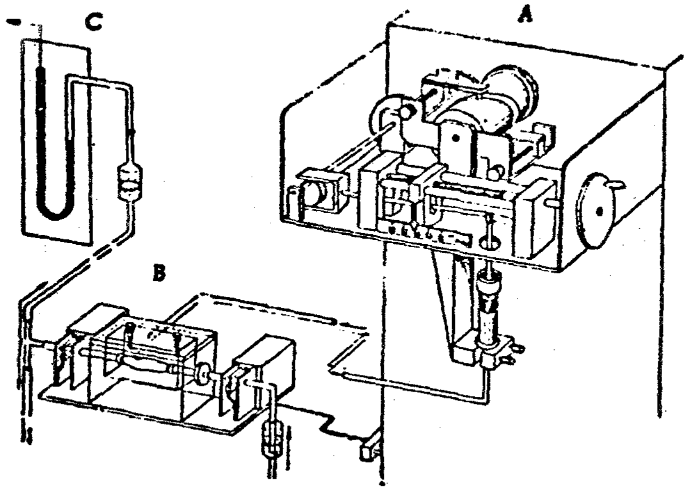

Fig. 3. General view of apparatus PN-2 (semi-diagrammatic). A, B, and C as in Fig. 1a.

All the mechanical and electrical components of the drive assembly (Fig. 3A) are mounted in a vertical housing. To ensure maximum accessibility, the cam and contact mechanism are built onto a removable unit attached to the front wall of the housing, and enclosed by a covering of transparent plastic.

Building the apparatus in separate units allows the working head to be brought right up to the blood vessel, and thus reduces the volume of blood outside the body to as little as 6-8 ml. Therefore the blood spends a comparatively short time in the artificial circuit, and there is no need to make provision for maintaining the temperature. It was shown that with a flow rate of 20-25 ml/minute the temperature does not fall by more than 0.5-1°C.

Before the experiment, the tubes which will contain blood are filled with warm physiological saline. After injecting heparin and inserting the canulae into the appropriate vessels the valves of the pump are opened. The saline is replaced by blood. The manometer 15 (see Fig. 3, C and Fig. 1a) records the collateral arterial pressure (with the pump disconnected from the drive). An appropriate pulsation frequency is chosen by engaging a suitable gear ratio, and the indicator on scale 19 is set to the required stroke volume.

After the pump has been switched on by pressing the "start" button, the output is gradually increased by turning the wheel on the control thread 18 until the average perfusion pressure is as nearly as possible equal to the previously recorded arterial pressure. The output per minute for the given perfusion pressure is then given by the product of the pulsation frequency and the readings shown on scale 19. If necessary, the amplitude of pulsation of the perfusion pressure can be regulated by changing the amount of air in the damping chamber 16.

After the experiment all the parts containing blood are washed out, first with water, then with sodium hydroxide, and finally with water again. Before the next experiment, physiological saline is passed through the apparatus for 10-15 minutes. If necessary the apparatus may be sterilized chemically by passing alcohol etc. through it. It is not necessary to filter the blood before it enters the vessels of the perfused organ. In an experiment lasting 4-7 hours, a single injection of 7.5-10 mg/kg of heparin (activity approx. 100 units per mg) is sufficient.

Many tests and experiments have shown that the apparatus described here satisfies the requirements for measuring flow resistance (vascular tone) outlined at the beginning of this article.

If the pump is used for other purposes, its action may be smoothed by introducing the required amount of air into the working head.

SUMMARY

A pump was designed for measurement of the resistance (tone) of vessels by the method of perfusion, by way of the stable minute blood volume, taken from the animal undergoing the measurement (autoperfusion), or from a donor. The pump maintains a stable output.

The pump provides the pulsation of perfusion pressure with the frequency of 90, 120, 150, 180, 240 and 300 per minute. When perfusion pressure is more than 300 Hg the pump automatically stops.

The apparatus consists of three separate units; the drive, the removable working head and the resistering mercury manometer.

This is attained by: a) ensuring rigidity of the hydraulic drive, and b) using distantly controlled external electromagnetic valves with a range of output regulation of from 3 to 120 ml per minute. The volume of blood contained in the pump is 6 to 8 ml.

LITERATURE CITED

[[1] V. M. Kalutin, "Recording vascular tone by an autoperfusion method." Fiziol. Zhur. SSSR 1958.