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A MEDIUM FOR PERFUSION OF INNERVATED BLOOD VESSELS

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Interest in drugs which lower blood pressure has increased with the use of these agents in treating human hypertension. To determine whether such drugs act by causing vasodilatation as the result of peripheral vascular action, perfused blood vessel preparations are usually employed. By perfusing limbs or organs which retain only nervous connexions with the remainder of the animal it is possible to examine on the perfused vessels not only the direct effects of drugs but also effects mediated by the nervous system.

Homologous blood is almost the only medium at present suitable for such perfusions, but, although it preserves the reactivity of the nervous connexions, it has disadvantages. The most important of these is that, for reasons of economy, it is usually necessary to recirculate the blood, so that any drug tested remains in the perfusate and may interfere with subsequent tests. Furthermore, mechanical trauma inseparable from recirculation and reoxygenation of the blood may lead to haemolysis, with the liberation of vasoactive substances (Chambliss, Demming, Wells, Cline, and Eckstan, 1950; Binet and Burstein, 1950).

This paper reports the use of a perfusion medium which maintains nervous reactivity, but which is without some of the disadvantages of homologous blood.

Preparation and Properties of Perfusion Medium

The medium consists of a suspension of red blood cells in Krebs–Henseleit Ringer solution containing 6% dextran.

Both human and sheep red cells have been used. The former were the residue from the preparation of plasma, and the latter were obtained from citrated sheep's blood recently collected at the slaughterhouse. The use of red cells from a different species does not seem to interfere with the vascular reactivity of the preparation in short-term experiments, provided that the cells are washed free of citrated plasma. This was done by centrifuging 250 ml. of cells in 1 litre of 0.9% (w/v) NaCl for 30 min. After a second wash with saline there was a final wash with the Ringer solution of Krebs and Henseleit (1932), buffered with bicarbonate to *p*H 7.4. The washed packed red cells were then suspended in Krebs–Henseleit solution containing 6% dextran (average mol. wt. 75,000) to give a packed cell volume of 40%. The medium was oxygenated by bubbling oxygen containing 5% CO_2 through it. After equilibration, the *p*H was checked with a calomel and glass electrode *p*H meter.

Oxygen Capacity and Availability.—The oxygen capacity of six samples oxygenated in this way was measured by a microvolumetric technique to be reported elsewhere (Saunders, unpublished); it averaged 18 vol. % with a range of 15–20 vol. %. The oxygen seemed to be readily available, since the venous effluent was considerably darker than the medium before perfusion.

Oedema Formation.—If the venous return remained unobstructed, no oedema of skin or muscle was observed in experiments lasting up to 3 hr. In the absence of dextran, however, oedema occurred within 15–30 min. Unlike other colloids, such as gum acacia, dextran does not appear to contain vasoconstrictor substances; no vasodilatation was produced by ACh (10 μg.) injected into the perfusate after the nervous tone had been removed by section of the nervous connexions (Fig. 1).

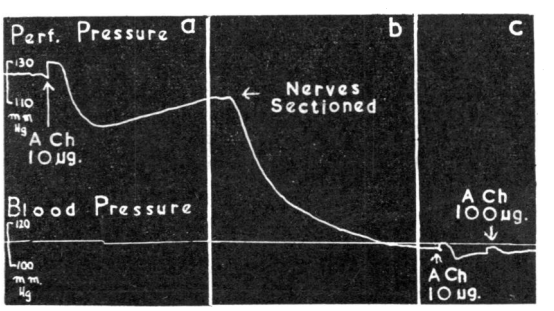

FIG. 1.—Rat, hind quarters perfused independently; simultaneous records of arterial blood pressure and perfusion pressure. (a) ACh 10 μg. injected into perfusate, followed by (b) division of nerve supply to limb, (c) ACh 10 and 100 μg. injected into perfusate.

Maintenance of Vascular Reactivity to Nervous Influences.—The hind-limbs of rabbits or the hind-quarters of rats, isolated from the rest of the circulation but with intact nervous connexions, were perfused by the method of Fastier and Smirk (1947). In this, the perfusion fluid is passed to the arterial supply of the perfused area by a constant-output rotary pump. Variation in the peripheral resistance gives rise to alterations in pressure which are recorded on a smoked drum with a Hg manometer.

It was consistently possible to demonstrate the presence of peripheral vascular tone of nervous origin in the perfused limb vessels. Section of the nerve supply to the limb was always followed by vasodilatation. For the maintenance of nervous reactivity it seems important that the *p*H should be kept between 7.2 and 7.4; alterations beyond this range resulted in a rapid loss of demonstrable tone from the perfused area.

The preservation of nervous reactivity was demonstrated by the injection of test substances into the general circulation of the animal. Raising or lowering the systemic blood pressure with intravenous injections of adrenaline or ACh gave rise to changes in the perfusion pressure of the perfused limb. When the systemic pressure of the rat or rabbit was raised by adrenaline, there was a fall in the perfusion pressure, whereas the fall of systemic blood pressure elicited by ACh was associated with a rise in the peripheral vascular tone of the perfused limb. Since the perfused area was isolated from the rest of the body except for nervous connexions, the changes in perfusion pressure produced by adrenaline or ACh could be mediated only through the nervous system.

Asphyxia, produced by clamping the trachea of rats, caused a rise in systemic blood pressure accompanied by a rise in the vascular tone of the perfused limb.

Use of Medium in Investigation of Vasodilator Actions

Results obtained with hexamethonium bromide illustrate the utility of the perfusion medium for the study of depressor agents of this type. Six experiments were performed on rabbits with the perfused hind-limb having only nervous connexion with the rest of the body. Hexamethonium bromide (1 to 5 mg.) dissolved in 0.1 ml. of Krebs–Henseleit solution at *p*H 7.4 did not produce vasodilatation in the perfused limb when injected into the fluid entering the perfusion cannula, whereas ACh (10 μg.) produced vasodilatation with a fall of perfusion pressure of 40 to 60 mm. Hg when given by the same route. When hexamethonium bromide (10 to 15 mg.) was injected intravenously, however, there was a fall of systemic blood pressure of about 40 mm. and a simultaneous fall in perfusion pressure of from 60 to 80 mm. Hg (Fig. 2). The fall in perfusion pressure was presumably

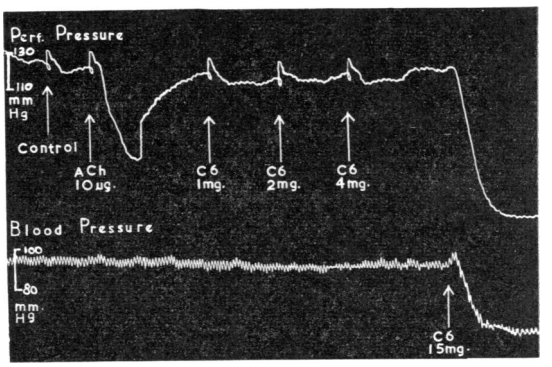

FIG. 2.—Rabbit, hind-limb perfused independently; simultaneous records of arterial blood pressure and perfusion pressure: Injection of ACh (10 μg.) and of hexamethonium bromide (1, 2, and 4 mg. C_6) into perfusate; injection of hexamethonium bromide intravenously (15 mg. C_6).

due to a decrease in nervous tone, since subsequent section of the nerves did not produce any additional fall in perfusion pressure.

Summary

1. A new perfusion medium is described. It is a suspension of washed human or sheep red cells in Krebs–Henseleit Ringer solution containing 6% dextran.

2. It preserves nervous reactivity in perfused isolated innervated blood vessel preparations, and may therefore facilitate the study of the actions of hypotensive drugs.

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