Upsala J Med Sci 78: 145–149, 1973

An in-vitro Method of Investigating the Rates of Transcapillary Exchange and Net Filtration by Single-injection Technique

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ABSTRACT

A method of preparing a cat's hind leg that is suitable for studying the rates of transcapillary solute and solvent movements is described. The preparation may be perfused at any desired venous pressure. A bolus may be injected into the capillary network without altering either the perfusion pressure or the flow rate (while continuously recording the weight of the isolated leg). A new method of calculating extractions is introduced. This method uses the estimate of the correlation between the relative concentrations of reference and test substances in the venous outflow.

INTRODUCTION

The Starling concept of paracapillary fluid circulation is well known. However, in studies of the movement of uncharged molecules from within the capillary to the extravascular space of the tissue, the effect of a transcapillary fluid flow on diffusion has all too frequently been ignored. One result of this omission has been that in explanations of the transcapillary separation between uncharged molecules of different sizes the possibility of the diffusion being modified by bulk flow has often been overlooked.

This report describes an experimental approach suitable for examining whether the effects of convection on diffusion may be disregarded in accounting for the transport of uncharged solutes in the capillary bed of a hind leg isolated from a cat.

METHODS

Preparation

Cats were anaesthetized with chloroform and Nembutal® (30 mg/kg body weight intravenously). An amount of heparin just sufficient to prevent coagulation (about 10 000 IU) was administered. After the paw was removed, a circular incision with a cautery was made in the skin just below the inguinal ligament and just above the greater trochanter. By blunt dissection, all muscular bundles were freed and cut down to the hip joint. Ligatures were carefully tied around each muscle to prevent bleeding. With the exception of the femoral artery and veins, all vessels were ligated separately.

After the leg was exarticulated, the animal was bled. Mean-while the femoral artery and vein of the leg were cannulated, the pareparation was laid on a balance (with an accuracy of 25 mg), and attached to the perfusion equipment, as shown in Fig. 1 *a* and *b*. From a reservoir the thermostated-controlled and oxygenated blood of the cat was circulated by a variable-speed peristaltic pump through the arterial and venous vessels, by-passing an injection loop, and returned to the reservoir, by-passing a sampling loop. The mean blood-perfusion flow was about 15 ml/min. Papaverin (usually around 60 mg) was introduced into the blood to produce a maximal dilatation of the blood vessels of the preparation, which was reached when an additional dose of papaverin did not further decrease the perfusion pressure.

The venous pressure depended on the lengths and diameters of the outflow catheters.

Apparatus

The experiment was started with the venous blood flowing through C (Fig. 1 *b*). When the leg weight was constant and the venous pressure stable, an injection was given by switching the arterial blood flow through the injection loop, which contained the bolus (tube A, Fig. 1 *b*). As soon as possible after the injection, the outflow was switched to route C–D–E for sampling. About 30 samples were taken during the venous passage of one bolus, which on the average lasted for 20–60 sec.

By changing the venous outflow route from C to F–D with E closed, the venous pressure increased. This increase was controlled by varying the length and/or the diameter of the catheter. This procedure caused the tissue to become oedematous. After a few seconds, when the leg weight increased at a constant speed and the venous pressure was again stable, a new bolus was injected and collected by opening E.

The bolus in the injection loop comprised a mixture of five parts of homologous blood, one part of glucose and raffinose (about 930 mM of each) and one part of 3 mg/ml Evans blue in physiological saline, which served to label the albumin. The injection loop contained about 1.3 ml.

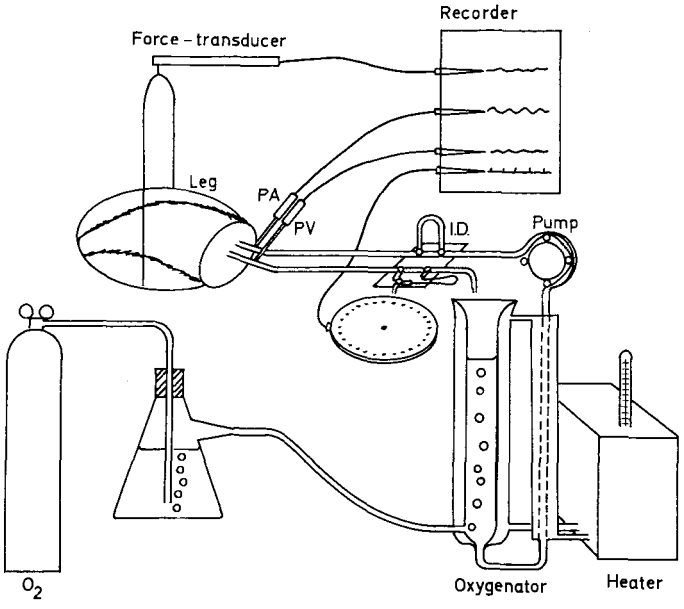

Fig. 1 a. A schematic drawing of the experimental set-up. The hind leg of a cat is placed on the balance and the arterial and venous cannulae are connected to the femoral artery and vein. Leg weight (oedema formation) and arterial and venous pressures (PA and PV) are measured, and the fraction collector is connected to the event marker on the recorder for measuring sampling time. The thermostate-controlled and oxygenated blood is circulated through the leg via the injection and sampling device (ID) shown in Fig. 1 *b*.

Analyses

Serial samples of the venous outflow, each about 250 µl in volume, were collected in small, weighed glass vials, which were then weighed again and immersed in 2.00 ml of physiological saline in centrifuge tubes. After the mixing, aliquots were analysed for glucose by a glucose-oxidase method (1, 6) and for raffinose by determining fructose after a Seliwanoff reaction (8). The remainder was centrifuged and the supernatant analysed for Evans blue at 610 nm in a Zeiss PMQ spectrophotometer.

The three measurements mentioned above were made on (*a*) blood samples drawn prior to the bolus injection ("blanks"), (*b*) the bolus itself, and (c) the serial samples from the venous outflow.

CALCULATIONS

The calculations were carried out with the help of a digital computer (CD 3600 or IBM 370) and were presented in the following forms:

1. Venous-outflow concentration curves, as shown in Fig. 2, in which the concentrations were given as relative to the concentrations in the injection solution (relative concentrations).

2. The relative concentrations of raffinose and glucose plotted against the relative concentration of albumin labelled with Evans blue for each sample, as shown in Fig. 3. The plots appeared to lie on straight lines, and the regression coefficients were calculated according to Bartlett (2). The lines pass close to the origin, which means that the regression coefficient equals c_d/c_r, where c_d is the concentration of the diffusible and c_r that of the reference substance.

3. Extractions calculated according to the formula $E = (c_r - c_d)/c_r$ or $1 - c_d/c_r$ (3, 4, 5). Thus, extraction can be calculated from the regression coefficient in Fig. 3. Extraction expresses the fraction of the total amount lost from the bolus or any part thereof, which was eliminated from the blood through the capillary wall.

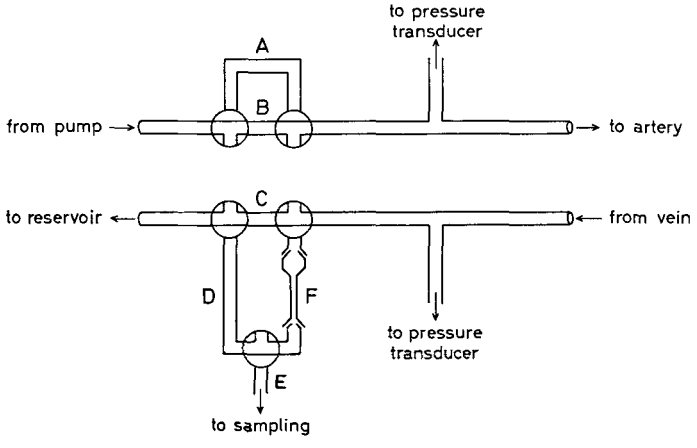

Fig. 1 b. The injection and sampling device. Upper catheter is "arterial". Blood goes via either route A (injection) or route B. Below the "venous" catheter is shown; Route C, free flow; Route C–D–E, sampling in free flow. Route F includes a thin exchangeable catheter producing venous congestion with or without sampling.

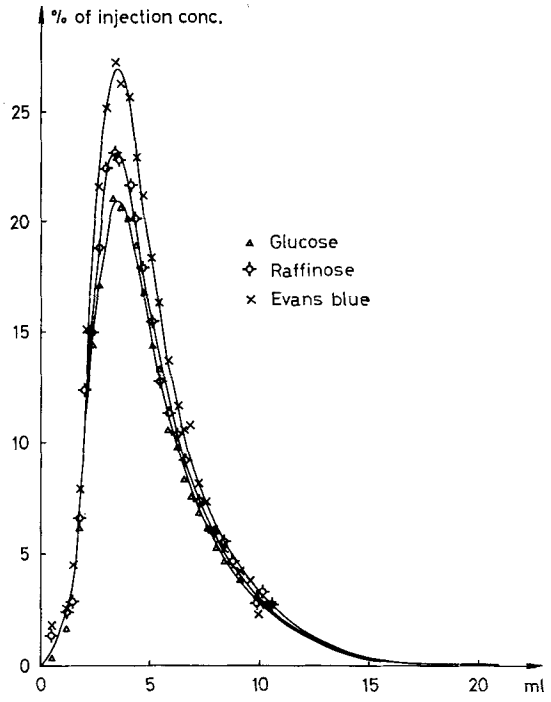

Fig. 2. The relative venous concentrations of albumin (tagged with Evans blue), raffinose and glucose in balanced-weight conditions (no net filtration), presented according to Chinard and Crone (4, 5, 6).

DISCUSSION

Methods

The preparation combines some of the qualities of those previously described (5, 7). One important problem is whether or not the perfused organ has suffered from the operation technique. Fortunately, most preparations were successful, in the sense that they were in weight balance (no oedema formation). This balance could be maintained for hours, provided the blood was well oxygenated. Occasionally, however, some preparations showed a slow but steady oedema formation, even when the venous pressure was negative. These preparations were discarded.

In order to obtain a homogeneous distribution of the bolus within the arterial vessel leading to an organ, the injection has to be fairly rapid. When this is given by a syringe, such a rapid injection is accompanied by a transient change in both blood pressure and flow. It cannot be safely assumed that this has any effect on the exchange between capillary and tissue. When the injection is given by a separate injection loop, such transients are avoided or at least made small enough not to be detected by the arterial-pressure transducer.

Although the bolus concentration is 540 mM and might therefore be expected to cause a loss of water from the tissue, only in one instance was there even a slight leg-weight decrease (about 3.4×10^{-3} g/sec, 100 g tissue). The explanation of this might be that the reflection coefficient for raffinose and glucose are so low as to have little osmotic effect across a capillary wall. These sugars might rapidly disappear from the blood stream, and the bolus thus behaves as if it were virtually isotonic in the exchange capillaries.

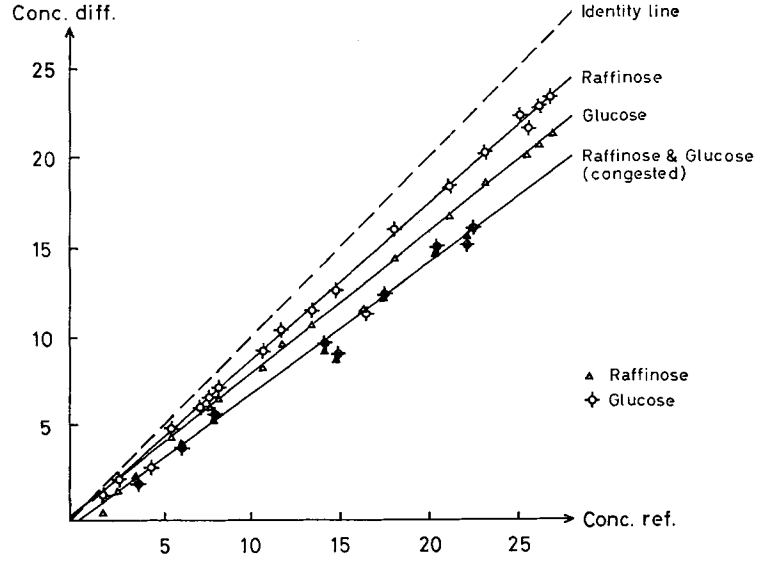

Fig. 3. The relative concentration of the diffusible molecule (conc. diff.) plotted against the relative concentration of the reference (conc. ref.). The continuous lines are the best fit of straight lines calculated according to Bartlett (3). The angles of inclination of these lines are used to calculate the extractions. Note that during rapid oedema formation (raffinose and glucose (congested)) the extrations of raffinose and glucose are of about the same magnitude.

Calculations

The slope of the regression line between reference and test-solute concentrations in the venous outflow is $\Delta c_d/\Delta c_r$. This ratio differs from the ratio c_d/c_r if the line does not pass through the origin.

There are three possibilities for c_d not to be zero when c_r is zero:

1. The diffusible substance passes back from the tissue to the blood (back diffusion or back transport). If this blood-tissue-blood transport were slower or faster than the intravascular passage of the bolus through the exchange vessels, there would be a time-lag between the venous appearance of the reference and test substance (compare the results in ref. (9)). In this case, the c_d/c_r ratios would not fall on a straight line but would form a loop. Fig. 3, however, showed no sign of such a loop.

2. The reference substance follows an axial stream in the blood, whereas the test solute does not. In that case, there would also be a different appearance time between the "faster" reference solute and the test solute. For the substances used, this possibility seems excluded.

3. The base-line in Fig. 2 has not been determined correctly. This would appear as a constant addition to the regression line (a parallel displacement of the regression line), if the concentration ratios fall on a straight line. In that case, the calculated ratio $\Delta c_d/\Delta c_r$ would probably be a more reliable estimate of the extraction than the ratio c_d/c_r.

The advantage of this extraction-estimate method (regression method) is its simplicity. It further gives information about the constancy of the extraction, it rules out the errors in concentration measurements at the beginning and the end of the venous-outflow-concentration curves, as these points bear little weight in calculating the regression line, and lastly it involves the possibility of excluding errors in base-line determinations.

Whatever method is used for calculating extractions, the values obtained will be erroneously low if the reference substance leaks out from the vessels. In that case, the concentration of the reference solute in the venous outflow must be corrected by multiplying by a correction factor (1 + the lost fraction of the reference substance). Whether or not such a leak of the reference substance used in the work described in this paper occurs will be further investigated.

Preliminary results

In the balanced-weight condition (when the perfused leg suffers no net change in weight during the bolus passage), the relative amounts of glucose and raffinose lost to the tissue seem proportional to their diffusion coefficients in free solution and the results seem to confirm those of Crone (5) (Figs. 2 and 3). In the experiment cited here, the (raffinose/glucose) extraction ratio amounted to 0.65, which equals the free diffusion coefficient ratio ($0.434/0.673 = 0.645$).

On the other hand, when there is venous congestion, the loss of both raffinose and glucose is increased. With increasing speed of oedema formation, raffinose is lost relatively more quickly than glucose, until the differences in their relative concentrations in the venous outflow finally vanish (Fig. 3).

The preparation described here and the regression method of calculate extractions is to be used in further studies of the transcapillary transport of raffinose and glucose, both in stable weight conditions and during oedema formation.

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Faculty, Uppsala University, the Swedish Medical Research Council (Project No. 14X-629) and the National Institutes of Health (Grant No. 5 RO1 HE 12960-08).

The authors also wish to express their gratitude to miss Carin Ferner for valuable technical assistance. This paper was translated by Neil Tomkinson.

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Received February 17, 1972
Revised Mars 15, 1973

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