

The Permeability of Capillaries in Various Organs as Determined by Use of the 'Indicator Diffusion' Method

By

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Abstract

CRONE, C. *The permeability of capillaries in various organs as determined by use of the 'Indicator Diffusion' method.* Acta physiol. scand. 1963. 58. 292—305. — The theory of a single injection technique, the 'Indicator Diffusion' method, for quantitative studies of capillary permeability is developed. It is shown that the permeability of a capillary area can be expressed by three parameters: the initial extraction (E) of test substances added in a single injection to the blood flowing to an organ, the blood flow (\dot{Q}) and the surface area (A) of the capillaries. The equation relating these figures is: $P = (\dot{Q}/A) \times \log_e 1/(1-E)$. The permeability coefficients of capillaries in kidney, liver, lung, brain and hind limb to inulin and sucrose are reported. It is found that the permeability of capillaries varies considerably from organ to organ. It is questioned whether the pore model adequately describes the functional characteristics of the capillaries in the muscles. The existence of pores should result in a pronounced deviation of the ratio between the permeability coefficients for sucrose and inulin from the ratio between the free diffusion coefficients. This was not found to be the case.

One of the most important activities of the vascular system occurs in the capillaries. Yet, our knowledge about the membrane across which the exchange between blood and interstitial fluid takes place is scanty. While many biological structures of very small size have been submitted to direct physiological study in later years, the capillaries have not yet yielded to a direct attack. Thus our knowledge of the permeability characteristics of the capillary wall has still to be deduced from experiments on a mass of capillaries. In the part

of capillary physiology dealing with permeability, quantitative studies have been advanced considerably by Pappenheimer and his coworkers in the last decade (PAPPENHEIMER 1953, RENKIN and PAPPENHEIMER 1957). This article is concerned with such quantitative studies and gives data for the permeability of the capillaries to two polar non-electrolytes (inulin and sucrose) in brain, lung, liver, kidney and hind limb.

Measurement of the permeability of capillaries *in situ* implies that the test substances come in contact with the capillary membrane. In actual experiments difficulties arise from the fact that two processes occur almost simultaneously: the test substances injected are diluted in the blood and diffusion occurs through the capillary wall. An experimental principle by which this problem may be solved was developed by CHINARD, VOSBURGH and ENNS (1955) and ANTHONISEN and CRONE (1956). These authors made a single injection into an afferent artery of an organ of a diffusible test substance together with a non-diffusible reference substance, which was assumed to remain in the capillaries. Immediately after the injection blood was rapidly collected from the vein draining the organ. The concentration of the reference substance in the individual samples is a measure of the degree of dilution at any time. The extent to which the concentration of the diffusible substance is less than the concentration of the reference substance is a measure of the loss of the test substance.

The loss thus determined must bear some relation to the permeability of the capillaries. It was for some time thought that the loss was a direct indicator of the permeability. However, this is not so; for the loss is not proportional to the permeability coefficient of the capillaries to the test substance. Nonetheless it is possible to derive a simple expression for the relation between permeability coefficient and the loss, as shown below. With this type of analysis the 'Indicator Diffusion' method, as the experimental approach will be called becomes useful for characterizing the physical processes of transcapillary exchange.

Recent investigations with the electron microscope show pronounced morphological differences of the capillary walls in different organs (BENNETT, LUFT and HAMPTON 1959, FLOREY 1961). This demonstration of their variability is borne out by the present work which gives evidence for differences in the permeability of capillaries in brain, lung, liver, kidney and the hind limbs. Inulin and sucrose were chosen as test substances because they are inert, easy to determine, do not penetrate the cells and are of appropriate molecular size (14.5 and 4.40 Å radius respectively).

Theory

Fig. 1 shows a time-concentration curve of the type obtained in the experiments. The curve for the concentration of the test substance (c_{test}) would be identical with that of the reference substance ($c_{\text{ref.}}$) if there were no loss

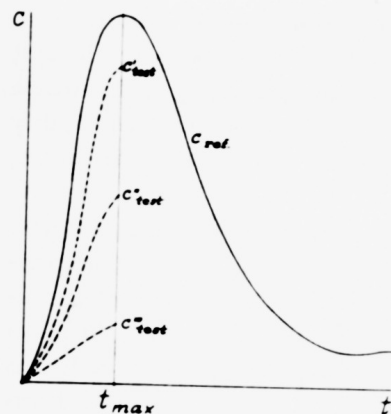


Fig. 1. The concentration variations in the effluent blood after a square wave injection of a mixture of substances into the afferent vessel of an organ. c_{ref} : non-dialysable reference substance, c_{test} : diffusible test substances.

during passage through the capillaries. The curve c_{test} indicates the actual concentration of the test substance(s), and the fractional loss is given by $(c_{ref} - c_{test})/c_{ref}$. (As it is impracticable to inject equimolar quantities of reference and test substances, the concentrations are expressed as fractions of those in the solution injected.) While the intravascular concentration is rising steeply the rate of outward diffusion is greatest. When the concentration is falling the net-diffusion decreases. The observations are made under conditions of continuous change and it is necessary to decide which samples can be used for estimating the average initial extraction. Only the rising part of the curve is considered in these calculations involving 3–5 samples from each experiment.

The initial extraction (E) of a test substance is not only dependent on the permeability of the capillaries, but also on the surface area of the capillaries and on the rate of blood flow.

The permeability (P) of a membrane to a given substance is the amount of substance which passes unit area in unit time for unit concentration difference across the membrane. Or, expressed in symbols:

$$P = \frac{dS}{dt} \times \frac{1}{A \times \Delta c} \quad (1)$$

dS/dt , the amount of substance passing the membrane in unit time, is determined as $\dot{Q} \times E \times c_a$, where \dot{Q} is the volume of blood passing through one gramme of tissue per second. E is the fractional reduction of the arterial concentration of the test substance during the conversion of arterial to venous blood. A is the surface of the capillaries in one gramme of tissue. Δc is the average concentration difference across the capillary wall.

The main difficulty in measuring the permeability of inaccessible biological membranes is the determination of Δc . An expression for Δc can, however, be derived if certain approximations are made. During the initial rapid rise of

intracapillary concentration, that outside is increasing comparatively slowly. The mean concentration difference (Δc) is therefore assumed to be equal to the mean intracapillary concentration. The mean intracapillary concentration can be expressed in terms of the concentration of the test substance at the inlet, c_a , and the concentration of the test substance at the outlet, c_v . The test substance passes through the capillary wall passively and the concentration therefore falls exponentially from the arterial to the venous end of the capillary. Thus, the mean concentration is given by $(c_a - c_v)/(\log_e c_a - \log_e c_v)$. E is by definition equal to $(c_a - c_v)/c_a$. By inserting in equation (1) the following relation is obtained:

$$P = \frac{\dot{Q}}{A} \times \log_e \frac{1}{1 - E} \quad (2)$$

By means of this expression it is now possible to calculate the capillary permeability from data obtained by the 'Indicator Diffusion' method. The term, E , is based on figures obtained from the experiments. It is not practicable to measure the tissue perfusion, \dot{Q} , because the experiments last less than a minute; and therefore values for this term were selected from the publications of other workers. The capillary surface area, A , was calculated from various anatomical measurements of capillary density in different tissues (see *Appendix* for further details).

Experimental Technique

The experiments were performed on mongrel dogs which were fasted overnight. Anaesthesia was obtained by intravenous injection of Sodium Pentobarbitone (Nembutal), *c.* 25 mg/kg. A cannula was inserted into the afferent vessel of the organ concerned without interfering with the blood supply to the organ. Another cannula fitted to a polythene catheter was introduced into the efferent vessel just before the injection. The injection took 1—2 seconds, and usually 2—5 ml of solution were used. The out-flowing blood was collected in small heparinized glass tubes mounted on a slowly moving Kymograph (ASMUSSEN and NIELSEN 1952). Usually 0.3—0.5 ml blood was collected in each sample. The solution injected contained Evans' Blue Dye in amounts between 2—5 mg depending on the dilution volume (greatest in experiments on lungs). A solution of inulin or sucrose was mixed with the dye solution. When the test substance was inulin sodium chloride was added to obtain isosmolarity. An aliquot of the injection solution was diluted in heparinized blood taken from the animal immediately before the injection. This mixture was later analyzed and thus the exact concentrations in the injection solution were determined. Immediately after the experiment the small test tubes were stoppered with corks covered with paraffin wax and rotated gently. The determinations of the concentration of the dye and of the test substances were carried out on blood rather than on plasma. Thus it was unnecessary to use correction factors to account for material lost in the erythrocytes.

Determination of concentration of dye and of test substances:

a) *Evans' Blue Dye:* 0.2 ml blood was diluted with 0.8 ml 0.9 % sodium chloride, centrifuged and the concentration in the supernatant was determined by way of a

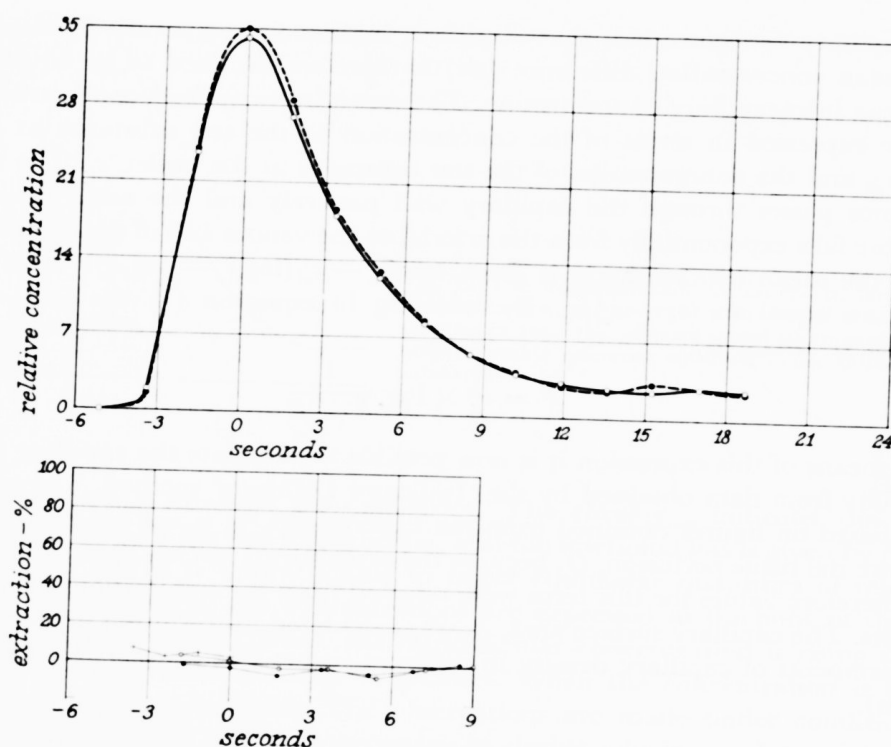


Fig. 2. *Inulin experiment on brain.* The upper part of the figure shows time-concentration curves of Evans' Blue Dye and of inulin. Sampling from the superior sagittal sinus after injection into the common carotid artery. Evans' Blue Dye: ○—○, inulin: ●-----●. The time for maximum concentration is arbitrarily called 0-time. The lower part of the figure shows the extraction of inulin in separate samples from three experiments.

spectrophotometer (Beckman model DU). Semimicro cuvettes were used because the amounts of supernatant were small. The cuvettes were cleaned between each reading as the remaining fluid after emptying a semimicro cuvette is a large proportion of the total volume.

b) *Inulin and sucrose* were analyzed as described by BOJESSEN (1952). The volumes originally proposed by him were reduced to one tenth, and the hydrolysis and colour development took place in 2 ml glass ampoules the tips of which were sealed after introduction of reagent and sample. Inulin and sucrose added to blood in known amounts were recovered to 96.8 and 97.0 per cent respectively, with standard deviations of 0.56 mg/100 ml and 0.40 mg/100 ml.

Calculation and treatment of data: All concentrations are expressed relatively, i. e.: that in each sample is divided by that in the injection fluid. For each sample the relative concentration of the test substance is divided by that of the reference substance giving a value between 0 and 1. By subtracting this value from 1 the extraction of the test substance in that particular sample is obtained. The coefficient of variation of this final value is estimated to be less than 3 %.

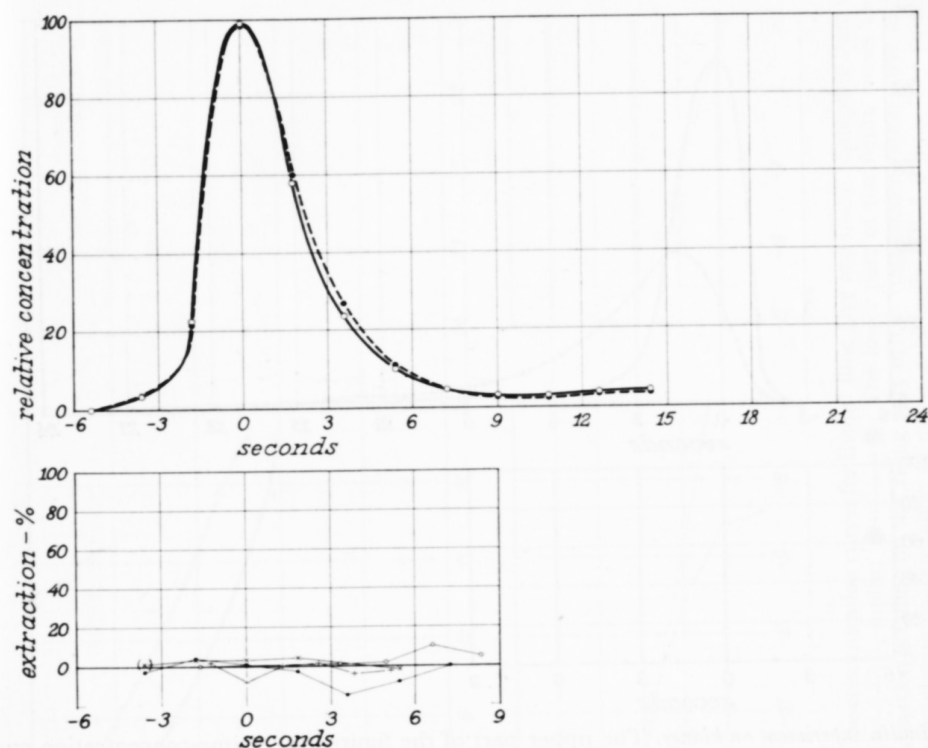


Fig. 3. *Sucrose experiment on brain.* The upper part of the curve shows time-concentration curves for Evans' Blue Dye and for sucrose. Evans' Blue Dye: ○—○, sucrose: ●---●. The lower part of the figure shows the extraction of sucrose in separate samples from four different experiments. The point in brackets represents a sample in which the concentration of dye was very low (extinction < 0.100).

Results

1. Brain.

Inulin. The results of an experiment are given in fig. 2. The curve for the test substance and the reference substance fall closely together, indicating that the brain capillaries are highly impermeable to polysaccharides. Three experiments were done and the extraction of inulin in each sample is shown in the lower part of Fig. 2. The mean extraction was 2.5 % (S. D.: 3.5, $n = 8$). In two experiments on human subjects a mean extraction of 0.5 % was found (the test solution was injected in the common carotid artery and cerebral venous blood was collected from a needle inserted in the bulb of the internal jugular vein).

Sucrose. Similar results were obtained with sucrose which is also a polar molecule but much smaller than inulin. Fig. 3 shows that even such small molecules are not lost appreciably from the blood during the passage through the brain.

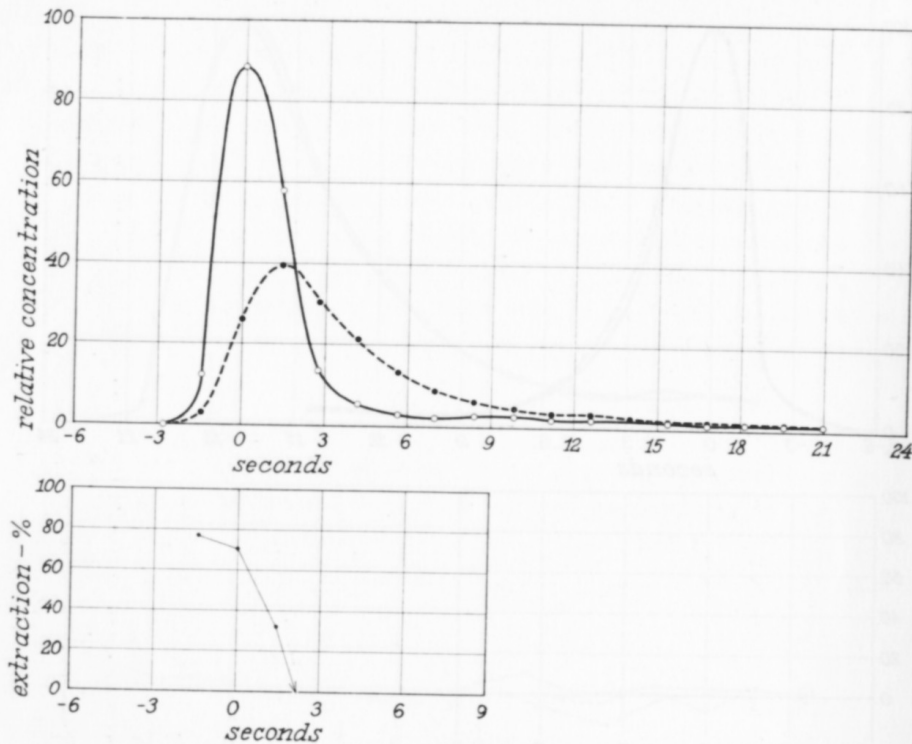


Fig. 4. *Inulin experiment on kidney.* The upper part of the figure shows time-concentration curves for Evans' Blue Dye and inulin. Sampling from a catheter in the renal vein after injection into the renal artery. Evans' Blue Dye: \circ — \circ . Inulin: \bullet ----- \bullet . The lower part of the figure shows the extraction of inulin in the separate samples.

Fig. 3 also shows the extraction in the individual samples. The mean extraction in the initial phase was 0.4 % (S. D.: 4.0, $n = 8$). One of the experiments was carried out in an animal which had sustained two periods of severe anoxia provoked by inhalation of pure nitrogen in 1.5 min. The experiment was performed immediately after the second period of anoxia. Contrary to what had been expected no change was observed in the extraction of sucrose. Histological examination showed swollen, chromatolytic ganglion cells in the cortex and in the basal ganglia. These observations confirmed that the brain had been severely anoxic.

2. *Kidney.*

FREIS *et al.* (1958) published an extensive series of investigations of the permeability of the renal capillaries to inulin. Similar results were obtained in the experiment illustrated in Fig. 4. The high initial loss of inulin is followed very quickly by a falling extraction and a net return occurs within a few seconds. The mean extraction in the two initial samples was 73.1 % (the above mentioned authors found an average loss of 79 %).

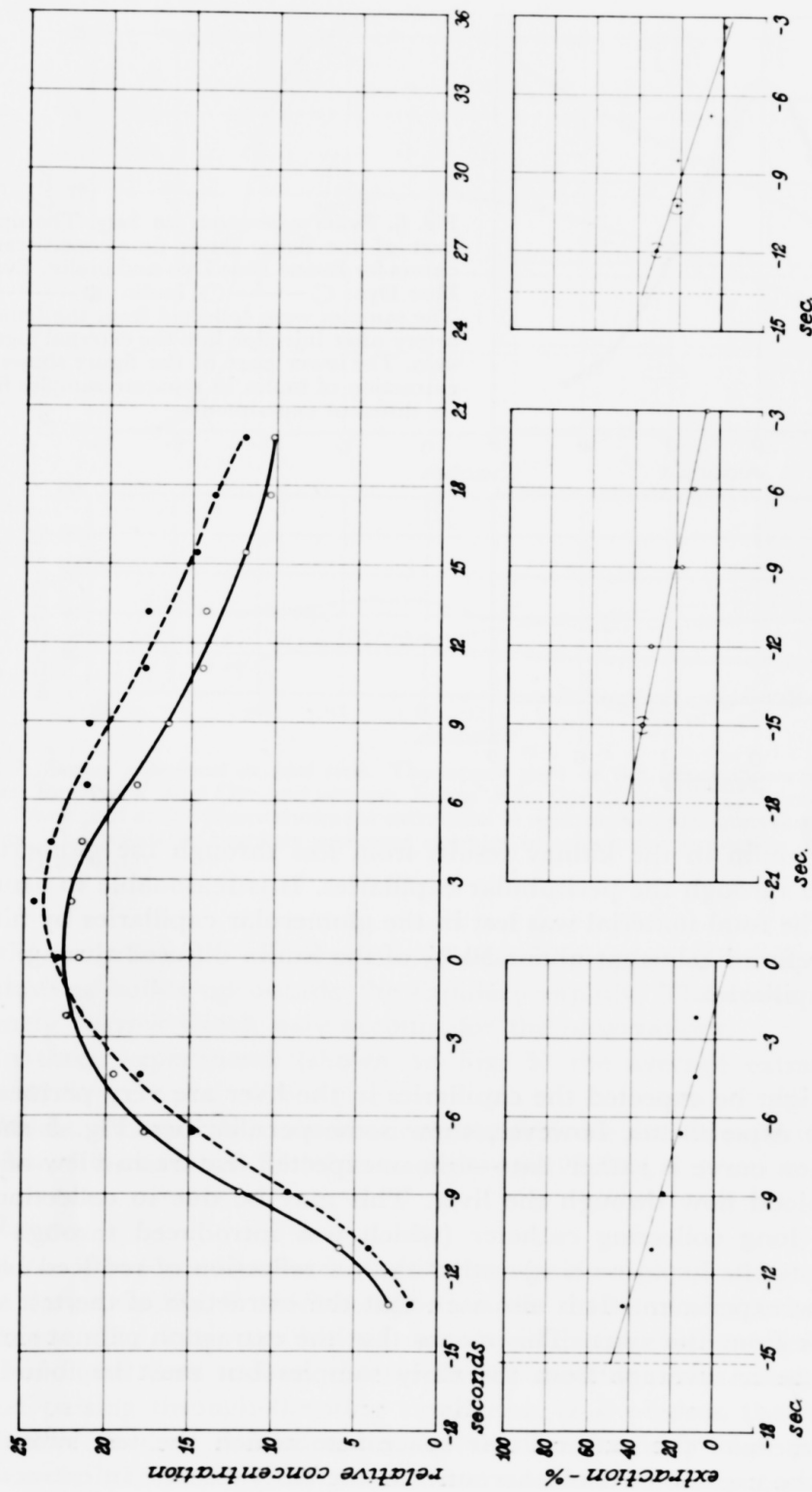


Fig. 5. *Inulin experiment on liver.* The upper part of the figure shows time-concentration curves for Evans' Blue Dye and inulin. Sampling from a hepatic vein after injection into the portal vein. Evans' Blue Dye: \circ — \circ , inulin: \bullet — \bullet . The lower part of the figure shows the extraction in separate samples from three different experiments. The extraction is falling on the rising part of the time-concentration curve and the initial extraction is found by extrapolation (see text). The time for maximum concentration of dye is arbitrarily called 0-time. The points in brackets represent samples in which the concentration of dye was very low (extinction < 0.100).

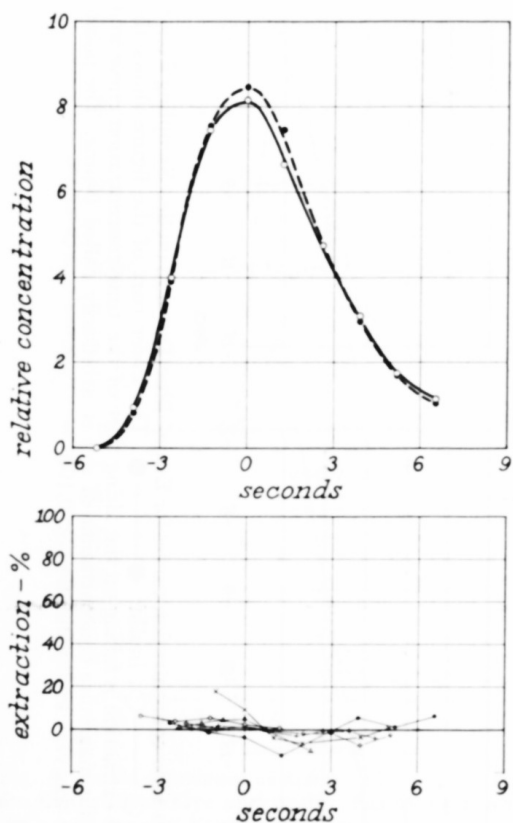


Fig. 6. *Inulin experiments on lung.* The upper part of the figure shows time-concentration curves for Evans' Blue Dye and inulin. Evans' Blue Dye: \circ — \circ , inulin: \bullet — \bullet . The samples were collected from the femoral artery after injection into the external jugular vein. The lower part of the figure shows the extraction of inulin in separate samples from six different experiments.

The loss of inulin in the kidney results from loss through the glomerular membrane and through the peritubular capillaries. It is reasonable to assume that 20 % of the total material was lost in the glomerular capillaries by filtration. It is therefore likely that about 50 % of the inulin diffused through the peritubular capillaries.

3. Liver.

Inulin. As might be expected the capillaries in the liver are very permeable to inulin. The experiments, however, show some peculiarities. Fig. 5 shows that the dilution curve is rather flat — an unexpected feature in view of the high rate of blood flow through the liver. This may be due to collection of blood from a long collecting catheter (which was introduced through the jugular vein into the hepatic veins) rather than a reflection of reduced blood flow during the experiment. It is also seen that the extraction of the test substance declines from the start. This means that the extraction cannot simply be calculated as an average from the early samples but must be found by extrapolation.

The narrowness of the extracellular space into which the test substance diffuses from the capillaries could account for the early decline in extraction.

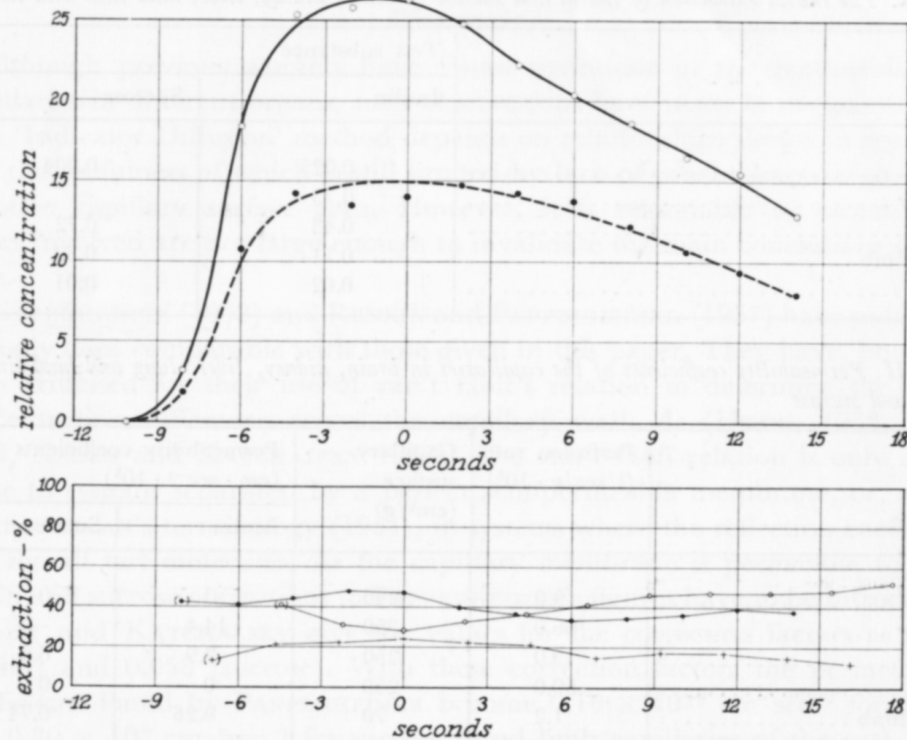


Fig. 7. Sucrose experiment on hind limb. The upper part of the figure shows time concentration curves for Evans' Blue Dye and sucrose. Evans' Blue Dye: \circ — \circ , sucrose: \bullet — \bullet . The lower part of the figure shows the extraction in separate samples from three different experiments. The points in brackets represent samples in which the extinction of Evans' Blue Dye was below 0.100.

If the extravascular distribution volume is very small an appreciable concentration builds up outside the capillary rapidly. The space of Disse is extremely narrow which may account for the observations.

In three experiments (shown on Fig. 5) the average extraction of inulin was 43.3 %.

Sucrose. Comparable observations were made in three experiments with sucrose. The average initial extraction was 72.3 %.

4. Lung.

Inulin. The loss of inulin during its passage through the lungs was negligible as is shown on Fig. 6 which summarizes the results of 6 experiments.

Sucrose. The lung capillaries were also impermeable to sucrose. The high rate of blood flow reduces the fractional extraction of a substance from the blood passing through the lung capillaries. It is obvious that the 'Indicator Diffusion' method is inadequate for tissues with very high perfusion rates. However, it is possible to demonstrate a loss of certain substances from the

Table I. The initial extraction of inulin and sucrose in brain, kidney, liver, hind limb and lung

Organ	Test substance	
	Inulin	Sucrose
Brain	0.025	0.004
Kidney	0.73	—
Liver	0.43	0.72
Hind limb	0.11	0.33
Lung	0.02	0.01

Table II. Permeability coefficients of the capillaries in brain, kidney, liver, lung and hind limb to inulin and sucrose

Organ	Perfusion rate (l/sec/g · 10 ⁶)	Capillary surface (cm ² /g)	Permeability coefficients (cm · sec ⁻¹ · 10 ⁵)	
			Inulin	Sucrose
Brain	9.0	240	0	0
Kidney	66.0	350	14.4	—
Liver	13.0	250	2.9	6.6
Lung	50.0	250	0	0
Hind limb	1.2	70	0.26	0.74

lung capillaries. Thus ANTHONISEN and CRONE (1956) found a high initial extraction of ethanol and CHINARD *et al.* (1955) of D₂O and THO.

5. Hind limb.

Inulin. The mean initial extraction was 10.7 % (S. D.: 6.6, $n = 21$). While a decline in extraction was found in experiments on other organs in the hind limb it remained constant for a long period, i. e. for at least 30 sec. This may be explained by the functionally large extravascular distribution space in a resting muscle: many capillaries do not function simultaneously so that the mean intercapillary distance is very high.

Sucrose. Fig. 7 shows the results from experiments with sucrose. The average extraction was 33.4 % (S. D.: 11.5, $n = 9$). The lower half of the figure shows that the extraction remains constant throughout the period of observation. While in the liver the flatness of the time-concentration curve was attributed to catheter artefacts the broad curve in the hind limb experiments is obviously due to the low rate of blood flow through resting muscle.

As mentioned earlier one of the purposes of this work has been to characterize the capillary wall in terms of permeability coefficients. The application of formula (2) to the average extractions tabulated in Table I gives the figures shown in Table II.

Discussion

Although previous workers have found variations in the permeability of capillaries in different organs, such observations have never been quantitative. The 'Indicator Diffusion' method depends on relationships shown in equation (2), the usefulness of which is still limited by lack of precise knowledge of the effective capillary surface area. However, it is reasonable to assume that errors involved are not large enough to invalidate the main conclusions drawn here.

PAPPENHEIMER (1953) and RENKIN and PAPPENHEIMER (1957) have published the only data comparable with those given in this paper. They have, however, been criticised for their use of van't Hoff's relation to determine the mean concentration difference across the capillary wall, Δc (USSING 1953, GRIM 1953, KEDEM and KATCHALSKY 1957). The van't Hoff relation is only applicable to systems separated by a perfect semipermeable membrane, or, stated in STAVERMAN's terminology (1951), in systems where the reflection coefficient is 1 for all test molecules. As the capillary membrane is permeable to both inulin and sucrose corrections to PAPPENHEIMER's figures have to be introduced. KEDEM and KATCHALSKY give the values for the correction factors as 0.375 (inulin) and 0.058 (sucrose). With these correction factors the permeability coefficients found by PAPPENHEIMER become $0.18 \times 10^{-5} \text{ cm} \cdot \text{sec}^{-1}$ for inulin and $0.30 \times 10^{-5} \text{ cm} \cdot \text{sec}^{-1}$ for sucrose (hind limb capillaries of the cat).

The agreement between these corrected figures and the figures given in table 2 for the permeability of the hind limb capillaries in the dog is interesting, considering that the results have been obtained with two fundamentally different experimental approaches based on different analyses of the problem. The relation between the permeability coefficients is seen to differ only slightly from that of the diffusion coefficients. The logical consequence of this finding is that the theory of 'restricted diffusion', originally advanced by PAPPENHEIMER (1953) must be reconsidered (CRONE 1963).

RENKIN (1959) derived an expression for 'tissue permeability' formally analogous to equation (2). No direct comparison can, however, be made between Renkin's figures and those presented here because of conceptual differences in the meaning of 'extraction' in Renkin's deduction and in the present analysis. Renkin determines the extraction in the steady state where the concentration of test substance outside the capillary wall is not necessarily insignificant. With the 'Indicator Diffusion' method determinations are made in a non-steady state and extracapillary concentrations are thus of much less importance. This means that the 'Indicator Diffusion' method gives true capillary permeability.

The finding that the brain capillaries are impermeable to inulin and sucrose raises the interesting question of how the brain is able to take up glucose from the blood in sufficient quantities. The difference in molecular

diameter between sucrose and glucose is only a few Ångström Units. The chemical structure of both molecules is similar, both being highly polar with great tendency to form hydrogen bonds with the water molecules in the surroundings. These considerations lead to the conclusion (CRONE 1960) that a special transfer mechanism must exist in the brain capillaries by means of which the passage of glucose into the brain tissue is facilitated. Experimental proof of this deduction will be given in a later paper.

Appendix

Determination of capillary surface areas from anatomical data

1. *Brain.* According to KROGH (1929) the capillary length in 1 mm³ of grey and white matter is 1,100—1,400 mm and 300—400 mm respectively (rat brain). CRAIGIE (1938), who also worked with brains from rats, found lengths of about 1,000—1,100 mm in the cortex. COBB (1932) found 1,000 mm as the average length in cortex of man. As the blood samples in the present work contained mainly cortical blood, 1,000 mm capillary length per mm³ was taken as a reasonable average figure. This gives a capillary surface of 240 cm²/g tissue.

2. *Muscle.* KROGH (1929) found a capillary surface of 520 cm²/cm³ resting muscle in a dog. This figure was authoritatively criticised by RENKIN and PAPPENHEIMER (1957) as being far too high. It would mean a capillary blood volume of 10.6 % of the total volume of the muscles. They found 70 cm²/g muscle from capillary counts on freeze sections. This latter figure was used in the present work.

3. *Lung.* ROUGHTON (1945) calculated that the surface area of the capillaries of the lung in man was 38 m², which means that the capillary surface per g of tissue is 250 cm², assuming a lung weight of 1,500 g.

4. *Kidney.* In a letter to the author professor VON KÜGELGEN kindly informed me that he has found a total surface area of 3.48 m² for the peritubular capillaries in a dog's kidney (weight: 152 g). As the cortex weight is approximately 70 % of the total kidney tissue mass then the capillary surface area in the cortex is about 350 cm²/g (technique: KÜGELGEN, KUHLO, KUHLO and OTTO, 1959).

5. *Liver.* No data could be found concerning the surface area of these capillaries. By analogy with the values from other parenchymatous organs a tentative value of 250 cm²/g was used in the present work.

P. EGGLETON gives estimates of various capillary surfaces in a chapter in FREY-WYSSLING's book: 'Deformation and Flow in biological systems' (1952). The following values were given: Brain: 265 cm²/g, Muscle: 166 cm²/g, Kidney: 134 cm²/g and Liver: 133 cm²/g. The order of magnitude of these figures is seen to correspond quite well with those given above but no information of how the author obtained these figures is given.

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