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HEART CAPILLARY PERMEABILITY TO LIPID-INSOLUBLE MOLECULES

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SUMMARY

1. A study has been made, in the isolated, beating dog heart perfused with blood, of the transcapillary exchange of the following substances: [^{3}H]water, ^{22}Na, ^{86}Rb, ^{36}Cl, [^{14}C]urea, [^{3}H]glycerol, [^{3}H]glucose, [^{14}C]sucrose and [^{3}H]inulin.

2. The method used to study the exchange was the 'indicator diffusion' technique. It consists in a rapid arterial injection of a mixture containing a diffusible and a non-diffusible molecule, followed by a rapid split collection of the venous outflow, up to 30 sec. The fractional extraction, E, of the diffusible substance was obtained by comparing the relative concentrations of both tracers in injected medium and in each venous sample.

3. E for [^{3}H]water was the highest ($0{\cdot}90 \pm 0{\cdot}03$), and it did not vary with flow. All other molecules had values for E that decreased as flow increased.

4. Capillary permeability constant, P, was estimated from $PS = -F \ln (1-E)$, in which S is the surface area of exchange and F is the blood perfusion rate. To test the validity of the equation, E was measured at different blood perfusion rates. It was found that the equation did not apply at relatively low flows for the more diffusible substances.

5. The average values of P estimated for inulin, sucrose, glucose, glycerol and urea were 0·27, 0·8, 1·0, 1·5 and $3{\cdot}1 \times 10^{5}$ (cm/sec), respectively. The ratio P/D (in which D is the free diffusion in water constant) was the same for all these substances. This can be interpreted as showing that if pores exist in the capillary endothelium, they must be larger than 80–100 Å diameter. It is concluded that the pores could actually be the intercellular slits as previously suggested by electron microscope studies. Endothelial cell participation in the exchange appears to be small except for [^{3}H]water.

INTRODUCTION

The capillary membrane is a composite barrier in which endothelial plasma membranes alternate with intercellular slits. Even though it is accepted from morphological data that capillaries are different in various tissues (Bennett, Luft & Hampton, 1959) a general physiological description of the processes that occur at this barrier can be stated (Landis & Pappenheimer, 1963). Lipid-soluble molecules pass through the cells, whereas water, ions and lipid-insoluble substances smaller than albumin pass through the interstices. The pathway for water, ions and non-electrolytes has been characterized by Pappenheimer, Renkin & Borrero (1951), in the resting skeletal muscle, by means of an osmotic transient technique, as pores of molecular dimensions that offer a variable degree of hindrance to the diffusion of the substances. This effect was termed 'restricted diffusion'. Vargas & Johnson (1964, 1967) using a similar technique suggested that the same effect is present in the rabbit heart.

The following consideration shows that this problem needs further investigation. The size of the pore for lipid-insoluble molecules in the skeletal muscle was originally estimated to be 30 Å radius, based on measurement of osmotic transients (Pappenheimer *et al.* 1951). However, the value was later raised to 40–45 Å after necessary theoretical corrections of their analysis (Landis & Pappenheimer, 1963). The validity of this correction has been questioned (Vargas & Johnson, 1964). By means of a tracer diffusion technique, Crone (1963*b*) found that the pores in muscle, if they exist, should be larger because he was unable to demonstrate any diffusion restriction to inulin and sucrose. Recently Karnowsky (1967) has raised objections to a rigid interpretation of the pore theory as originally proposed. On the basis of electron microscopic evidence he postulates that intercellular slits at cell junctions are the morphological equivalent of the pores. In myocardial capillaries water diffusion has been shown to be exceedingly fast compared with the diffusion of ions of similar size, suggesting that the endothelial cell membrane is also available for the passage of water as well as for lipophilic substances (Yudilevich & Alvarez, 1967).

The present work was undertaken to reconsider the problem of an adequate model of the capillary membrane, and is based on studies with the 'indicator diffusion' technique in the isolated, beating dog heart perfused with blood. The 'indicator diffusion' technique consists in measuring the fractional extraction of a labelled diffusible molecule, in a single passage through the microcirculation (Crone, 1963*a*; Martín de Julián & Yudilevich, 1964). From the fractional extraction the capillary permeability constants can be estimated from the mathematical model for transcapillary

exchange proposed by Renkin (1959) and Crone (1963*a*). Since it has been argued (Vargas & Johnson, 1964; Chinard, Enns, Goresky & Nolan, 1965) that capillary permeability measurements obtained in this way may be invalidated by the occurrence of flow-limitation to the passage of the diffusing molecules, we have used a wide range of perfusion flows. Estimates of permeability constant were obtained for inulin, sucrose, glucose, glycerol, urea, Cl^-, Rb^+, Na^+ and water.

METHODS

Animal preparation. The capillary bed of the spontaneously beating dog heart was perfused following techniques described previously (Yudilevich & Martín de Julián, 1965). In a penthobarbitone anaesthetized animal the heart was isolated but kept *in situ*. The phrenic and vagus nerves were bilaterally sectioned. Blood flowed from an elevated reservoir through plastic tubing to the brachiocephalic artery, the aorta, and then into the coronary arteries. From the right ventricle the venous coronary blood flowed through tubing, inserted through the right auricle, into a receptacle from which blood was pumped into the perfusion reservoir. The temperature of the blood was kept at about 37–38° C and CO_2–O_2 mixture was continually bubbling into it. The heart was kept warm and moist. The left ventricle was perforated with a cannula to have the heart contracting but performing no external work. Electrogram and arterial perfusion pressure were recorded by means of a Grass polygraph. The arterial pressures were changed by raising or lowering the reservoir in a range from 25 to 100 mm Hg. In this way various venous flow rates were obtained. The heart contraction rate ranged between 60 and 90 per minute and it did not vary with changes in perfusion pressure.

The perfusion medium was blood diluted to an haematocrit of 10–20 %. In most experiments the dilution was made with a solution containing polyvinylpyrrolidone (35 g/l.) and NaCl (9 g/l.). In a few experiments, blood was diluted with fresh plasma. Heparin (5000 i.u./l.) was always used as anticoagulant.

Measurement of capillary permeability. The 'indicator diffusion' method of Crone (1963*a*) and Martín de Julián & Yudilevich (1964) was used to measure the fractional extraction (E) of a diffusible molecule. The mathematical expression relating capillary permeability (P) and experimentally measured variables is (Renkin, 1959; Crone, 1963*a*)

$$PS = -F\ln(1-E),$$

where S is the total capillary area contained in 100 g of heart and F is the blood flow rate in front of that area. As defined by Martín de Julián & Yudilevich (1964), extraction (E) of the diffusible molecule is

$$E = \lim_{t\to 0} 1 - c(t)/C(t),$$

where $c(t)$ and $C(t)$ are the venous concentrations of the diffusible and non-diffusible labelled molecules, respectively, expressed as fraction of the injected dose.

Experimentally, F was measured from the volume of perfusate collected in a given time at the venous side of the heart preparation. To estimate E, $C(t)$ and $c(t)$ were measured in successive venous samples obtained every fraction of a second immediately after a rapid (1–2 sec) injection of a mixture of diffusible and non-diffusible labelled substances. The injection was made into the arterial stream, close to the brachiocephalic artery. Collection of the venous outflow was made by means of a mechanical sampling device described below. Thirty samples were collected in separate tubes in 7–30 sec depending on the flow.

As seen in Fig. 1, the function $c(t)/C(t)$ was such that to get E the zero time limit could be obtained from the average of the first six to twelve samples. This approach appears to be

more straightforward than that of Martín de Julián & Yudilevich (1964), in which E is calculated from the complete concentration versus time function obtained in a larger time span with samples at longer intervals.

In each heart preparation only one mixture of labelled molecules was tested. Two to five 'indicator diffusion' experiments were successively performed to get E at various perfusion flows. The mixtures of molecules that were used are shown in Table 1.

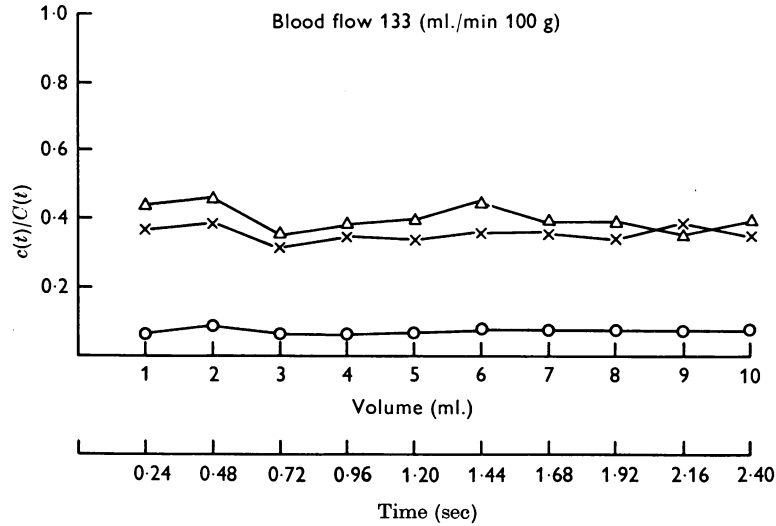

Fig. 1. Diffusible ($c(t)$) to non-diffusible ($C(t)$) venous concentration ratio against accumulated blood volume. In this experiment three diffusible labelled molecules were present in the injected mixture ($\triangle$ $^{36}Cl^-$, $\times$ [^{14}C]urea and $\bigcirc$ [^{3}H]water) that also contained [^{59}Fe]siderophilin which is a non-diffusible reference tracer. The time after the appearance of the first venous sample that contained radioactivity was taken as the zero time. The fractional extraction (E) was estimated for each molecule from 1 minus the average of its concentration ratios.

Sampling device. This consisted of two lucite pieces. One was a disc with thirty holes in a circular array, in which 16 mm diameter test-tubes could be placed hanging by their lips. The edge of this piece had a groove to keep in place a thread coiled around it. The second lucite piece was a ring with its upper face pierced by thirty coalescent funnel-shaped holes, and its lower face carved to fit into the test-tube lips. To operate, the thread was tied to the shaft of a variable rate reduction drive (Sigmamotor pump). The sampling rate could then be adjusted at will. Equal volumes were collected in each tube. In this set of experiments the adjustment was such that the volume of the samples was from 1 to 2 ml. depending on the perfusion flow rates, that is to say, the collection time for each sample varied to keep the sample volume constant.

Test molecules and radioactivity analytical procedures (see Table 1). All test molecules were labelled with a radioactive isotope. They were obtained from New England Nuclear Corp., except tritiated water which was supplied by the Comisión Nacional de Energía Atómica, Argentina. In most experiments [^{59}Fe]siderophilin was used as the reference non-diffusible tracer (Yudilevich & Martín de Julián, 1965); in some [^{51}Cr]haemoglobin was used instead. The diffusible molecules tested were [^{3}H]inulin, [^{14}C]sucrose, [^{3}H]glucose, [^{3}H]glycerol, [^{14}C]urea, $^{22}Na^+$, $^{36}Cl^-$, $^{86}Rb^+$ and tritiated water (here called HTO). Rubidium was used as a

tracer for K (Kahn, 1963). The molecules were combined in eight different mixtures at doses that are shown in Table 1.

The counting procedures to separate the radioactivity of each tracer in a given mixture followed methods already described (Yudilevich & Martín de Julián, 1963, 1965; Alvarez & Yudilevich, 1967). Table 1 gives the type of radiation of each isotope and the region of its energy spectrum at which it was counted. Cross-contamination in general existed, and appropriate corrections were performed.

TABLE 1. Composition of the eight different mixtures of tracers used. The energy of the β and γ spectra at which the radioactivity detection system was set are in the third column. In the row of each isotope is the counting condition at which it was counted with the best efficiency

Mixture	Dose (μc)	Energy (MeV)
[^{59}Fe]siderophilin	2	Integrated γ
$^{36}Cl^-$	2	0·710 β
[^{14}C]sucrose	2	0·155 β
[^{3}H]inulin	20	0·018 β
[^{59}Fe]siderophilin	2	Integrated γ
$^{36}Cl^-$	2	0·710 β
[^{14}C]sucrose	2	0·155 β
[^{3}H]glucose	20	0·018 β
[^{59}Fe]siderophilin	2	Integrated γ
$^{36}Cl^-$	2	0·710 β
[^{14}C]urea	2	0·155 β
[^{3}H]water	20	0·018 β
[^{59}Fe]siderophilin	2	Integrated γ
$^{36}Cl^-$	2	0·710 β
[^{14}C]urea	2	0·155 β
[^{3}H]glycerol	20	0·018 β
[^{59}Fe]siderophilin	2	Integrated γ
[^{14}C]urea	2	0·155 β
[^{3}H]glycerol	20	0·018 β
[^{59}Fe]siderophilin	2	Integrated γ
[^{14}C]sucrose	2	0·155 β
[^{3}H]glucose	20	0·018 β
[^{51}Cr]haemoglobin	20	0·32 γ
$^{86}Rb^+$	15	0·60 γ
[^{51}Cr]haemoglobin	20	0·32 γ
$^{22}Na^+$	2	0·40 γ
		0·54 β
[^{3}H]glucose	20	0·018 β

Gamma counting was done in a Tracerlab automatic well type scintillation spectrometer using aliquots of 0·5–1·0 ml. whole blood. Beta counting was done in a Packard automatic liquid scintillation spectrometer in samples prepared from 0·2 ml. whole blood extracted with 3 ml. ethanol. After centrifugation, the ethanol phase was mixed with a scintillation mixture. This procedure eliminates the reference labelled proteins (Alvarez & Yudilevich, 1967).

When inulin was in the mixture a different procedure was followed to prepare the sample for beta counting because inulin is precipitated by ethanol. An aliquot of 0·2 ml. plasma was precipitated with 3 ml. ethanol, and rinsed once with 3 ml. ethanol. The precipitate was dissolved in 0·5 ml. Hyamine 10 × (Packard), and added to the scintillation mixture.

TABLE 2. Fractional tracer extractions (× 100) versus blood flow

Tracer	Flow rate (ml./min.100 g) < 20	20–40	40–80	80–150	150–250	> 250
Inulin (0·18)	37 ± 4 (2)	26 ± 7 (3)	—	—	—	—
Sucrose (0·55)	64 ± 7 (2)	51 ± 7 (5)	35 ± 8 (12)	—	—	—
Glucose (0·68)	74 ± 8 (2)	57 ± 10 (6)	43 ± 8 (8)	—	—	—
Glycerol (0·89)	—	—	38 ± 8 (3)	26 ± 4 (5)	22 ± 5 (6)	14 ± 5 (4)
Urea (1·45)	—	—	57 ± 4 (2)	39 ± 9 (9)	41 ± 10 (7)	27 ± 7 (7)
Na^+ (1·33)	83 ± 8 (3)	77 ± 9 (5)	67 ± 11 (5)	54 ± 6 (5)	46 ± 10 (4)	—
Cl^- (2·03)	—	73 ± 2 (4)	58 ± 9 (9)	51 ± 9 (9)	49 ± 11 (8)	48 ± 7 (4)
Rb^+ (2·10)	84 ± 5 (4)	78 ± 2 (6)	71 ± 7 (6)	58 ± 12 (4)	54 ± 5 (3)	—
Water (2·36)	—	—	88 ± 6 (4)	92 ± 2 (6)	93 ± 1 (3)	93 (1)

The average fractional extractions (× 100), ± S.E., and the number of observations in parentheses, are given for all tracer molecules. In parentheses after each tracer are the coefficients of free diffusion in water (D) at 25° C, expressed in $(cm^2/sec) \times 10^5$. They were taken from the following references: inulin (Kruhøffer, 1946); sucrose, glucose, urea and tritiated water (Renkin, 1954); glycerol (Villegas, Caputo & Villegas, 1962); Na^+, Rb^+ and Cl^- from the *American Institute of Physics Handbook*. New York: McGraw (1957).

RESULTS

Fractional extraction of tracer. The results for all molecules are pooled in Table 2. Six flow ranges were chosen to group all indicator diffusion experiments in all hearts. This means that the S.E. of the average reflects the flow distribution in each range and the statistical variation of the measurement itself.

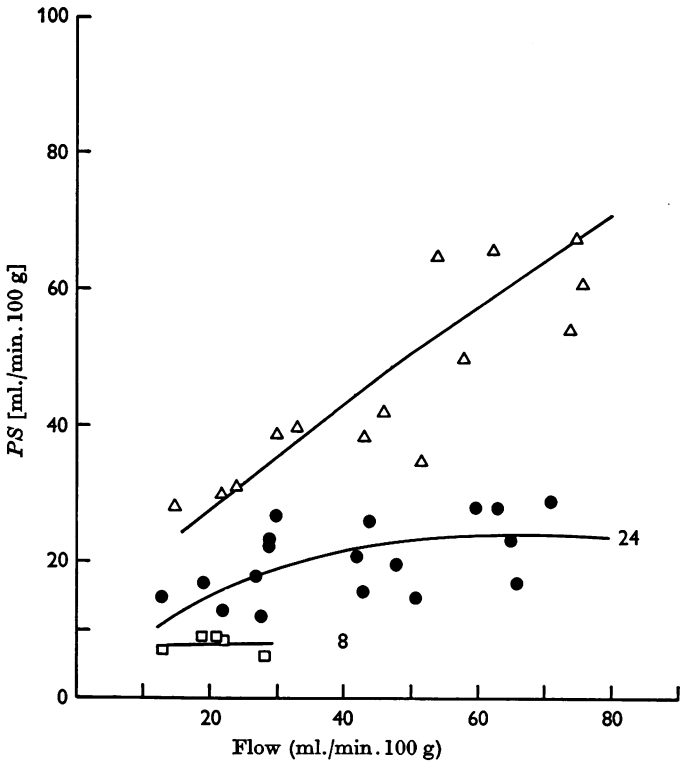

Fig. 2. Permeability-surface product *PS*, versus blood flow relationship for the experiments in which the molecules were simultaneously tested. *PS* for inulin (□) and sucrose (●) reached a relatively constant value at 8 and 24 ml./min.100 g, respectively. $^{36}Cl^-$ is represented by open triangles.

It can be seen that the fractional extraction for HTO did not depend on the perfusion flow; the total average is 0·91 ± 0·03. On the other hand, *E* for all the other molecules decreased as flow increased. At low flows *E* for Rb^+, Cl^- and Na^+ tended to a maximal value, which approached the extraction of water. The electrolytes and urea extractions were similar at every flow level, and the data for this group were compared with those for the group with smaller diffusion coefficients, comprising glycerol, glucose

TABLE 3. Permeability–surface product versus blood flow

Tracer	Flow rate (ml./min. 100 g)					
	< 20	20–40	40–80	80–150	150–250	> 250
Inulin	7·6 ± 1·3 (2)	8·0 ± 1·6 (3)	—	—	—	—
Sucrose	16 ± 2 (2)	21 ± 6 (5)	24 ± 7 (12)	—	—	—
Glucose	26 ± 2 (2)	30 ± 5 (6)	31 ± 12 (8)	—	—	—
Glycerol	—	—	27 ± 2 (3)	35 ± 4 (5)	42 ± 7 (6)	45 ± 13 (4)
Urea	—	—	71 ± 34 (2)	67 ± 20 (9)	97 ± 30 (7)	92 ± 29 (7)
Na^+	29 ± 4 (3)	46 ± 7 (5)	67 ± 12 (5)	91 ± 8 (5)	114 ± 25 (4)	—
Cl^-	—	35 ± 4 (4)	51 ± 12 (9)	89 ± 21 (9)	135 ± 29 (8)	204 ± 65 (4)
Rb^+	28 ± 10 (4)	50 ± 25 (6)	72 ± 12 (6)	106 ± 25 (4)	143 ± 31 (3)	—
Water	—	—	150 ± 50 (4)	280 ± 67 (6)	567 ± 100 (3)	800 (1)

PS average ± S.E. and number of observations in parentheses are given for each flow range.

and sucrose. It can be seen that the latter group had smaller extractions than the former. The difference was more pronounced at the highest flows. Inulin, the least diffusible molecule used in our experiments, had a fractional extraction which was about half that of sucrose.

Capillary permeability. Permeability-surface products, estimated from the fractional extractions, appeared to be blood-flow dependent for most molecules studied. Figure 2 shows a correlation of *PS* versus flow in the experiments in which the injected mixture contained either sucrose and $^{36}Cl^-$ or these plus inulin. It can be seen that *PS* for Cl^- increased with *F*

TABLE 4. Capillary permeability constants

	PS (ml./min. 100 g)	$P \times 10^5$ (cm/sec)	$D \times 10^5$ (cm²/sec)	*P*/*D* (1/cm)
Inulin	8 ± 1·2 (5)	0·27 ± 0·04	0·18	1·5
Sucrose	24 ± 7 (12)	0·8 ± 0·2	0·55	1·5
Glucose	31 ± 9 (14)	1·0 ± 0·3	0·68	1·5
Glycerol	45 ± 7 (10)	1·5 ± 0·3	0·89	1·6
Urea	92 ± 30 (14)	3·1 ± 1·0	1·45	2·1
Na^+	> 114	> 3·8	1·33	> 2·9
Cl^-	> 204	> 6·8	2·03	> 3·4
Rb^+	> 143	> 4·8	2·10	> 2·3

Capillary permeability constants (*P*) were estimated from the highest *PS* values assuming *S* to be 500 cm²/g (Schafer & Johnson, 1964). The coefficients of free diffusion (*D*) are those shown in Table 2.

at all flows, whereas that for sucrose reached a plateau of about 24 ml./min. 100 g when the flow was greater than 40 ml./min. 100 g. *PS* for inulin was not affected by the flow rate within the range studied, and it averaged 8 ml./min. 100 g. The *PS* versus flow analysis done with the data from all diffusion experiments is given in Table 3. It appears that in addition to inulin and sucrose, an almost constant *PS* value was attained by glucose, glycerol and urea at 31, 45 and 92 ml./min. 100 g, respectively. It is evident that the higher the *PS* the higher the flow that was needed to reach these constant values. For the more diffusible molecules *PS* was still increasing at the highest flow.

For the molecules for which permeability-surface products became independent of flow, a capillary permeability constant can be computed assuming *S* equal to 500 cm²/g (Schafer & Johnson, 1964). For the rest (except HTO) only lower limit values can be estimated. The results are shown in Table 4, together with the coefficient of free diffusion in water, *D*. The ratios of *P* to *D* were relatively constant (1·5–2·1) for inulin, sucrose, glucose, glycerol and urea. For Na, Cl and Rb ions, the lower limits estimated for *P*/*D* were higher.

DISCUSSION

Effect of flow on the measurement of capillary permeability. The mathematical expression used here to relate capillary permeability to surface area, flow and fractional extraction, has not been adequately proved to be a valid description of transcapillary exchange kinetics, although it has been widely used. In the original work, correlation of ^{42}K clearance versus flow in skeletal muscle failed to fit the theoretical model from which that expression was derived (Renkin, 1959). The argument advanced was that the capillary surface area perfused could change as the perfusion pressure changed. However, in an artificial kidney with a fixed area of cellophane tubing (Wolf, Remp, Kiley & Currie, 1961) the equation was shown to apply (Renkin, 1967). We have used here, as an approach to test the validity of the equation in the coronary circulation, the correlation of PS and F for various molecules studied simultaneously. The fact that, in our heart preparation, PS can be independent of perfusion flow for inulin and sucrose, while the simultaneously measured PS for Cl^- is increasing, indicates that the capillary surface area S is the same at all flows.

The other critical assumption in the derivation of the equation is that the tracer concentration on the outside of the capillary membrane is zero (Renkin, 1959; Crone, 1963*a*). It can be suggested that this assumption is not valid when fractional extractions of tracer are high. This could be the explanation for the finding that PS increases with flow in a range of flows at which extractions even though decreasing are still relatively high. In our experiments for inulin, sucrose, glucose, glycerol and urea, PS became constant at approximate extractions of 0·37, 0·51, 0·43, 0·26, 0·41 respectively.

Among the various ways that have been suggested to measure fractional extractions, the indicator diffusion technique is the most suitable as it provides a very early measurement after the tracer injection (Crone, 1963*a*; Martín de Julián & Yudilevich, 1964; Yudilevich, Renkin, Alvarez & Bravo, 1968). However, back diffusion of tracer from the extracapillary space can occur in one capillary passage time. This would explain the underestimation of PS at low flows observed here. A model for transcapillary exchange, proposed by Johnson & Wilson (1967), attempts to introduce extravascular tracer concentration as a new variable in the model, but no experimental evidence for its applicability was offered.

Capillary permeability. The literature offers few opportunities for comparing the capillary permeability constants we have calculated with other estimates for the heart or other organs. In the case of the heart the comparison is complicated by the fact that the preparations are not alike. This is important since it has been found that heart capillary permeability may

be greatly influenced by experimental conditions (Stubbs & Widdas, 1959; Sutherland & Young, 1966; Young, 1968). Furthermore, comparison of results in the contracting heart with investigations in resting skeletal muscle is complicated by the influence of contractility on transcapillary flux of water and solutes (Stubbs & Widdas, 1959; Young, 1968).

As seen in Table 5, our results for inulin and sucrose agree with those reported by Crone (1963*b*) for skeletal muscle in the intact dog. Both data were obtained by the indicator diffusion technique. The ratio P/D is the same for the two molecules in each organ. This means that no restricted

TABLE 5. Comparison of capillary permeability constants ($\times 10^5$ cm/sec)

	Indicator diffusion			Osmotic transient		Tissue sampling
	Heart	Skeletal muscle	Brain	Heart	Skeletal muscle	Heart
Inulin	0·26	0·26	0	0·54	0·3	0·4
Sucrose	0·80	0·74	0	5·9	4	11
Glucose	1·0	—	0·16	9·7	6	—
Glycerol	1·4	—	0·21	—	—	—
Urea	3·1	—	0·44	—	14	—
Na^+	>3·8	—	—	—	—	—
Cl^-	>6·8	—	—	—	—	—
Rb^+	>4·8	—	—	—	—	—

Permeability constants presented in this Table were grouped according to the method employed for their measurement. Indicator diffusion data for the heart are those of Table 4 in this paper; for skeletal muscle (dog) it was obtained from Crone (1963*a*), and for the brain (dog) from Crone (1965). Permeabilities obtained by means of osmotic transient methods for the heart (rabbit) are from Vargas & Johnson (1967), and for skeletal muscle (cat) from Landis & Pappenheimer (1963). Tissue sampling after tracer administration is the technique used by Schafer & Johnson (1964) in the rabbit heart.

diffusion appears to exist. The Table shows that the osmotic techniques give inulin values that are similar to those estimated from indicator diffusion techniques while permeabilities for sucrose, glucose and urea are several times larger if measured by the former technique. The ratio P/D is the larger the smaller the molecule. This fact has been taken as evidence for the presence of a pore structure in the capillary barrier, these pores being about 30–45 Å radius (Landis & Pappenheimer, 1963).

The theoretical analysis used to compute permeability constants from osmotic experiments has been criticized on theoretical grounds several times (Ussing, 1953; Grim, 1953; Kedem & Katchalsky, 1958). These criticisms were based on the omission of the osmotic reflexion coefficients σ, in Pappenheimer's use of the van't Hoff relation. An independent estimate of σ for these experiments is still lacking.

Vargas & Johnson (1964) estimated σ for some non-electrolytes from osmotic transients in the excised rabbit heart, perfused with non-blood physiological solutions. Later they developed the theory to estimate

permeability constants from the same experiments (Vargas & Johnson, 1967). Their values are shown in Table 5, and are also consistent with Pappenheimer's pore model for the capillary membrane. Unfortunately, five correcting factors were necessary in the analysis of the raw data, which weakens their conclusions, since these factors had to be arbitrarily evaluated.

Permeability constants for inulin and sucrose have also been measured by Schafer & Johnson (1964) in the rabbit heart, perfused, with Ringer solution, by following the tracer kinetics of tissue exchange by means of tissue sampling. Their results are in Table 5. This work also led to the same 30–45 Å pore model. Very recently Young (1968) studied capillary permeability in the excised rat heart. However, this is an extremely 'leaky' preparation that has capillaries freely permeable to bovine plasma albumin (Sutherland & Young, 1966). Therefore this study cannot be considered here.

The difficulties encountered in comparing diffusion and osmotic data constitute a general problem in the study of biological membranes (Hanai, Haydon & Redwood, 1966). Our indicator diffusion data show that all the non-electrolytes ranging from urea (r = 2·8 Å) to inulin (r = 12–15 Å) pass across the capillary membrane of the heart at rates that are proportional to their coefficients of free diffusion. According to Landis & Pappenheimer (1963), these results can be explained if pores larger than 50–60 Å in radius were the pathway for these molecules. From the ratio P/D (Table 4) an estimate of the areas available for diffusion of these molecules can be made if a thickness of membrane is assumed. According to electron microscope observations, this can be taken as 100–400 Å, which is the depth of the intercellular junction from the luminal to the tissue surfaces (Karnowsky, 1967). The areas for diffusion so computed range between 0·001 and 0·01 % of the total endothelial area, which is comparable with the 0·02 % values histologically derived by Karnowsky (1967) for the intercellular area of the endothelium in the heart.

The results for the transcapillary diffusion of water and of Na and Rb ions confirm previous work which showed the fast rate at which water, in comparison with those ions, diffuses through the heart capillary barrier. HTO probably crosses the endothelial cells whereas Na, Rb and Cl ions cross mainly through the intercellular gaps (Yudilevich & Alvarez, 1967). Following the pore hypothesis it can be suggested that very small pores, which do not allow the passage of ions but do allow the passage of water, might exist in the endothelial cell plasma (Ussing, 1968). However, other physicochemical membrane models could explain here, as with any plasma membrane, the specially high permeability to water. That some cell permeability to the ions possibly exists is suggested by the larger P/D of

the ions compared with that for the non-electrolytes. However, it must be emphasized that chloride ion permeability appears to be similar to that of the cations, which contrasts with the permeability properties of the membrane of dog erythrocytes.

In conclusion, to arrive at a definitive model for the capillary endothelium as a physiological barrier appears to be impossible with the investigations available at the moment. However, from the diffusion experiments reported here the following model can be proposed for the heart capillary endothelium. Intercellular clefts are the pathway for lipid-insoluble molecules, and they represent 0·001–0·01 % of the total capillary area. The interspace must be wider than 80–100 Å. It remains to be studied by means of diffusion techniques how the permeability of lipid-insoluble molecules larger than inulin is gradually reduced in order to estimate its functional size. The plasma membrane of the endothelial cell appears to have no permeability for hydrophilic non-electrolytes and small ions, and a high permeability to water and lipophilic molecules. If this model were true for every organ, it could be speculated that the degree of tightness of the intercellular junctions could offer an effective means of controlling the blood tissue exchange of lipid-insoluble molecules. An example that supports such a hypothesis is the brain capillary bed which shows values for capillary permeability (Crone, 1965) lower than that of any other organ (see Table 5) and it is also known to have tight intercellular junctions (Reese & Karnowsky, 1967). At the other extreme is the liver (Yudilevich *et al.* 1968), in which the sinusoids present extremely low hindrance to the diffusion of large molecules.

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REFERENCES

ALVAREZ, O. A. & YUDILEVICH, D. L. (1967). Capillary permeability and tissue exchange of water and electrolytes in the stomach. *Am. J. Physiol.* **213**, 315–322.

BENNETT, H. S., LUFT, J. H. & HAMPTON, J. C. (1959). Morphological classifications of vertebrate blood capillaries. *Am. J. Physiol.* **196**, 381–390.

CHINARD, F. P., ENNS, T., GORESKY, C. A. & NOLAN, M. F. (1965). Renal transit times and distribution volumes of T-1824, creatinine and water. *Am. J. Physiol.* **209**, 243–252.

CRONE, C. (1963*a*). The permeability of capillaries in various organs as determined by use of the 'indicator diffusion' method. *Acta physiol. scand.* **58**, 292–305.

CRONE, C. (1963*b*). Does 'restricted diffusion' occur in muscle capillaries? *Proc. Soc. exp. Biol. Med.* **112**, 453–455.

CRONE, C. (1965). The permeability of brain capillaries to non-electrolytes. *Acta physiol. scand.* **64**, 407–417.

GRIM, E. (1953). Relation between pressure and concentration differences across membranes permeable to solute and solvent. *Proc. Soc. exp. Biol. Med.* **83**, 195–200.

HANAI, T., HAYDON, D. A. & REDWOOD, W. R. (1966). The water permeability of artificial bimolecular leaflets: a comparison of radio-tracer and osmotic methods. *Ann. N.Y. Acad. Sci.* **137**, 731–739.

JOHNSON, J. A. & WILSON, T. A. (1967). A model of capillary exchange. *Am. J. Physiol.* **210**, 1299–1303.

KAHN, J. B. (1963). The entry of rubidium into human erythrocytes. *J. Pharmac. exp. Ther.* **136**, 197–204.

KARNOWSKY, M. J. (1967). The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. *J. cell Biol.* **35**, 213–236.

KEDEM, O. & KATCHALSKY, A. (1958). Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochim. biophys. Acta* **27**, 229–246.

KRUHØFFER, P. (1946). The significance of diffusion and convection for distribution of solutes in interstitial space. *Acta physiol. scand.* **11**, 37–47.

LANDIS, E. M. & PAPPENHEIMER, J. R. (1963). Exchange of substances through the capillary walls. In *Handbook of Physiology*, section 2, Circulation, vol. II, pp. 961–1043, ed. HAMILTON, W. F. & DOW, P. Washington, D.C.: American Physiological Society.

MARTÍN DE JULIÁN, P. & YUDILEVICH, D. L. (1964). A theory for the quantification of transcapillary exchange by tracer dilution curves. *Am. J. Physiol.* **207**, 162–168.

PAPPENHEIMER, J. R., RENKIN, E. M. & BORRERO, L. M. (1951). Filtration, diffusion and molecular sieving through peripheral capillary membranes. *Am. J. Physiol.* **167**, 13–46.

REESE, T. S. & KARNOWSKY, M. J. (1967). Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J. cell Biol.* **34**, 207–217.

RENKIN, E. M. (1954). Filtration, diffusion and molecular sieving through porous cellulose membranes. *J. gen. Physiol.* **38**, 225–243.

RENKIN, E. M. (1959). Transport of potassium-42 from blood to tissue in isolated mammalian skeletal muscles. *Am. J. Physiol.* **197**, 1205–1210.

RENKIN, E. M. (1967). Blood flow and transcapillary exchange in skeletal and cardiac muscle. In *Coronary Circulation and Energetics of the Myocardium*, ed. MARCHETTI, G. & TACCARDI, B., pp. 18–30. Basel and N.Y.: Karger.

SCHAFER, D. E. & JOHNSON, J. A. (1964). Permeability of mammalian heart capillaries to sucrose and inulin. *Am. J. Physiol.* **206**, 985–991.

STUBBS, J. & WIDDAS, W. F. (1959). Extravascular fluid losses in the perfused isolated rabbit heart. *J. Physiol.* **148**, 393–402.

SUTHERLAND, T. M. & YOUNG, D. A. B. (1966). Increased permeability of the capillaries of the rat heart to plasma albumin with asphyxiation and with perfusion. *J. Physiol.* **183**, 112–122.

USSING, H. H. (1953). Transport through biological membranes. *A. Rev. Physiol.* **15**, 1–20.

USSING, H. H. (1968). The role of the basement membrane in epithelial and endothelial permeability. *Transactions of VI International Diabetes Congress, Stockholm.* (In the Press.)

VARGAS, F. F. & JOHNSON, J. A. (1964). An estimate of reflection coefficient for rabbit heart capillaries. *J. gen. Physiol.* **47**, 667–677.

VARGAS, F. F. & JOHNSON, J. A. (1967). Permeability of rabbit heart capillaries to non-electrolytes. *Am. J. Physiol.* **213**, 87–93.

VILLEGAS, R., CAPUTO, C. & VILLEGAS, L. (1962). Diffusion barriers in the squid nerve fiber. *J. gen. Physiol.* **46**, 245–255.

WOLF, A. V., REMP, P. G., KILEY, J. E. & CURRIE, G. D. (1951). Artificial kidney function: kinetic of hemodialysis. *J. clin. Invest.* **30**, 1062–1070.

YOUNG, D. A. B. (1968). Factors controlling the washout of the interstitial space of the isolated, perfused rat heart. *J. Physiol.* **196**, 747–759.

YUDILEVICH, D. L. & ALVAREZ, O. A. (1967). Water, sodium and thiourea transcapillary diffusion in the dog heart. *Am. J. Physiol.* **213**, 308–314.

YUDILEVICH, D. L. & MARTÍN DE JULIÁN, P. (1963). A method to process the data when several mixtures of elements are to be resolved in their components. Application to mixtures of ^{59}Fe, ^{22}Na, ^{86}Rb and ^{131}I. *Int. J. appl. Radiat. Isotopes* **14**, 563–570.

YUDILEVICH, D. L. & MARTÍN DE JULIÁN, P. (1965). Potassium, sodium and iodide transcapillary exchange in the dog heart. *Am. J. Physiol.* **208**, 959–967.

YUDILEVICH, D. L., RENKIN, E. M., ALVAREZ, O. A. & BRAVO, I. (1968). Fractional extraction and transcapillary exchange during continuous and instantaneous tracer administration. *Circulation Res.* **23**, 325–336.