

The Permeability of Single Capillaries to Potassium Ions

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ABSTRACT This paper reports a description of methods for determining the diffusional permeability to potassium ions of single capillaries in the frog mesentery. By means of micropipettes, injections or infusions were delivered into a single capillary. The subsequent concentration variations in and about the capillary were followed with K^+ -sensitive microelectrodes. A theoretical analysis is provided which gives a quantitative frame of reference for evaluating the observed time-concentration curves in terms of capillary permeability. The advantage of single capillary studies is that the surface area through which diffusion occurs is known as is the concentration difference across the capillary membrane. Three different techniques are: (a) the "single injection" method which represents an application of the indicator diffusion technique where a high- K^+ bolus is injected into a single capillary; (b) the "sack" method which determines the rate of K^+ disappearance from within and immediately outside an occluded capillary segment, after a brief increase in intracapillary K^+ concentration; and (c) the "interstitial diffusion" method which records time and spatial distribution of K^+ in the interstitial space after a step-change in intracapillary K^+ concentration. The methods gave an average potassium permeability of the capillary membrane of $67 \times 10^{-5} \text{ cm s}^{-1}$ (SD: 23, $n = 26$) at room temperature.

These figures are clearly higher than those previously reported in mammalian capillary studies using whole-organ techniques. In terms of the Pappenheimer pore model, this estimate of capillary permeability is consistent with the behavior of a membrane with a thickness of $1.0 \mu\text{m}$ which possesses equivalent pores with a radius of 110 \AA , a fractional pore area of 0.3%, and a pore density of $8 \mu\text{m}^{-2}$.

INTRODUCTION

So far determinations of the permeability of capillaries to various ions and non-electrolytes have been carried out in whole-organ experiments in which the response of the organ to intraarterial injections or infusions of test solutes (indicators) was analyzed. The analysis of such input-output or black box experiments rests upon specific mathematical models of the capillary bed and its surroundings. The models which have been employed are essentially "single capillary" models; i.e., it is assumed that the organ consists of a multitude of similar units of a central capillary surrounded by a tissue cylinder. This is probably not a realistic assumption because heterogeneity of perfusion, capillary length, and possibly permeability is likely to be present. Despite the limitations

of the commonly employed indirect methods, many data have been obtained over the last 25 yr, but it would be desirable to check results from whole organ experiments against studies on single capillaries.

In 1971 Walker developed a potassium-sensitive microelectrode with a tip diameter of 1 μm . We have taken advantage of this new tool to investigate K^+ permeability of the rather large capillaries in the mesentery of the frog. A brief description of the first results obtained with this method was given by Crone and Friedman (1976).

The aim of the present investigation was twofold: (a) to develop the analytical tools necessary for determination of transcapillary diffusion in a single capillary under specified geometric conditions; and (b) to do experiments under well-defined conditions and thus obtain data for diffusive permeability based on an analysis requiring fewer assumptions than those implied in whole-organ experiments.

METHODS

Experimental Approaches

The permeability of the capillary wall was determined by three different experimental methods: (a) the "single injection" method; (b) the "sack" method; and (c) the "interstitial diffusion" method.

SINGLE-INJECTION EXPERIMENTS In view of the many applications of this method in whole-organ experiments (Crone, 1970), a special interest attaches to the application of the method to a single capillary. The general setup for the experiments is illustrated in Fig. 1 where it is seen that the method requires the insertion of three micropipettes into the microcirculation, an injection pipette, and two microelectrodes. In practice a single pulse of a high-potassium solution is delivered to the proximal end of a capillary segment. Two microelectrodes separated by 400–1000 μm are situated in the same vessel downstream from the injection site. The first electrode indicates the input to the capillary segment under study, while the second measures the output after the bolus has traveled through the length of the capillary. From the rising part of the curves recorded at the two electrodes, the extraction (E) was determined as the difference in area relative to the area recorded at the first electrode. The flow (F) was determined as the interelectrode capillary volume, $\pi r^2 \cdot L$, divided by the difference in appearance time ($t_2 - t_1$) of the bolus front at the two electrodes (cf. Fig. 4). The interelectrode capillary surface area (A) through which potassium has diffused is $2\pi \cdot rL$. The permeability of the capillary segment is then calculated from $P_c = -(F/A)\ln(1 - E)$. By inserting the appropriate geometrical values for F and A , the expression reduces to

$$P_c = -\frac{r}{2(t_2 - t_1)} \ln(1 - E). \quad (1A)$$

This expression is derived under the assumption of a small or negligible extravascular concentration during the rising phase of the potassium bolus. The surface of the mesentery is constantly flushed with normal frog Ringer's solution (superfusion).

SACK METHOD The design of the experiment is illustrated in Fig. 2a. A brief intravascular infusion of high- K^+ Ringer is given into a single capillary, of which the proximal end is closed with a glass microrod. Seconds later, when the infusion pressure increment is reduced to zero, the distal end of the capillary is rapidly occluded to form a sack. One electrode was placed inside the capillary beforehand, and another was placed on the outside (cf. Fig. 2b).

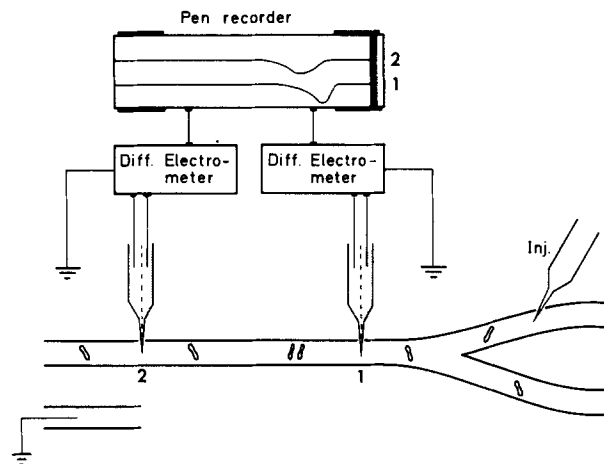


FIGURE 1. Schematic representation of the single-injection method. A K^+ bolus is injected into a capillary, and the concentration of K^+ within the capillary is monitored downstream by two K^+ -sensitive microelectrodes. The response of the electrodes is fed into two differential electrometers and recorded on a double-pen recorder.

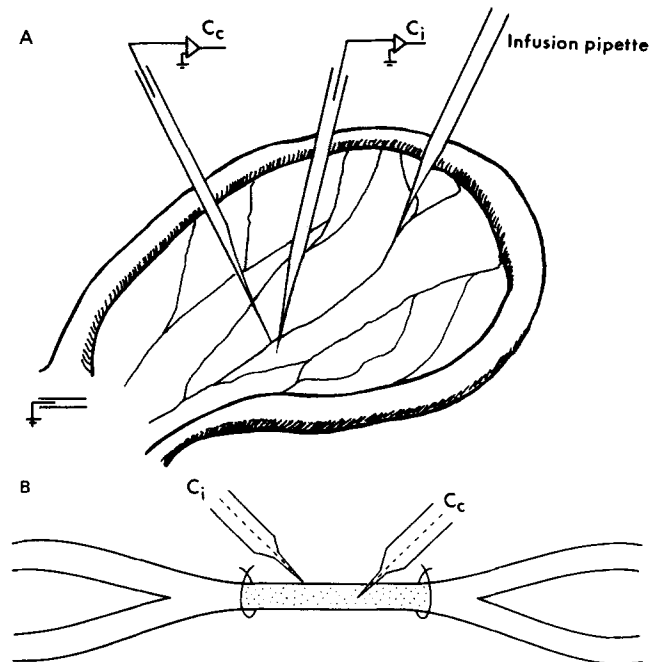


FIGURE 2. (A) The sack method. A capillary is filled with an isotonic Ringer's solution containing an elevated K^+ concentration. The diffusion of K^+ across the capillary wall is followed by use of two K^+ -sensitive microelectrodes, one within the capillary lumen and the other in the interstitium just outside the capillary wall (the closing rods are not shown). (B) Close-up of electrode positions in the sack experiment. The microrods which convert a segment of the capillary into a closed sack are symbolized by the two ligatures.

The permeability coefficient of the endothelial wall can be derived from the time-course of the intravascular concentration, $C_c(t)$, and the extravascular concentration very close to the capillary $C_i(0,t)$ during outward diffusion from the closed capillary segment.

The rate of loss of mass per unit length from the capillary to the interstitium is given by

$$\pi r^2 \frac{dC_c(t)}{dt} = -2\pi r P_c [C_c(t) - C_i(0,t)]$$

or

$$\frac{dC_c(t)}{dt} = -\Delta C(t) \frac{2P_c}{r},$$

where $\Delta C(t) = C_c(t) - C_i(0,t)$. The capillary permeability P_c can then be determined from the relation

$$P_c = -\frac{(dC_c/dt) r}{\Delta C(t) 2}. \quad (1B)$$

In practice, the differential quotient appearing in this expression can be replaced by a difference quotient, or it can be determined from graphical analysis. As seen from Eq. 1B the capillary permeability can be determined at any time during the discharge of the sack. At large values of time, however, both ΔC and dC_c/dt are small (cf. Fig. 5) so that the best evaluation can be made at short times. Usually, the determination of P_c was made at $t = 0$. This method has the advantage that no specific assumptions concerning the time-course of C_c and $C_i(0,t)$ are necessary because they are both measured experimentally.

In the particular case where the extravascular concentration can be neglected, the intravascular concentration decays exponentially with a time constant equal to $r/2P_c$. This situation would occur when the efficiency of the superfusion was sufficient to keep the concentration on the outside of the capillary equal to the superperfusate concentration. This, however, could not be achieved due to the relatively low permeability of the mesothelium covering the interstitial tissue.

INTERSTITIAL-DIFFUSION METHOD In this experimental approach, an intravascular infusion of high-K⁺ Ringer is started at $t = 0$. The flow rate is kept sufficiently high to ensure a constant intravascular concentration longitudinally. The interstitial concentration $C_i(x,t)$ is measured at different positions (x) in the interstitial space of the mesentery (cf. Fig. 3). Thus, the capillary need not be punctured by an electrode. The infusion is continued until the system has reached steady state, i.e., until $C_i(x,t)$ becomes constant. Then it is terminated, and the decay of $C_i(x,t)$ towards the superfusate concentration is recorded. The interstitial concentration at any point is the result of a balance between the supply of potassium ions through the capillary wall, the diffusional transport away from the capillary, and the loss through the upper mesothelial layer. The interstitial concentration can therefore under specified conditions yield information about the capillary permeability. This method was applied in two versions: steady state and transient. In the steady-state version, the information is extracted from the spatial dependence of the steady-state concentration. In the transient version of the method, the time-dependence of the concentration at a given position is used.

If $C_i(x,\infty)$ is the steady-state potassium concentration at various distances, x and C_c is the concentration of potassium in the perfused capillary, then the capillary permeability (P_c) can be found from the equation

$$C_i(x, \infty) = C_c \frac{1}{1 + \frac{D_i}{P_c \cdot \lambda}} \exp(-x/\lambda), \quad (1C)$$

where D_i is the diffusion coefficient of potassium in the mesentery, and λ is the characteristic length of the exponential concentration profile in the mesentery at steady state.

Both of these quantities are determined experimentally.

An account of the mathematical analysis of the interstitial-diffusion method is given in the Appendix, which also contains a list of the symbols used in this article.

A long capillary which lies reasonably isolated in the mesentery is preferentially chosen for investigation because the method requires that measurements be performed at various distances from the perfused capillary without the disturbance from the sink action of other flowing capillaries which would affect the final concentration arrived at in the interstitium.

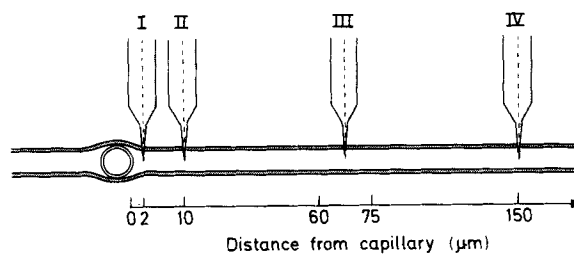


FIGURE 3. Schematic representation of the interstitial-diffusion method. The capillary is perfused at high flow rates with isotonic 50 mM K^+ frog Ringer's solution. K^+ -sensitive microelectrodes detect changes in interstitial K^+ concentration at various distances from the capillary.

The rate of perfusion should be high to satisfy the requirement that the fall of concentration in the longitudinal direction be minimal. This was ascertained in separate experiments.

After a measurement, time was allowed for the capillary content to equilibrate with the interstitial fluid and with the superfusate. Throughout the experiments and in the pauses between experiments, the upper surface of the mesentery was flushed with a constant, liberal flow of superfusate, to remove potassium ions (in excess of 2 mM) and to counteract any stagnant layers on the surface of the mesentery. The interstitial electrode was then repositioned further away from the capillary, and perfusion with the high-potassium Ringer's solution was resumed.

Preparation

All experiments were carried out on mesenteric capillaries of *Rana temporaria* at ambient temperature (20–24°C). The animals had been kept for varying periods (weeks to months) at 4°C. The animals were delivered in the autumn, and the experiments were carried out at various times during the year. Their weight was around 40 g. Anesthesia was established by placing the frogs in 5% urethane or 0.5% MS 222 solution (tricaine methanesulfonate, Sandoz Pharmaceuticals, E. Hanover, N. J.) until the mouth respiration stopped. They were then rinsed under water and the abdominal cavity opened in the right hand side by gentle use of a hot cautery needle to minimize bleeding. The frog

was then placed on its back on a special tray and the mesentery was carefully spread over the polished surface of a short Perspex pillar for transillumination. Great care was taken not to kink or twist the supplying or draining vessels. This part of the procedure proved to be crucial for securing a normal circulation. Any touching of the intestine was done with cotton-covered forceps soaked in frog Ringer's solution.

The exposed mesentery was kept moist by continuous superfusion with frog Ringer's solution. The duration of the experiments was generally such that the mesentery was exposed for 2-4 h. Most experiments were performed on male frogs inasmuch as it was difficult to expose the mesentery when oviducts full of eggs were present.

A M5 Wild dissecting microscope allowing magnification up to $\times 125$ (Wild Heerbrugg Instruments, Inc., Farmingdale, N. Y.) was used for cannulation and for positioning of the potassium-sensitive microelectrodes. The experiments were performed on arteriolar, mid- and venular capillaries with most data collected on midcapillaries. Capillary diameter and length were measured by means of a calibrated eyepiece micrometer to an accuracy of probably not more than 3-5 μm due to the fact that the border between capillary wall and tissue is poorly defined.

Microtools used in this study included microperfusion pipettes, potassium-sensitive microelectrodes, and glass microrods. Microperfusion pipettes were made from 1.8-mm OD Pyrex tubing (Corning 233010, Corning Glass Works, Science Products Div., Corning, N. Y.) pulled in a vertical microelectrode puller to produce a tip of approximately 1 μm . They were beveled on a rotating grinding stone (Walter Klotz, München) to produce a bevel with an angle of about 20° . In the process the tip diameter was allowed to increase to 3-7 μm . The pipettes were filled immediately before the experiments with isotonic high-potassium Ringer's solution via a thin polyethylene tubing introduced into the back end of the pipette, the stem of which was about 8 cm long. The solution had previously been passed through a Millipore filter with 0.22 μm mesh size (Millipore Corp., Bedford, Mass.) to remove particles.

Single-barreled or double-barreled liquid ion-exchange electrodes were prepared according to the method used by Zeuthen et al. (1974) based on the principle described by Walker (1971). Single-barreled pipettes were pulled from the same Corning glass as used for the infusion pipettes, drawn to tip diameters less than 1 μm . The back end of the pipette was exposed to the vapor of dichlorodimethylsilane (BDH no. 28197, BDH Chemicals, Ltd., Poole, England) for 60 s. The next day the pipettes were baked in an oven at 100°C for 1 h. The tip of the siliconed barrel was then filled from behind with liquid ion-exchanger (Corning 47317) by means of a thin glass capillary connected to a syringe. Then the tip region was examined microscopically to verify the absence of air bubbles. When present they were removed by gentle warming of the outside of the tip region by means of a wire heated by an electric current. The shaft was then filled with 150 mM KCl solution. Double-barreled pipettes were prepared in a puller which could accommodate two glass tubes side-by-side. During a first period of heating, the holder was rotated 360° , then 180° in the opposite direction to glue the two tubes together. In a second heating the double-electrode was pulled conventionally to a tip diameter of approximately 1 μm . During the mounting the glass tubes were slightly off-set lengthwise so that one of them got a longer stem. This permitted one of the two micropipettes to be siliconed alone. This barrel was then filled by the same procedure as described for the single-barreled type. Finally, the non-siliconed barrel was filled with a reference 150 mM KCl-solution. The resistance of the ion-selective barrel was 10^9 - 10^{10} ohms.

To cannulate the capillaries was difficult due to the fact that the mesentery contains numerous strands of fine fibrils which create a rather dense network resisting the penetration by the microcannula. It was therefore necessary to place a holding glass rod in the immediate neighborhood of the point of cannulation to keep the mesentery

fixed. The glass microrods were made from unfilled micropipettes. The tip was brought close to a microflame where another glass rod was heated to red heat. By gently touching the sealed pipette with the heated glass rod, the end of the shaft of the pipette could be bent to an angle of approximately 60° with the stem.

Electrical Circuit and Calibration of the K⁺-Sensitive Electrodes

The potassium activity at the tip of the double-barreled electrodes was measured from the DC-potential arising between the two barrels, whereas the activity at the tip of the single-barreled electrode was measured as the DC potential arising between it and a reference electrode in contact with the tissue via a polyethylene tubing with 150 mM KCl solidified in 2% agar. Ag⁺/AgCl connections were both used in the polyethylene tubing, the reference barrel, and the K⁺-sensitive barrel. A Keithley 604 differential electrometer (Keithley Instruments, Cleveland, Ohio) with input resistance above 10¹⁴ Ω was used for the amplification of the signals. The output from the electrometer was fed into a 2-channel pen recorder, either a fast Brush 220 recorder (Gould Inc., Inst. Sys. Div., Cleveland, Ohio) or a slower Kipp-Zonen BD9 recorder (Kipp-Zonen, Delft, Holland), depending on recording speed requirement. The fastest response time was obtained with the shield of the input lead following the signal. This system had a time constant of 20–40 ms. Without this capacity-reducing arrangement, the system had a time constant of 200–400 ms. The electrodes were calibrated before and after the experiments by measuring the relationship between DC-potential and K⁺-concentration in Ringer's solutions containing 2, 4, 6, 8, 10, 16, 25, 50, 75, and 100 mM potassium. The double-barreled electrodes typically gave a 46–48 mV change for a 10-fold change in potassium concentration while the single-barreled electrodes had a slightly better Nernstian slope with a 52–54 mV change for a 10-fold change in potassium concentration. When double-barreled electrodes were used, the electrical potential between the reference barrel and a reference electrode on the surface of the mesentery was recorded through a NF1-Bioelectric amplifier (General Microwave Corp., Farmingdale, N. Y.). No DC-potential deflections (within 1 mV) were observed when the perfusion started or ended. Similarly, no measurable potential difference was recorded across the capillary wall.

General

In the single-injection experiments the injection cannula was placed in one of two merging capillaries so that a pulse of high-K⁺ Ringer's solution could be given into flowing blood. Under these circumstances the experimental capillary was exposed to a mixture of blood and high-K⁺ Ringer's solution. After the injection the capillary was rapidly cleared from injectate by the freely flowing blood.

The fact that the superfusate did contain 2 mM potassium, a concentration similar to that of frog plasma and interstitial fluid, means that all experimentally induced changes of potassium concentration within capillaries or in the interstitium represented increments of potassium concentration above a 2 mM background value which had to be subtracted.

The efficiency of the superfusion to clear excess potassium from injected capillaries was determined in experiments where a high-potassium solution was infused into a capillary while the potassium concentration was monitored on the surface of the mesentery above that capillary. The effect of the high-potassium infusion could not be detected on the surface of the mesentery under these circumstances.

The pressure rise inside the capillary due to the injection was also investigated in separate experiments with the Servo-Null system (Intaglietta et al., 1970) for pressure measurement in micro-vessels. In five experiments where a standard injection was

given, maximal pressure increments of 3–5-cm H₂O were seen. This pressure was considered to have no effect on the potassium movement across the capillary wall.

Normal frog Ringer's solution was prepared to provide the following concentrations (mM): Na⁺, 110.7; K⁺, 2.0; Ca⁺⁺, 1.8; Cl⁻, 116.4; and HCO₃⁻, 1.2. The osmolality was 215 mosmol l⁻¹. The perfusion solution consisted of normal frog Ringer's solution in which the K⁺-concentration was increased by substituting KCl for NaCl, to a K⁺-concentration of about 50 mM for sack-diffusion and interstitial-diffusion experiments, and 75–150 mM KCl in single-injection experiments. Bovine serum albumin (Sigma no. A-4503, Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 1.25%. Superfusion solution consisted of normal frog Ringer's solution with bovine serum albumin added to provide the same concentration. Calibration solutions were prepared from normal frog Ringer's solution with KCl substituted for NaCl.

In the illustrations, the curves shown are the result of re-drawings of the original recordings indicating the potential variations due to variations in potassium concentration at the electrode tip. The potentials were converted to concentrations by means of calibration curves constructed for each micro-electrode. From these results fully drawn curves were then made as exemplified in some of the illustrations.

RESULTS

Single-Injection Experiments

Fig. 4 gives an example of time-concentration curves which were obtained in these experiments. The appearance of the bolus at each electrode is followed by a fast and steep rise in concentration. It is seen that the rise of potassium concentration at the down-stream electrode occurs with a very short delay after the upstream response representing the capillary transit time. The area before the peak under the second curve is clearly lower than the corresponding area under the first because of the outward diffusion of potassium in the intervening capillary segment.

Table I shows the results from experiments on 10 different capillaries with several determinations made on each capillary. When successive determinations were performed, the intracapillary electrodes were kept in position. After the capillary had been cleared by free flowing blood and the excess potassium had been removed via the superfusate, another injection was performed.

It is seen that the permeability coefficients range from $32 \times 10^{-5} \text{ cm} \cdot \text{s}^{-1}$ to $127 \times 10^{-5} \text{ cm} \cdot \text{s}^{-1}$ with a mean value of $74 \times 10^{-5} \text{ cm} \cdot \text{s}^{-1}$ (SD = 31). The scatter of results with the single-injection technique was greater than with the other two methods. The cause of this is probably the flow determination which is the most uncertain part of this procedure.

The potassium movement after the bolus injection begins as unidirectional outward diffusion which later is followed by return of most of the externally located potassium back to the capillary. This effect was clearly seen if the permeability calculations included areas of the curves after the peak. A continuous fall in the calculated permeability was found with incorporation of later parts of the curves.

The results from the present series of experiments are lower than those originally reported by Crone and Friedman (1976) which is explained by technical difficulties during the initial trials with the technique.

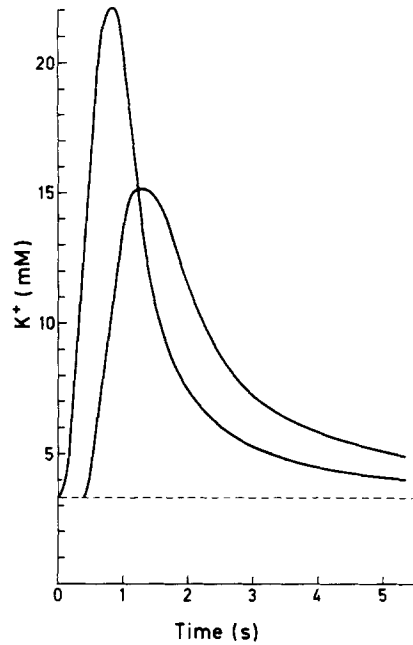


FIGURE 4. Single-injection experiment. A K^+ bolus is injected upstream into a capillary. The figure shows time-concentration curves recorded downstream by two K^+ -sensitive microelectrodes inserted into the same capillary with an interelectrode distance of $960 \mu\text{m}$. The dashed line indicates the physiological background K^+ concentration.

TABLE I
 K^+ PERMEABILITY OF SINGLE CAPILLARIES DETERMINED WITH THE
SINGLE INJECTION TECHNIQUE

Capillary	Radius μm	P_e $10^{-3} \text{ cm s}^{-1}$	Determinations n
1	15	110	9
2	10	75	2
3	14	40	2
4	13	54	2
5	16	58	9
6	11	84	3
7	10	127	4
8	13	60	3
9	18	32	5
10	12	102	5

This table lists values of K^+ permeabilities determined by repeated injections of an isotonic high- K^+ frog Ringer's solution into 10 capillaries from different frogs. n represents number of determinations on the same capillary.

Sack Experiments

The results of one of these experiments are illustrated in Fig. 5. A brief pulse, lasting for about 4 s was used to augment the capillary content of potassium. It was found that the concentration on the outside of the capillary rose rapidly

from the beginning of the perfusion and, far from being negligible, reached about 50% of the intravascular concentration within a few seconds. Had the efficiency of the superfusate in clearing interstitial potassium been high, the decay of intravascular potassium concentration would have followed a mono-exponential course. However, the intravascular and the immediately extravascular potassium concentrations converged to the same value quite rapidly signifying a significant delay in potassium removal from the extracapillary region. The delay is caused by the diffusional transport in the interstitium and by the diffusional resistance in the mesothelial layer of the mesentery.

Table II shows the results from experiments on eight different capillaries. The mean estimate of $P_K = 68 \times 10^{-5} \text{ cm} \cdot \text{s}^{-1}$ (SD = 12).

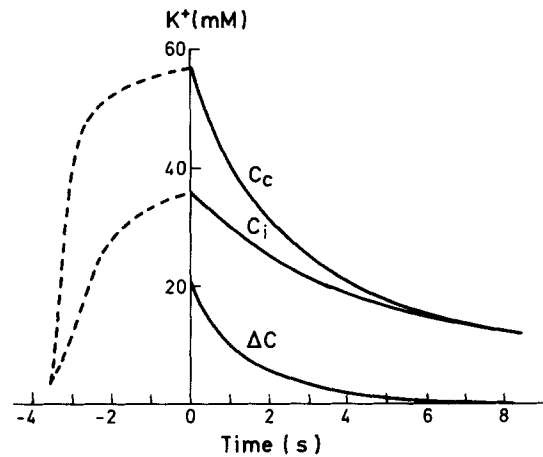


FIGURE 5. Sack experiment. The figure shows the rise and fall of K^+ concentration within (C_c) and just outside the capillary (C_i) during a brief infusion of isotonic 60 mM K^+ -Ringer's solution and after formation of a capillary sack by occluding microrods. The lower curve (ΔC) shows the time-course of the transcapillary K^+ concentration gradient.

To evaluate the results, it is essential that the concentration within the sack be uniform, i.e., that no longitudinal gradients are present. This assumption is justified by the finding that the maximal intravascular concentration always matched that of the perfusate. This is also a test for proper placement of the electrode tip.

Interstitial-Diffusion Experiments

STEADY-STATE METHOD. The results of an experiment are illustrated in Fig. 6 where the concentrations in the interstitial space at three distances from the capillary are represented over time. It is seen that the final concentration reached is lower the longer the distance from the perfused capillary. Also, the attainment of steady state, as expected, occurs with greater delay.

A prerequisite for arriving at the capillary permeability is knowledge of the 'decay-time constant' through the mesothelium (τ_m). This constant was determined when the steady-state concentration had been reached, by stopping the

intracapillary perfusion and letting the mesothelium 'discharge' its extra potassium content through the upper mesothelial surface.

Two sets of information were thus obtained from these experiments: the steady-state concentration at various distances from the perfused capillary, and

TABLE II
 K^+ PERMEABILITY OF SINGLE CAPILLARIES DETERMINED WITH THE
 SACK TECHNIQUE

Capillary	Radius μm	P_e $10^{-5} \text{ cm s}^{-1}$	Determinations n
1	14	62	1
2	19	57	1
3	11	70	4
4	13	63	1
5	14	73	1
6	10	73	4
7	8	53	5
8	10	92	7

This table lists values of K^+ permeabilities determined from the efflux of potassium from a closed capillary segment after injection of an isotonic high- K^+ frog Ringer's solution into eight capillaries from different frogs. n represents number of determinations on the same capillary.

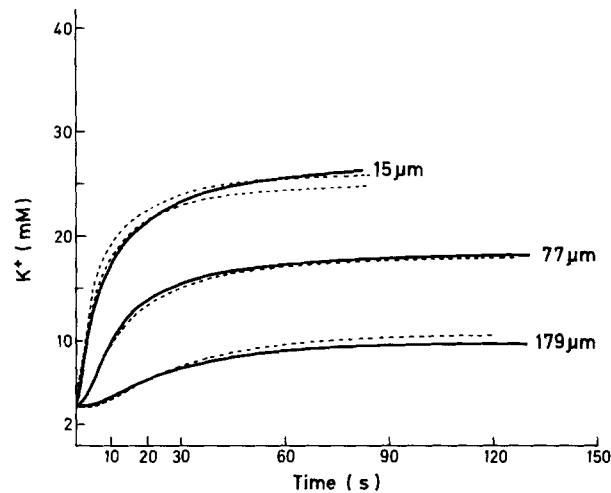


FIGURE 6. Interstitial-diffusion method. The figure shows the rise of interstitial K^+ concentration at three distances from a capillary continuously perfused with an isotonic Ringer's solution containing 50 mM K^+ . The dotted lines refer to results from curve-fitting procedures in the transient-state analysis (for further details see text).

the decay-time constant for diffusion through the mesothelium. As explained in the Appendix, the parameter λ ($= \sqrt{D_i \cdot \tau_m}$) can be obtained from a plot of the logarithm of the steady-state concentration vs. distance (cf. Fig. 7). The slope of this line defines λ . When τ_m is known, D_i can be calculated. The value of the potassium concentration extrapolated to $x = 0$ gives the concentration at

the surface of the capillary, $C_i(0, \infty)$ at steady state. Because, according to Eq. 1C, $C_i(0, \infty) = C_c / (1 + D_i / P_c \lambda)$, P_c can now be calculated as the only remaining unknown.

Table III contains the results from eight successful experiments with the steady-state version of the interstitial-diffusion method. The mean estimate of P_K was $62 \times 10^{-5} \text{ cm} \cdot \text{s}^{-1}$ (SD = 21).

TRANSIENT METHOD In the Appendix it is explained how the time-course which the interstitial potassium concentration follows during attainment of a steady-state concentration contains information about the capillary permeability. The expression relating the various key parameters is given in Eq. 9a in the Appendix. The parameters P_c and D_i were determined by a least squares fit to

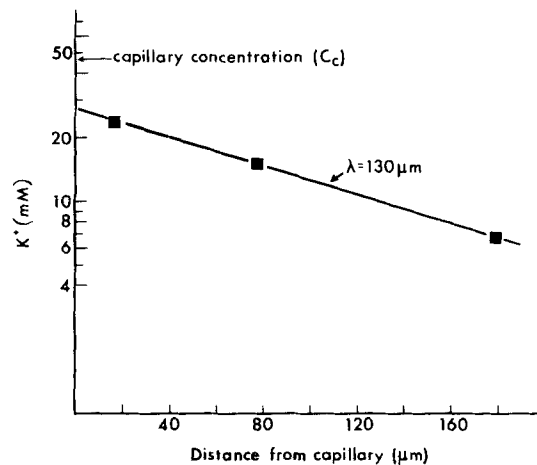


FIGURE 7. Steady-state concentrations of K^+ within the interstitium of the mesentery measured at three distances ($15 \mu\text{m}$, $77 \mu\text{m}$, and $179 \mu\text{m}$) from the capillary. The capillary is continuously perfused with an isotonic Ringer's solution containing $50 \text{ mM } K^+$. $\lambda = \sqrt{D_i \cdot \tau_m}$ (cf. list of symbols). The figure shows that there is a concentration drop across the capillary wall which in the steady state is about 40% of the intracapillary concentration.

the time-course of rise of interstitial potassium concentration. An example is seen in Fig. 6, where the dotted lines indicate the fitted concentrations whereas the fully drawn curves indicate the actual experimental results.

The results from eight successful experiments are shown in Table III. The mean estimate of P_K was $54 \times 10^{-5} \text{ cm} \cdot \text{s}^{-1}$ (SD = 16). The transient results were obtained in the same experiments which gave the steady-state results; thus, the table allows a comparison of the correspondence of data obtained with the two versions of the interstitial-diffusion method. As seen from the table, there was, in most cases, good agreement between the results obtained from the two versions of the interstitial-diffusion method.

The analysis of both steady-state results and the results from the transient state contains the diffusion coefficient of potassium in the interstitial tissue of the mesentery. The mean values were $0.70 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ and 0.63×10^{-5}

$\text{cm}^2 \cdot \text{s}^{-1}$, respectively. At 20°C the free diffusion coefficient of KCl at infinite dilution is $1.8 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ (Robinson and Stokes, 1959). Thus, the rate of diffusion in the interstitial space is reduced by a factor of 3 compared to free diffusion in water. One might have expected a smaller reduction due to the noncellular composition of the mesentery. However, electron microscope studies of the preparation disclosed the presence of numerous fine fibrils in the connective tissue. The cross-sectioned fibrils had diameters of approximately 50 nm and appeared to lie in bundles. These fibrillar strands suggest that tortuosity factors contribute in this relatively cell-free tissue.

When the results obtained with the three methods are compared, it is found that they do not differ systematically from each other. The individual results

TABLE III
 K^+ PERMEABILITY OF SINGLE CAPILLARIES DETERMINED WITH THE
 TWO VERSIONS OF THE INTERSTITIAL DIFFUSION TECHNIQUE

Capillary	Radius μm	τ_m s	Steady state		Transient state	
			P_c $10^{-5} \text{ cm s}^{-1}$	D_i $10^{-5} \text{ cm}^2 \text{ s}^{-1}$	P_c $10^{-5} \text{ cm s}^{-1}$	D_i $10^{-5} \text{ cm}^2 \text{ s}^{-1}$
1	11	90-96	60	0.37	62	0.5
2	11	50-70	83	0.9	83	0.7
3	17	45-55	47	0.49	45	0.43
4	23	46-48	50	1.3	50	1.3
5	13	40	46	0.43	40	0.5
6	11	25-37	37	0.27	43	0.52
7	13	46-49	98	0.32	70	0.35
8	10	40-41	73	1.6	40	0.7
Mean values		51	62	0.71	54	0.63

This table lists values of K^+ permeabilities (P_c) obtained from the increment of interstitial potassium concentration during perfusion of a nearby capillary using either the transient or the final steady-state K^+ concentration. τ_m is the time constant for the decay of interstitial K^+ concentration after arrest of capillary perfusion. The values indicate the lowest and highest values of time constants in successive determinations at various distances from the perfused capillary. D_i is the interstitial diffusion coefficient of K^+ determined as described in the text.

from the 26 capillaries studied are compiled in one histogram shown in Fig. 8. When the capillaries are considered to belong to one population, a mean value for P_K of $67 \times 10^{-5} \text{ cm} \cdot \text{s}^{-1}$ (SD = 23) is calculated.

DISCUSSION

The main interest of this study from an experimental point of view consists in having demonstrated the feasibility of measuring the permeability of a single capillary. We have described three approaches: one in which the permeability is deduced from a determination of the initial loss of material across a known capillary surface area (single-injection experiments); one in which the flux is determined under conditions where the transcapillary concentration difference is known (sack experiments); and one in which the permeability was deduced from the events taking place in the interstitium surrounding the capillary after a known step input into the vessel (interstitial-diffusion method).

Single-Injection Experiments

The advantage of this method compared with its application in whole organ experiments (Crone 1963*a*) is that the transcapillary loss occurs through a well-defined capillary surface area. It might be criticized that in the modeling the concentration of test solute immediately outside the capillary wall is disregarded. It is possible, however, to evaluate the magnitude of error of this omission in the calculation of permeabilities. It can be seen from Fig. 4 that the upslope time of the bolus is about 1 s. During this time the extravascular concentration next to the capillary wall would have increased to a maximal level of 30% of the intravascular concentration (cf. Fig. 5) with an average of 15% for the entire upslope period. The extraction (E) is, therefore, underestimated by a similar percentage. For a typical value of extraction of 0.4–0.5, the permeability would be underestimated by about 12%. It is, of course, somewhat arbitrary to

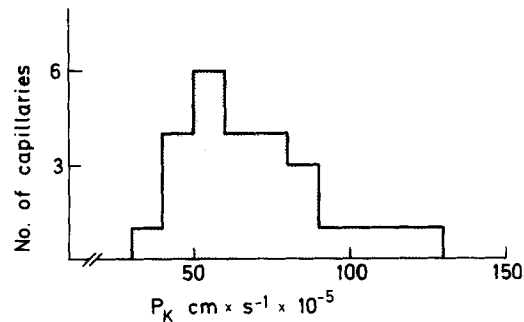


FIGURE 8. Histogram of values of the K^+ permeability of the walls of 26 capillaries. Results were obtained with three different experimental approaches as described in the text. Inasmuch as there are two versions of the external-diffusion method, mean values of the two determinations on the same capillary were used. The average value for potassium permeability from all experiments was $P_K = 67 \times 10^{-5} \text{ cm s}^{-1}$ (SD = 23).

integrate the areas from appearance to peak time. This procedure was chosen to diminish the effect of 'noise' at the low early increments in potassium concentration. To decide whether the underestimation of the permeability could be larger than 10–15% would require experiments with external electrodes placed on the outside of the capillary directly opposite the internal electrodes. Even if this could be done, there would remain the problem of possible discrete openings in the wall, in which case the external electrode would be located at undefined distances from such openings anyway.

Another uncertainty of the single-injection technique concerns the determination of the capillary flow during the passage of the bolus. Inasmuch as the passage time is determined from the difference in time between the bolus arrival at the two electrodes, which is less than 1 s, inaccuracies are unavoidable. The derivation of Eq. 1*A* requires that extraction (E) and flow (F) be properly matched at all times. Because the flow increment due to the bolus injection is rapid at first and then decays, it is not possible to obtain a perfect match, even

on the upslope. The nonsteady flow situation would mean that the difference in appearance time would overestimate flow. But again since the peak of the upslope is reached so fast this error is considered to be small. To the extent that it occurs it would lead to a slight overestimation of the permeability, thus counteracting the effect of the neglected outside-concentration.

Another source of error is connected with the placement of the electrodes. Because of the limitations of the resolving power of the dissecting microscopes, one operates at the limit of resolution when deciding whether an electrode tip is safely within the capillary lumen or just on the outside of the capillary. It is immediately clear that the worst combination of placement errors would be a correctly placed proximal electrode with the distal one just on the outside of the capillary. Such a situation, which can be difficult to discover, would lead to gross overestimations of the permeability.

The advantage of the method is that the capillary is perfused with normal frog blood between the experiments because the injection pipette was placed so as to permit free flow between injections. Also, the injectate is mixed with frog plasma, which may be advantageous.

If there are problems defining the free capillary surface area through which diffusion occurs, these would be minimal in the single injection situation where probably the whole of the capillary would allow unidirectional outward diffusion right at the beginning. In the two other methods the small space between capillary and the covering mesothelium might be 'filled' and thus effectively reduce the capillary surface area through which diffusion occurs since in these cases the 'transport situation' lasts longer.

Of the three methods employed the single injection technique proved to be the most difficult to execute, but it may have its place in studies on single capillaries in other organs where sack or interstitial-diffusion methods would be difficult to employ.

Sack Method

This method is comparatively free from sources of error. It requires that the sack be reasonably long so that damage due to the pressure of the closing rods be as insignificant as possible relative to the large area of intervening capillary surface. There is, however, a potential problem concerning leaks around the tip of the electrode.

The mathematics describing the wash-out sequence is fitted directly to the experimental conditions with proper consideration of the outside concentration and allowance for nonexponential conditions. Errors can arise because of the fast rate with which the transcapillary concentration difference decays. A few seconds after the sac was closed, ΔC decayed to a value of a few millimolars (cf. Fig. 5). The capillary permeability can, in practice, best be determined from the most early readings (within the first few seconds) where ΔC is largest.

Interstitial-Diffusion Method

Since the internal concentration in the perfused capillary is known, the method only requires placement of an interstitial electrode. This gives the method a

special appeal because the preceding methods which require intracapillary measurements are technically more difficult.

Irrespective of this attractive feature there are shortcomings which need comments.

STEADY-STATE METHOD Two parameters are needed: the decay time-constant through the mesothelium (τ_m) and the characteristic diffusion length in the interstitium (λ). The determination of τ_m presents no difficulty. However, the determination of λ requires that steady-state readings be made at various radial distances from the perfused capillary.

It is therefore important that other vessels perfusing the mesentery do not act as sinks, which requires that a relatively isolated capillary be chosen for the experiment. Also, the mathematics presupposes uniform mesentery thickness and mesothelial permeability. The thickness is, however, not quite uniform, and this introduces an uncertainty in the determination of D_i . It can only be determined with an accuracy of 20% if the characteristic diffusion length is determined with an accuracy of 10%. The values of P_c were, however, rather insensitive to this variation. This is so, because an excessively low value of λ (and therefore of D_i) results in a steeper slope in the above-mentioned plot. This leads to an overestimation of $C_i(0, \infty)$, the extrapolated concentration on the outside of the capillary wall at steady state. This reciprocal relationship tends to maintain P_c relatively constant. To illustrate this, we found that a variation of the value of D_i with a factor of 2 only resulted in 10–20% variation of P_c .

The steady-state method thus yields fairly precise values of P_c , whereas the determination of D_i is less reliable.

As seen from Fig. 6, it takes at least 1 min to reach a steady-state which means that the capillary perfusion is long-lasting. Any possible increase of the capillary permeability due to the high-potassium solution has the best possibilities to develop in this approach. However, the permeabilities were not higher than those obtained with the other methods, which is an indication that the effect of the high-potassium solution on permeability is small.

It is obvious that the method requires a correct indication of the attainment of a steady state. Small underestimates in the steady-state concentrations affect the results linearly. It is, of course, somewhat difficult to define exactly when a 'creeping' concentration (cf. Fig. 6) reaches its final value.

TRANSIENT METHOD In general, the problems encountered here are the same. Fortunately, this version is quite sensitive to variations in D_i . To obtain P_c and D_i , the following procedure was adopted: (a) P_c and D_i determined from the steady-state results were used as starting figures in an iteration procedure for curve fitting; (b) D_i was then adjusted to give optimal correspondence between experimentally observed and calculated transients.

To the extent that the figure for P_c determined from steady-state analysis is accurate, the transient analysis determines D_i with an accuracy of about 20%. In some cases where agreement between measured and 'fitted' data could not be obtained for small and long extracapillary distances, P_c had to be changed to obtain a good fit.

In most cases, the difference between measured and fitted concentrations was kept within 5–30% in fits on curves obtained at three different distances from the same capillary (cf. Fig. 6).

The values for P_c and D_i given in Table III illustrate the point raised here. It is seen that the values of P_c derived from the steady-state or the transient version of the interstitial-diffusion method differ very little, whereas quite large differences are seen in the values for D_i .

The transient method can be used when an additional check on the values of D_i is required. The uncertainty with which P_c and D_i are determined was smaller than the scatter of these quantities from capillary to capillary.

General Comments

An obvious problem touching upon our—as well as most other—studies of exposed or isolated tissues is the question of whether the exteriorization has changed the structure under investigation. The dissection of the frog was done as gently as possible with minimal touching of the intestine, but the exposure of the mesentery to new conditions such as gas tensions different from those in the abdomen, incident light, and perhaps, slight traction might elicit responses changing the permeability. We did often observe that the intestine immediately after exposure was quite pale, only to become reddish 1 min or so after exposure to the atmosphere. Perhaps a chain of events is started leading with time to an inflammatory state. However, we could not identify any trend in the permeability data suggesting, for example, an increase with time.

Another general problem is the possible effect of the anesthesia on the capillaries. The frog was placed in 5% urethane solution which diffused through the skin covering the abdomen so that the abdominal cavity concentration probably would exceed that in the rest of the frog. However, some of the experiments were done under MS 222 anesthesia without clear differences between the results. In the experiments, solutions containing potassium ions in quite high concentration, about 50–100 mM, were injected. It cannot be completely ruled out that such a high concentration exerts physiological effects on the endothelium. According to Funaki (1958), the endothelial cell has a membrane potential of -50 mV and would probably be depolarized under the influence of a high-potassium solution. This might induce intracellular effects. It is almost certain that the perfusion with a high-potassium solution with a Donnan product of KCl different from the normal, will lead to a net movement of KCl with water into the cell, thus creating a slight swelling of the endothelial cell. To what extent this interferes with the capillary permeability is unknown. In the short-lasting pulse-type experiments with normal perfusion in between, the effects would supposedly be small. In the longer-lasting experiments with the interstitial-diffusion method, time is ample for water shifts to occur. However, we could not find any systematic trend in the data between the methods. But the problem may be a real one and, therefore, should be looked into with morphological methods.

We were worried about the possibility that the exteriorization of the mesentery might lead to edema development with changes of the geometry of the

mesentery. Inasmuch as the mathematical analysis of the interstitial-diffusion methods rests upon the assumption that there is vertical 'instantaneous' mixing, pronounced and irregular swelling of the mesentery would upset this condition. In a few light microscopical studies of the mesenteries fixed after various times of exposure, gross edema was never present. What we saw was the collection of fluid immediately outside the larger vessels; never did extra fluid visibly accumulate around the capillaries or in the interstitial tissue.

Inasmuch as the data reported in this paper refer to the permeability of the capillary to a single ion, potassium, it may be asked whether the conditions are such that the results reflect the permeability of this ion alone? If the wall offered various degrees of restriction to the permeation of different ions, particularly Na^+ , K^+ , and Cl^- , the movement of any particular ion would not be independent of that of the others. When a high-potassium solution is suddenly introduced into a capillary, a rearrangement of several intra- and extracapillary ions takes place. Potassium ions leave the capillary mainly in exchange for sodium ions. The course of events depends mainly on differences in the degree of diffusion restriction of these three ions. Transient diffusion potentials would have arisen between the electrode in the capillary and that in the interstitium if there were significant selective restriction of permeation of Na^+ , K^+ , and Cl^- . In that transient potential variations were not seen, we assume that the permeability data reflect permeability of the potassium ion proper.

Final Considerations and Implications

The current paradigm in capillary physiology as regards an equivalent model of the capillary membrane is the so-called Pappenheimer pore model (1953). This model in its original version described the capillary membrane as being analogous to an inert $0.5\text{-}\mu\text{m}$ thick membrane perforated by cylindrical pores or rectangular slits of molecular dimensions constituting less than a thousandth of the surface area. The original model has been revised (with preservation of the fundamental idea) to include a transcellular pathway for water movement in the so-called parallel-pathway model (Pappenheimer, 1970; Johnson, 1970; Lifson, 1970) for which experimental support has been provided by Effros (1974), by Curry et al. (1976) and by Wangensteen et al. (1977). According to the revised model, water moves through an exclusive pathway (cells) as well as through a channel (pore) shared by water and small hydrophilic molecules and ions. There is some disagreement as to whether or not the diffusion restriction for small ions and molecules proposed by Pappenheimer et al. (1951) for continuous capillaries does occur, or whether diffusion restriction is only seen for large molecules (Crone, 1963*b*; Curry et al., 1976; Paaske, 1977).

Can our results from the frog mesentery be interpreted in terms of the pore model despite the fact that the potassium permeabilities were about 20 times higher than those previously reported for potassium or ions of similar size (Alvarez and Yudilevich, 1969; Sheehan and Renkin, 1972; Stray-Pedersen and Steen, 1975; Tancredi et al., 1975; Guller et al., 1975)? Previous data were obtained on mammalian muscle (striated or heart) with whole-organ technique,

whereas ours derive from a segment of a single capillary, albeit also one of the continuous type (Bennett et al., 1959) and from a different species. A difference in morphology exists inasmuch as the mammalian muscle capillary has a diameter of 4 μm whereas the capillaries in the frog's mesentery have diameters between 10–35 μm . If the size of the endothelial cells were similar between the species, there would, however, be the same junctional area per cm^2 irrespective of the diameter of the capillary.

Apparently, this difference in morphology alone cannot explain our findings. Can they be fitted to a numerically revised Pappenheimer model? Our answer is yes, based on the following reasoning. To obtain an equivalent pore radius, Pappenheimer combined data about water diffusion and water filtration through the muscle capillary. It is possible to carry out the same calculations on the frog capillary because the water filtration coefficient (L_p) of this capillary is known from the studies of Michel et al. (1974), whereas our data allow an estimate of the water diffusional permeability of the pores alone. To arrive at this figure we make two assumptions: (a) the potassium permeation through the capillary takes place only via the pores, i.e., the K^+ permeability of the endothelial cells can be ignored; and (b) K^+ diffusion through the pores is unrestricted. Under these assumptions the water permeability of the pores is given as:

$$P_{\text{H}_2\text{O}}^p = P_K \cdot \frac{D_{\text{H}_2\text{O}}}{D_K}$$

Insertion of appropriate figures ($D_{\text{H}_2\text{O}}$ at 20°C = $2.15 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$, and $D_K = 1.80 \times 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$) gives a figure for $P_{\text{H}_2\text{O}}^p$ of $80 \times 10^{-5} \text{ cm} \cdot \text{s}^{-1}$.

It is important to realize that this argument does not preclude water diffusion through the endothelial cells which undoubtedly occurs. However, for the calculation of an equivalent pore radius, only that fraction of the water diffusion which occurs via the pores is relevant.

By means of the Poiseuille and Fick equations, an equivalent pore radius and a density of pores per unit area is obtained:

$$L_p^p = N \cdot \frac{\pi \cdot \rho^4}{8 \cdot \eta \cdot \Delta x} \quad (\text{filtration})$$

and

$$P_{\text{H}_2\text{O}}^p = \frac{D_{\text{H}_2\text{O}}}{\Delta x} \cdot \pi \cdot \rho^2 \cdot N, \quad (\text{diffusion})$$

where N is number of pores per unit of area, η is the viscosity of water at 20°C, ρ is the equivalent pore radius and Δx is the length of the pore pathway. In Michel et al. (1974), the average value for L_p is $60 \cdot 10^{-8} \text{ cm} \cdot \text{s}^{-1} (\text{cm} \cdot \text{H}_2\text{O})^{-1}$. Curry et al. (1976) have provided evidence that $\cong 90\%$ of the filtration occurs via pores. This reduces the appropriate L_p^p to $54 \cdot 10^{-8} \text{ cm} \cdot \text{s}^{-1} (\text{cm} \cdot \text{H}_2\text{O})^{-1}$.

Insertion of the various values and constants in the equations leads to an equivalent pore radius of 110 Å, a pore density of 8 μm^{-2} , and, in consequence, a fractional pore area of 0.3%, if the pore path length, Δx , is taken to be 1.0

μm . The latest figures for the mammalian muscle capillary were: pore radius, 40–45 Å; pore density, $10 \mu\text{m}^{-2}$; and fractional area, 0.15% with a diffusion path length of 0.5 μm (Landis and Pappenheimer, 1963).

The greatest difference between the two sets of data is the difference in pore dimension. The main explanation for the large pore radius obtained for the frog capillary is the 20-times larger filtration coefficient of these capillaries than the one obtained for muscle capillaries. Inasmuch as the filtration data from Michel's group correspond quite well with those from Landis' original experiments (1927), there is reason to believe that this figure describes accurately the nature of the capillary porosity. Michel (1977), from determinations of reflection coefficients of macromolecules in the same capillaries, deduced pore radii of 50 Å. Mason et al. (1977) proposed pore radii of 100–150 Å on the same capillaries. Wangenstein et al. (1977) report equivalent pore radii of 100 Å in the rabbit lung capillary.

The calculation of equivalent pore radii rests on the assumption that there is only one class of pores (disregarding the pores in the endothelial cells) and that filtration takes place by Poiseuille flow in the pores. Particularly the first requirement may seem unrealistic in that the presence of large pores (leaks) repeatedly has been inferred. Although they are very few in number, they have strong effects upon the filtration coefficient while the effect on diffusional permeability is small. If the presence of such leaks is demonstrated in the frog mesenteric capillary, the filtration coefficient relevant for the calculation of pore size goes down, and a smaller equivalent pore radius would result. Unfortunately, the problem of a single-sized pore system vs. a dual-class system is unsolved, and for this reason, the experimentally determined coefficients are still phenomenological.

Our result that the equivalent pore is so large raises the question of the anatomical location of it.

Pappenheimer (1953) was not explicit about this aspect of his hypothesis, but was inclined to accept the suggestion of Chambers and Zweifach (1948) that the intercellular junctions were the equivalent water-filled pores. Later writers have been more definite in their support of this hypothesis (Ohori, 1963; Karnovsky, 1968; Perl, 1971; Trap-Jensen and Lassen, 1971). However, since the proposal of Simionescu et al. (1975) that the Pappenheimer pores might in fact be construed as temporary transcellular channels formed by fusion of neighboring intraendothelial vesicles, a new alternative has been introduced into the debate. The so-called neck-region of vesicles attached to the membrane surface has the right dimension with its 80–100 Å radius, but definite proof that fused vesicles may serve as a main diffusion pathway for small molecules and ions is still lacking. We would, however, like to entertain the idea that in the frog mesentery the Pappenheimer pores include such temporary channels because this possibility avoids the difficulty of explaining the high values of P_K and L_p by intercellular junctions which look similar to those in mammalian muscle capillaries. The endothelium of the capillaries in the frog mesentery is loaded with vesicles, and many intraendothelial fusions are seen (Bundgaard and Frøkjær-Jensen, unpublished). What this means in terms of function is still unknown. On the other hand Lassen and Trap-Jensen (1970) calculated that

only a small fraction of the circumference of tight junctions between endothelial cells need be open in order to explain permeability data from experiments on mammalian muscle. Therefore, the higher permeability found in the frog mesentery could also be due to a larger fraction of the junction being open.

At the present time the possibility of the pores being constituted by intercellular junctions and (or) transendothelial channels cannot be properly evaluated, but future exploration of both these possibilities seems indicated.

Capillary physiology has now reached an interesting cross-road where valuable information awaits to be harvested on single vessels from organs which have been submitted to whole-organ analysis. Only by such a direct comparison can we get insight into the reality of permeabilities obtained from whole-organ experiments. The micro-electrode technique seems well suited for this task.

LIST OF SYMBOLS

Symbol	Quantity	Unit
A	Capillary surface area between measuring electrodes	cm^2
C_c	Intracapillary K^+ concentration	mM
$C_i(0, t)$	Interstitial K^+ concentration on the outside of capillary	mM
$C_i(x, t)$	Interstitial K^+ concentration with respect to distance from the capillary (x) and time (t)	mM
C_s	K^+ concentration in superfusate	mM
D_i	K^+ diffusion coefficient in interstitium	$\text{cm}^2 \cdot \text{s}^{-1}$
$D_{H_2O}^{20}$	Self-diffusion coefficient for water at 20°C: 2.15×10^{-5}	$\text{cm}^2 \cdot \text{s}^{-1}$
D_K^{20}	Free K^+ ion-diffusion coefficient at 20°C: 1.8×10^{-5}	$\text{cm}^2 \cdot \text{s}^{-1}$
E	Extraction or fractional loss of material through capillary wall	Dimensionless
F	Flow in a single capillary	$\text{cm}^3 \cdot \text{s}^{-1}$
$G(x)$	Diffusive flux of excess K^+ in the interstitium	$\text{mol cm}^{-2} \cdot \text{s}^{-1}$
L	Interelectrode distance	cm
L_p	Average filtration coefficient of capillary wall	$\text{cm s}^{-1} (\text{cmH}_2\text{O})^{-1}$
N	Number of pores per 1 cm^2 of capillary surface area	cm^{-2}
P_c	Permeability coefficient of the capillary wall	cm s^{-1}
P_m	Permeability coefficient of the mesothelial layer	cm s^{-1}
h	P_c/D_i	cm^{-1}
r	Radius of capillary	cm
x	Coordinate in mesenterial plane perpendicular to capillary	cm
Δx	Diffusion path length through capillary wall	cm
ϵ	Mean thickness of mesentery	cm
η	Viscosity of water ($\approx 10^{-5}$) at room temperature	$\text{cm H}_2\text{O s}$
λ	Characteristic diffusion length within mesentery ($\sqrt{D_i \cdot \tau_m}$)	cm
ρ	Equivalent pore radius	Å units, 10^{-8} cm
τ_m	Decay time constant for transport through mesothelium	s

APPENDIX

Interstitial-Diffusion Method

In this method, the capillary is perfused with a potassium-rich Ringer's solution, and the interstitial potassium concentration in response to this perfusion is measured. In the steady-state version of this method, the interstitial concentration is measured vs. distance from the capillary wall. In the transient version of the method, the interstitial concentration is recorded vs. time at different

distances from the capillary wall. Both versions can be used to infer values of the capillary permeability.

Qualitatively, the experiment involves the following processes, sketched in Fig. 9. Potassium ions permeate the capillary wall from perfusate to tissue. In the interstitium, there is a diffusional transport of potassium away from the capillary. Material which has diffused through the upper mesothelial surface is lost irreversibly to the superfusate. Thus, the interstitial concentration will always be below the concentration of the capillary perfusate.

In the quantitative analysis of this experimental situation we have assumed the semi-infinite slab-geometry shown in Fig. 9, which, together with the list of symbols defines the symbols used in the following. The basis for this reduction to an essentially one-dimensional geometry is that the perfusion rate is assumed to be so high that longitudinal concentration gradients can be neglected, and that the mesentery is so thin that concentration gradients perpendicular to the plane of the mesentery can be neglected. Let, for convenience, the symbol $G(x)$ represent the diffusive flux in the interstitium, i.e., $G(x) = -D_i \partial C_i / \partial x$. The change in interstitial concentration in time (dt) is

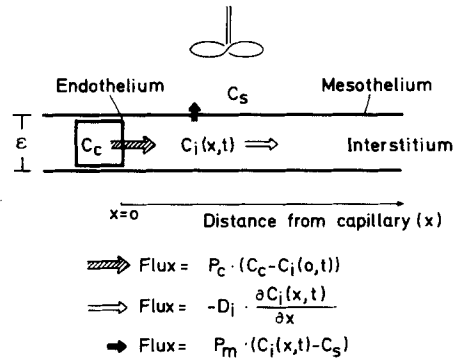


FIGURE 9. Simplified representation of system used for analysis of interstitial-diffusion data. The compartments represented are the capillary, interstitium, and superfusate. The K^+ concentration within the three compartments is C_c , $C_i(x, t)$, and C_s , respectively. The transport of K^+ from the capillary to the interstitium, within the interstitium and through the mesothelium to the superfusate sink is described by the three flux equations. The coordinate system and other symbols is defined in the list of symbols.

expressed by considering the change of mass in the interval $x/x + dx$ due to the diffusive flux $G(x)$ and the flux through the upper mesothelial layer (cf. Fig. 9):

$$dC_i \epsilon dx = \epsilon \{G(x) - G(x + dx)\} dt - P_m(C_i - C_s) dx dt. \quad (1a)$$

This expression can be rearranged to give

$$\frac{\partial C_i}{\partial t} = -\frac{\partial G}{\partial x} - \frac{(C_i - C_s)}{\tau_m}, \quad (2a)$$

where $\tau_m = \epsilon/P_m$. Since C_s is equal to the physiological K^+ -concentration in the body fluid of the frog, it disappears from the equation as only increments above the normal concentration are being dealt with. Correspondingly, the value of C_s , i.e., 2 mM, must be subtracted from measured concentrations before they are used for analyses. Inserting the expression for the diffusive flux into Eq. 2a yields the governing equation

$$\frac{\partial C_i}{\partial t} = D_i \frac{\partial^2 C_i}{\partial x^2} - \frac{C_i}{\tau_m}, \quad (3a)$$

which is an equation for diffusion in the presence of a volume sink. This equation forms the basis for the analyses of the steady-state and the transient version of the interstitial-diffusion methods.

STEADY-STATE METHOD The steady-state situation is characterized by a constant flux of potassium ions through the capillary wall and into the interstitium, where it gradually attenuates with distance due to diffusion through the upper mesothelial boundary. The general behavior of interstitial concentration in steady state can be found from Eq. 3a with the left hand side put to zero (steady state). The only physically meaningful solution to this equation is

$$C_i(x, \infty) = C_i(0, \infty) \exp(-x/\lambda), \quad (4a)$$

where $\lambda = \sqrt{D_i \tau_m}$ is the characteristic diffusion length in the interstitium. The exponential solution has the convenient property that the diffusive flux is given by

$$G(x) = D_i \frac{C_i(x, \infty)}{\lambda}. \quad (5a)$$

The interstitial concentration at the capillary wall, $C_i(0, \infty)$, can be determined from the requirement that, in the steady state, the flux through the capillary wall equals the diffusive flux at the wall, or, with the aid of Eq. 5a,

$$G(0) = \frac{D_i}{\lambda} C_i(0, \infty) = P_c \{C_c - C_i(0, \infty)\}, \quad (6a)$$

which can be solved for the concentration at the wall,

$$C_i(0, \infty) = C_c \frac{1}{1 + \frac{D_i}{\lambda P_c}}. \quad (7a)$$

The combining of Eqs. 7a and 4a gives an expression for the interstitial concentration,

$$C_i(x, \infty) = C_c \frac{1}{1 + \frac{D_i}{\lambda P_c}} \exp(-x/\lambda), \quad (8a)$$

which is similar to Eq. 1C quoted in Methods. The parameters can be determined in the following way. τ_m is determined from the decay of C_i from the steady-state value when the capillary perfusion is terminated. The time it takes for the diffusion process to equilibrate the interstitial concentration over a distance λ , is typically λ^2/D_i . In the present experiments, this time was less than or equal to τ_m . After a time of the order of τ_m , the diffusion term in Eq. 3a is thus negligible, and C_i decays exponentially with the time constant τ_m . λ is determined from a plot of the logarithm of the steady-state concentration vs. distance from capillary (cf. Fig. 7). According to the definition of the characteristic diffusion length λ , D_i can be calculated. The ratio between the concentrations inside and, just outside the capillary is given by the pre-exponential factor in Eq. 7a. In that D_i and λ are known, the capillary permeability coefficient P_c can be determined.

TRANSIENT METHOD The transient rise in interstitial concentration in response to an abrupt change in capillary potassium concentration can be found from Eq. 3a together with the boundary condition

$$G(0) = -D_i \left. \frac{\partial C_i}{\partial x} \right|_{x=0} = P_c (C_c - C_i).$$

The initial condition is that, at time zero, the interstitial concentration is zero and the capillary concentration is changed from zero to the value C_c . A solution was obtained employing the Laplace transform method together with a table of Laplace transforms (Carslaw and Jaeger, 1959; Appendix V no. 31). The result is:

$$\begin{aligned} \frac{C_i(x, t)}{C_c} = & \frac{1}{2} \left[\frac{h\lambda}{1+h\lambda} \exp(-x/\lambda) \operatorname{erfc}(x/(2\sqrt{D_i t}) - \sqrt{t/\tau_m}) \right. \\ & \left. + \frac{h\lambda}{h\lambda-1} \exp(x/\lambda) \operatorname{erfc}(x/(2\sqrt{D_i t}) + \sqrt{t/\tau_m}) \right] \\ & - \frac{h^2 \lambda^2}{h^2 \lambda^2 - 1} \exp(hx - t/\tau_m + h^2 D_i t) \operatorname{erfc}(x/(2\sqrt{D_i t}) + h\sqrt{D_i t}), \end{aligned} \quad (9a)$$

where $h = P_c/D_i$. Notice that, for $h\lambda \rightarrow 1$, the two last terms cancel. In practice, the parameters D_i and P_c can be determined from a fit of Eq. 9a to experimental values of $C_i(x, t)$ vs. time obtained at different electrode positions in the mesentery (cf. Fig. 6). For $\tau_m \rightarrow \infty$, i.e., in the steady state, the solution degenerates to the result obtained in Eq. 8a.

While the steady-state method extracts information from the situation when $t \gg \tau_m$, the transient method, in contrast, derives its information from the early period when $t \ll \tau_m$, i.e., from the initial time-course of the interstitial concentration.

Close to the capillary the interstitial concentration depends both upon P_c and upon D_i , i.e., upon potassium supplied from the capillary and upon the potassium transported further into the interstitium. Further away from the capillary, the concentration is mainly dependent on D_i since the rate of diffusion is rate-limiting for the transport in the interstitium. For $t \ll \tau_m$ and for small values of x ($x \ll \sqrt{D_i t}$), the only parameter determining C_i is the ratio P_c^2/D_i . Thus, measurements of the initial rise of interstitial concentration at different distances from the capillary provides another method for determination of P_c and D_i , once τ_m has been measured.

Comments on the Use of the Interstitial-Diffusion Method

Finally, we shall briefly justify that the assumptions behind this model are valid in the actual experimental situation. Typical values of the parameters are $P_c = 60 \cdot 10^{-5}$ cm s⁻¹, $D_i = 7 \cdot 10^{-6}$ cm² s⁻¹, $\tau_m = 50$ s, $\lambda = 190$ μ m, $\epsilon = 10$ μ m. It thus seen that the characteristic diffusion length (λ) is much larger than the diameter of the capillary and than the thickness of the mesentery. This is a necessary condition for treating the problem in one dimension only. It is also an obvious condition for obtaining meaningful experimental results.

An important assumption for using the semi-infinite geometry shown in Fig. 9 is that the sink action of neighbouring capillaries can be disregarded. In the experiments, the distance to the closest neighboring capillary was more than two times λ so that this assumption is justified.

The large value of λ is due to the large values found for τ_m . From the relation $P_m = \epsilon/\tau_m$ we find $P_m = 2 \cdot 10^{-5}$ cm s⁻¹ as a typical value of the mesothelial permeability in the frog. This value of the mesothelial permeability is very low compared to mesothelial permeabilities in mammalian preparations (Rasio, 1974). In principle, an apparent permeability of this magnitude could be due to an unstirred fluid layer beneath the mesentery. In a forthcoming paper (Frøkjær-Jensen and Christensen, 1978), we discuss this problem and show that the low value derived above, indeed, represents the true permeability of the mesothelium in the frog.

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