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WATER FLOW ACROSS THE WALLS OF SINGLE MUSCLE CAPILLARIES IN THE FROG, *RANA PIPIENS*

BY F. E. CURRY AND J. FRØKJAER-JENSEN*
From the Department of Human Physiology, School of Medicine, University of California, Davis, CA 95616, U.S.A.

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SUMMARY

1. Individual capillaries of the transilluminated frog muscle cutaneous pectoris were perfused with suspensions of human red cells in frog Ringer solution containing 1 g/dl bovine serum albumin. The modified Landis technique (Michel, Mason, Curry & Tooke, 1974) was used to measure hydraulic conductivities of the capillary wall. Sucrose osmotic reflexion coefficients of the capillary wall were measured in four capillaries when the superfusate contained 100 mM-sucrose. All experiments were made at 22–24 °C.

2. The hydraulic conductivity of arterial capillaries varied from 0·3 to $1{\cdot}26 \times 10^{-7}$ cm/(s cmH_2O) with a mean of $0{\cdot}79 \times 10^{-7}$ cm/(s cmH_2O) (six capillaries). The hydraulic conductivities of mid-capillaries varied from 0·43 to $1{\cdot}86 \times 10^{-7}$ cm/(s cmH_2O) with a mean value of $0{\cdot}72 \times 10^{-7}$ cm/(s cmH_2O) (six capillaries).

3. The mean reflexion coefficient to sucrose was $0{\cdot}12 \pm 0{\cdot}05$ (S.D.).

4. The measured reflexion coefficients to sucrose conform to the hypothesis that 90% of the transcapillary water flow crosses the capillary wall via the principal hydrophilic pathway. The remaining 10% crosses via an exclusive water pathway. The distribution of water flow is similar to that previously described in frog mesenteric capillaries.

5. The mean value of the hydraulic conductivity of frog muscle capillaries is about one-seventh the mean value of the hydraulic conductivity of frog mesenteric capillaries measured at the same temperature. The result conforms to the hypothesis that only a small fraction (mean 10%) of the area of junctional contact between adjacent endothelial cells is available for water and solute exchange in frog muscle capillaries. The hydraulic and diffusional conductances per unit length of open junction appear to be very similar when muscle capillaries are compared to mesenteric capillaries in the frog.

6. Our results lead us to speculate that structures within the intercellular junctions determine the extent of open junction and may modulate the hydraulic conductivity of both the principal water pathway and the exclusive water pathway.

* Dr Frøkjaer-Jensen was visiting from the Institute of Medical Physiology, Department A, The Panum Institute, University of Copenhagen, Denmark.

INTRODUCTION

The experiments described in this paper were designed to investigate pathways for water flow across the walls of frog muscle capillaries. These experiments were carried out after one of us measured the permeability coefficients to potassium ions (P_K) in frog muscle capillaries and found a mean P_K for arteriolar and mid-capillaries which was an order of magnitude smaller than the mean P_K in frog mesenteric capillaries (Frøkjaer-Jensen, 1982). A similar difference in electrical resistance of frog muscle compared to mesenteric capillaries has also been demonstrated (Olesen & Crone, 1983). The nature of the structures in the capillary wall responsible for the difference in permeability properties is not known. When examined using conventional transmission electron microscopy, the ultrastructure of the junctional pathways in the two types of capillaries is not significantly different (Frøkjaer-Jensen, 1982; Bundgaard & Frøkjaer-Jensen, 1982).

Our experiments were designed to test the hypothesis that the structures determining resistance to water flow are similar in muscle and mesenteric capillaries but that the area of membrane available for exchange is reduced in muscle capillaries. We tested the hypothesis by measuring the hydraulic conductivity (L_p) and sucrose osmotic reflexion coefficients (σ) of the capillary wall. The modified Landis micro-occlusion technique (Michel, Mason, Curry & Tooke 1974; Curry, Mason & Michel, 1976) was used. If the magnitude of L_p in muscle capillaries were found to be similar to that in mesenteric capillaries, our results would indicate that the resistance to water flow per unit area available for potassium ion diffusion is less in muscle capillaries than in mesenteric capillaries. The results would not conform to the hypothesis. On the other hand, if the fractional reduction in the L_p of muscle capillaries relative to the mesenteric capillaries were the same as the fractional reduction in potassium ion permeability coefficients, our results would conform to the hypothesis.

The structure of the water pathways was investigated further by measuring the osmotic reflexion coefficient to a small hydrophilic solute, sucrose, in muscle capillaries.

METHODS

The modified Landis micro-occlusion technique described by Michel *et al.* (1974) has been used to measure hydraulic conductivity in frog muscle capillaries. Refinements in the method are described in a recent review (Curry, Huxley & Sarelius, 1983). Modifications in the method related to the present study are given below.

Animals. Experiments were performed on small male frogs (*Rana pipiens*) 4–5 cm in length supplied by J. M. Hazen, VT. The animals were stored at 15 °C for up to 1 week before use. Experiments were carried out at room temperature (22–24 °C). Anaesthesia was established by placing the frogs in a 5 % urethane/tap water solution until mouth respiration stopped. Details of the dissection of the thin muscle cutaneous pectoris are given by Frøkjaer-Jensen (1982).

Microscopy. The muscle was pinned out over a short Leucite pillar for transillumination and viewed using a Leitz Laborlux II fixed-stage microscope. A trinocular attachment was used to view the preparation with a television camera and record the experiment on 1/2″ video tape for subsequent frame-by-frame analysis. All measurements were made using a × 20 long working distance objective. (N.A. = 0·57). The total magnification from microscope stage to television screen was × 800.

Micro-tools. A restraining rod was used to hold the muscle near the site of cannulation. Two

muscles contracted when the rod was placed on the tissue but in all other preparations the muscles remained stable during the experiment. Micropipette tips were bevelled to 8 μm diameter.

Solutions. The composition of the frog Ringer solution was (mM): NaCl, 111; KCl, 2·4; $MgSO_4$, 1·0; $CaCl_2$, 1·1; glucose, 5·5; $NaHCO_3$, 0·195 (air bubbled) (pH 7·4). The control superfusate was frog Ringer solution. The perfusate was frog Ringer solution containing 1 g/dl bovine serum albumin (Sigma, A4378). In osmotic transient experiments the superfusate contained 100 mM-sucrose. The perfusate contained a suspension of human red cells as flow markers.

Measurement of capillary pressure. Capillary pressure was measured directly in cannulated capillaries. The pressure in a water manometer was adjusted until there was no flow into or out of the micropipette as indicated by marker red cells in the capillary lumen. Transcapillary water flow per unit area (J_v/S) was calculated from the velocity of a marker cell (dl/dt) and distance l of the cell from the site of occlusion:

$$\frac{J_v}{S} = \frac{dl}{dt} \cdot \frac{1}{l} \cdot \frac{r}{2}. \quad (1)$$

The capillary radius (r) was measured directly from the image of the vessel on the television screen.

Vessel identification. Classification was on the basis of the direction of blood flow in the individual vessel and its neighbours. Vessels in a segment between divergent and convergent flow were called mid-capillaries. A segment upstream between the first and third divergence was an arterial capillary. A capillary down-stream between the first and third convergence was termed a venous capillary.

Vessel integrity. In five preliminary experiments the antihistaminic drug promethazine (0·3 ml of 0·2 μg/ml) was injected into the dorsal lymph sac 10 min before dissection. Promethazine did not prevent the increase in hydraulic conductivity in venous capillaries after micro-occlusion. Promethazine was not used in experiments in which only mid-capillaries and arterial capillaries were investigated. We tested the integrity of mid- and arterial capillaries by perfusing individual vessels with a 5% solution of FITC-150-Dextran (mol. wt. 150000) in the perfusate. A Balzers filter (450–495 nm), mounted over the end of a fibre optics light pipe, was used for excitation. No leakage of the tracer was detected by inspection for periods up to 10 min.

RESULTS

Micro-occlusion experiment without cannulation

In a preliminary series of experiments, we simply occluded twelve muscle capillaries, without cannulation, and recorded the subsequent movement of the frog erythrocytes. In contrast to the rapid initial filtration transients reported in a number of microvascular beds (Landis, 1927; Smaje, Zweifach & Intaglietta, 1970; Lee, Smaje & Zweifach, 1971; Gore, 1982), we observed very little *net* displacement of frog erythrocytes towards, or away from, the occlusion site in nine of the twelve vessels. Most cells oscillated around a mean position; the maximum displacement we observed in these vessels was less than 5 μm in a 250 μm segment of a capillary, 20 μm in diameter. Periodic displacements of 3–4 μm magnitude are expected to result from changes in capillary diameter (compliance) due to a pulse pressure of 2–3 cmH_2O in thin walled (0·5 μm) vessels (elastic modulus for the wall material close to 5×10^6 dyn/cm^2; Smaje, Fraser & Clough, 1980). We concluded that compliance effects were likely to obscure most of the net filtration in these simple micro-occlusion experiments.

The three vessels in which initial filtration was larger than reported above were all venous capillaries. Furthermore, the filtration rate in all three vessels increased with subseqent occlusions. These observations are consistent with the previous observation that the potassium permeability of venous capillaries in frog muscle may

increase during repeated experimentation (Frøkjaer-Jensen, 1982). We have not included venous capillaries in our sample reported in this paper.

Microcannulation: capillary pressure in frog muscle capillaries

The mean pressure (P_c) in nine arterial capillaries was $20{\cdot}1 \pm 4{\cdot}45$ (S.D.) cmH_2O with a range from 12 to 26 cmH_2O. The mean pressure in eleven mid-capillaries was $10{\cdot}5 \pm 4{\cdot}23$ cmH_2O with a range from 7 to 20 cmH_2O. The mean total protein

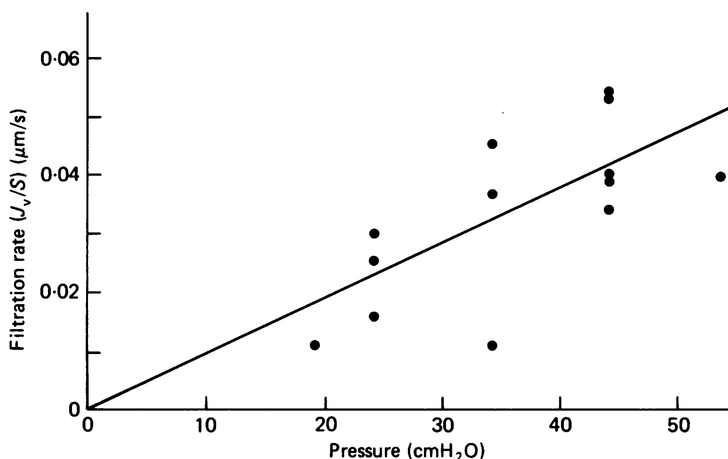

Fig. 1. The relationship between filtration rate per unit area of the capillary wall (J_v/S) and capillary pressure in a single perfused capillary of frog muscle. The regression line through the points has a slope equal to the hydraulic conductivity. In this arterial capillary (capillary 2, Table 1) the hydraulic conductivity is $0{\cdot}93 \times 10^{-7}$ cm/(s cmH_2O). The intercept on the abscissa is close to zero because the osmotic pressure of the perfusate was $3{\cdot}6$ cmH_2O.

concentration from eight samples of blood drawn by cardiac puncture in the same (early fall) population of frogs was $21{\cdot}9 \pm 6$ mg/ml. The plasma oncotic pressure corresponding to the latter concentration is less than 8 cmH_2O. These measurements indicated that the average driving force for transcapillary exchange in arterial capillaries is greater than 10 cmH_2O in superfused capillaries of frog muscle. The failure to observe significant filtration in occluded capillaries indicates that the L_p of arterial capillaries is less than 1×10^{-7} cm/(s cmH_2O). Direct measurement of L_p confirmed this result as shown below.

Measurement of hydraulic conductivity

Fig. 1 shows an experiment to measure the hydraulic conductivity in an arteriolar capillary perfused with frog Ringer solution containing 1 g/dl bovine serum albumin (BSA), osmotic pressure $3{\cdot}6$ cmH_2O. The superfusate was frog Ringer solution. Initial filtration rates $(J_v/S)_0$ were measured over a period of 5–10 s after occluding the capillary at pressures between 19 and 54 cmH_2O (see control, Fig. 2). The continuous line in Fig. 1 is the linear regression of $(J_v/S)_0$ on capillary pressure: the slope corresponds to an L_p of $0{\cdot}93 \pm 0{\cdot}33$ (S.D.) $\times 10^{-7}$ cm/(s cmH_2O). The intercept of the pressure axis is $0{\cdot}1 \pm 5{\cdot}1$ cmH_2O.

Reliable measurements of $(J_v/S)_0$ were made at capillary pressures between 40 and 50 cmH_2O. In most capillaries, L_p was calculated from the mean of four to six determinations using the relation, $L_p = (J_v/S)_0/(P_c - 3{\cdot}6)$. Table 1 summarizes the measurements of L_p in six arteriolar capillaries and six mid-capillaries. The mean L_p in the arteriolar vessels is $0{\cdot}79 \times 10^{-7}$ cm/(s cmH_2O) with a range from 0·3 to $1{\cdot}26 \times 10^{-7}$ cm/(s cmH_2O) and the mean L_p of mid-capillaries is 0·72 (range 0·43–1·86) $\times 10^{-7}$ cm/(s cmH_2O).

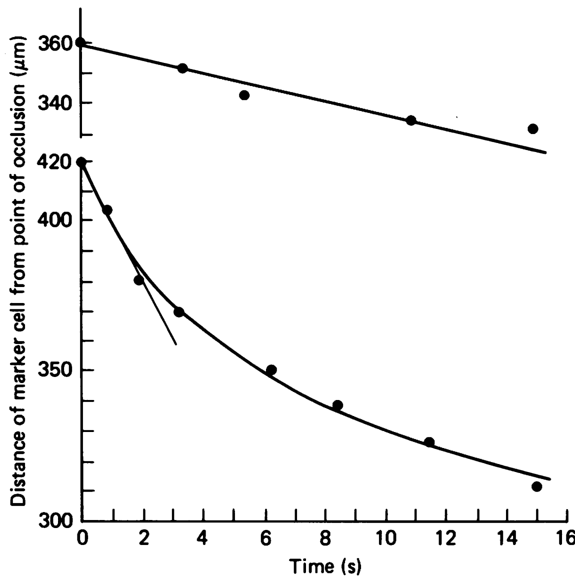

Fig. 2. The movement of red cells in the lumen of an occluded capillary to measure net fluid flow across the capillary wall. The ordinate is the distance in micrometres of the marker cell from the site of occlusion. The capillary was occluded at time zero. The perfusate was frog Ringer solution containing 1 g/dl albumin at a pressure of 50 cmH_2O. Upper curve: control filtration; superfusate is frog Ringer solution. Lower curve: muscle superfused with frog Ringer solution to which sucrose has been added at a concentration of 100 mM. The smooth curve and initial tangent were fitted to the data by eye.

Measurement of osmotic reflexion coefficients

To measure the osmotic reflexion coefficient we first determined the filtration rate as described above. The pressure was set to 40 or 50 cmH_2O and the filtration rate calculated from the cell trajectory following occlusion. The superfusate was frog Ringer solution and the perfusate contained no sucrose. Following four determinations of the initial filtration rate, the superfusate was changed to frog Ringer solution containing 100 mM-sucrose. The perfusion pressure was maintained throughout the experiment at 40 or 50 cmH_2O. The rapid perfusate flow ensured that sucrose which diffused into the capillary lumen was washed out rapidly by the sucrose-free perfusate. After at least 2 min of superfusion with 100 mM-sucrose, the filtration rates were measured from the first 2 s of the osmotic transients. As shown in Fig. 2, the initial filtration rates were significantly increased, being on average five to eight times larger than control. The increase in initial filtration rate $(\Delta J_v/S)_0$ measures the

osmotic flow across the capillary wall due to the 100 mM concentration difference between superfusate and perfusate. Osmotic reflexion coefficients were calculated as $(\Delta J_v/S)_0/(L_p RT\Delta C)$. ΔC is the sucrose concentration across the capillary wall and RT is the gas constant multiplied by absolute temperature.

Fig. 3 shows that the filtration rate, calculated by drawing tangents to the cell transient at 1–2 s intervals, decreased towards the control values after 10–15 s. This

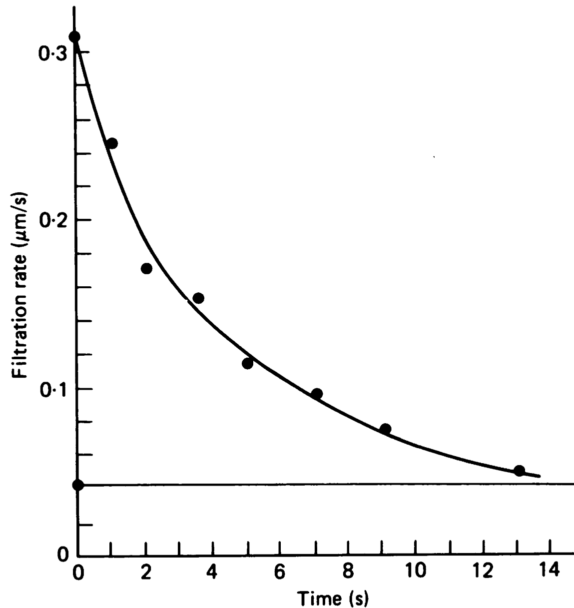

Fig. 3. The relationship between filtration rate per unit area of capillary wall and time after occlusion, from the data in Fig. 2. The marker cell velocity was estimated by drawing tangents to the smooth curves in Fig. 2. The lower line is the control filtration rate at a pressure of 50 cmH_2O. The upper line is the filtration rate with 100 mM-sucrose added to the superfusate. The distance between the curves is $L_p\sigma\ RT\Delta C$ where ΔC is the sucrose concentration across the capillary wall.

result indicates that the hydraulic conductivity of the capillary wall is not increased in the presence of hypertonic sucrose. We checked further for a change in hydraulic conductivity after the sucrose osmotic transient experiment by washing the tissue with sucrose-free Ringer solution perfusate for at least 2 min, and measuring a second control L_p. There was no increase in the control L_p after sucrose superfusion in the four capillaries studied. Individual values of $\sigma_{sucrose}$ are recorded in the final four rows of Table 1. The mean sucrose reflexion coefficient is $0{\cdot}12 \pm 0{\cdot}05$. The same mean value was measured in frog mesenteric capillaries (Curry *et al.* 1976).

Compliance effects

The interpretation of the initial cell displacement in terms of transcapillary water flow is compromised if an increase in capillary diameter causes shortening of the fluid column between marker cells and the site of occlusion. Fig. 4 shows the movement of a marker cell resulting from a step increase in pressure from 5 to 50 cmH_2O in an occluded capillary. The pressure step was complete within 0·2 s. After 0·5 s the

TABLE 1. Estimates of hydraulic conductivity and the osmotic reflexion coefficient to sucrose in single muscle capillaries

Capillary no.	Type	Diameter (μm)	$L_p \times 10^7$ cm/(s cmH_2O) Mean ± S.D. (n)	$\sigma_{sucrose}$ Mean ± S.D. (n)
1	Arterial capillary	16	0·94 (1)	—
2	Arterial	26	0·93 ± 0·33 (13)	—
3	Mid-capillary	16	0·59 ± 0·10 (3)	—
4	Arterial			
	Upper region	18	0·59 ± 0·2 (6)	—
	Lower region	18	0·77 ± 0·27 (6)	—
5	Mid-capillary	17	0·50 ± 0·23 (2)	—
6	Arterial	24	0·77 ± 0·20 (3)	—
7	Mid-capillary	24	0·61 ± 0·28 (8)	—
8	Mid-capillary	24	0·43 ± 0·14 (9)	—
9	Arterial capillary	25	0·30 ± 0·07 (7)	0·145 ± 0·05 (5)
10	Mid-capillary	23	1·86 ± 0·43 (13)	0·091 ± 0·03 (6)
11	Mid-capillary	25	0·39 ± 0·04 (3)	0·171 ± 0·09 (7)
12	Arterial	21	0·85 ± 0·05 (3)	0·074 ± 0·02 (8)
	Mean values		0·74 ± 0·41	0·121 ± 0·05

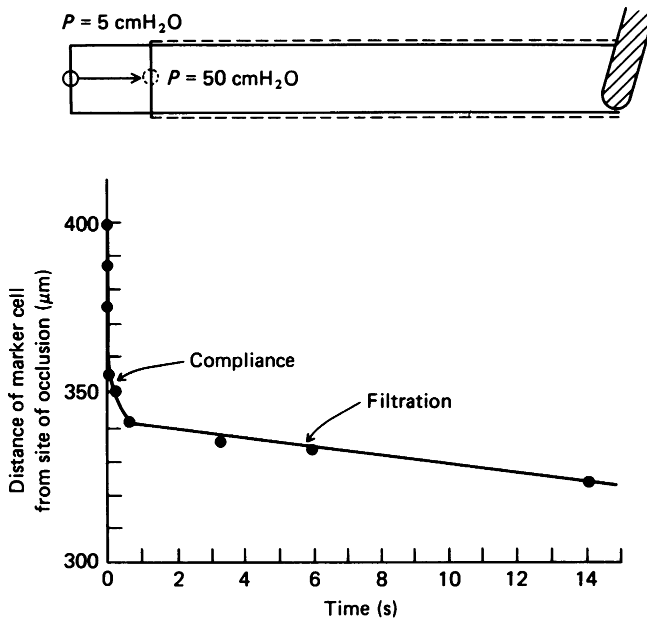

Fig. 4. The movement of marker red cells which results from compliance changes and filtration in a single muscle capillary. A step change in pressure from 5 to 50 cmH_2O was made at time zero. Top: schematic to demonstrate how the change in capillary diameter was measured from the shortening of the fluid column in an occluded capillary. Bottom: cell movement due to compliance occurs for 500 ms after the pressure step. Steady filtration is measured for more than 10 s after the initial compliance changes.

marker cell had jumped from an initial position 400 μm from the site of occlusion to a position 342 μm from the occlusion. Thereafter the cell continued in steady filtration for longer than 10 s. The initial diameter of the capillary measured from the television screen was 20 μm. Assuming there is negligible fluid loss from the capillary during the pressure step, the rapid shortening of the fluid column corresponds to an increase in the radius of a cylindrical capillary from 10 to 10·8 μm. The elastic modulus (E) of the capillary wall was calculated assuming the capillary could be treated as a thin-walled cylinder:

$$E = \frac{\Delta P \cdot r^2}{\Delta r \cdot h}, \qquad (2)$$

where ΔP is the pressure, r is capillary radius, Δr is the change in capillary radius and h is the thickness of the muscle capillary wall (0·39 μm, J. Frøkjaer-Jensen, unpublished observation). The calculated elastic modulus is $14{\cdot}1 \times 10^6$ dyn/cm^2. The cell trajectory in Fig. 4 indicates that cell movements during the first 0·5 s of the transient may over-estimate filtration due to compliance. However, our measurements of marker cell movement made over a period of 1–10 s after occlusion, measure filtration alone as compliance changes are negligible during this period.

DISCUSSION

The mean value of the hydraulic conductivity of frog muscle arterial and mid-capillaries, $0{\cdot}74 \times 10^{-7}$ cm/(s cmH_2O), is one seventh of the mean value for frog mesenteric capillaries measured at room temperature (22–24 °C) (Michel *et al.* 1974). The ratio of hydraulic conductivities is similar to the ratio of potassium ion permeability coefficients in muscle arterial capillaries ($8{\cdot}6 \times 10^{-5}$ cm/s; Frøkjaer-Jensen, 1982) compared to mesenteric capillaries (67×10^{-5} cm/s; Crone, Frøkjaer-Jensen, Friedman & Christensen, 1978). The simplest interpretation of the result is that the mean area for the exchange of water and small hydrophilic solutes in muscle capillaries is close to 15% of that in mesenteric capillaries. This conclusion is independent of specific assumptions about the mechanisms of water and solute transport; it implies that the molecular structures responsible for the resistance to water and solute movement in both muscle and mesenteric capillaries are similar.

The same conclusion about the similarity of molecular structures in the transcapillary pathways in frog muscle and mesenteric capillaries might be drawn from the measured reflexion coefficients to sucrose. The mean sucrose reflexion coefficients are the same in both types of vessel. Equality of σ_{sucrose} suggests identical structure of a common pathway for water and solute in the two capillary types. This argument is compromised because we have demonstrated previously that water crosses the walls of mesenteric capillary blood vessels by at least two pathways (Curry *et al.* 1976). The relative contribution of both pathways to the total water flux must be evaluated in order to draw conclusions about pathway structure. Before attempting a more detailed interpretation of the results, we shall examine the validity of the methods.

Measurement of hydraulic conductivity

Our estimates of hydraulic conductivity are amongst the smallest values reported for individually perfused vessels. They are similar in magnitude to estimates in single

capillaries in rat cremaster muscle measured by varying albumin oncotic pressure in the superfusate (Smaje *et al.* 1970). On the other hand, they are two orders of magnitude lower than the values obtained on individual rat intestinal muscle capillaries (Gore, 1982). The possible errors in our method are discussed below.

A fluid leak at the cannulation site could lead to an underestimate of L_p. We have carefully avoided this problem by ensuring that the volume of fluid, swept out as a marker cell moves through the micropipette shaft, corresponds to the volume displacement calculated from cell movement in the capillary. The pressure drop across the tips of the micropipettes during filtration has been estimated to be less than 1 cmH_2O (Michel *et al.* 1974) in mesenteric capillaries, and is likely to be even less in muscle capillaries due to the much lower filtration rate in these vessels. Two factors may make our estimates too high: over-estimate of mean axial water velocity by marker red cells, and compliance. We checked for a systematic error due to marker cells tracking on the centre line of a parabolic velocity profile by drawing frog cells back into the capillary lumen. In three capillaries no difference was found between the L_p estimated using large frog erythrocytes as flow markers and the L_p estimated using human erythrocytes in the same vessel.

Hydraulic conductivity will be over-estimated if movement of marker cells due to diameter changes is interpreted as transcapillary fluid flow. The elastic modulus measured in a frog muscle capillary is close to twice the value measured in other single capillaries (Gore, 1982). The muscle capillary wall appears slightly less compliant, over the pressure range investigated, than other vessels. Nevertheless, there are large potential errors in the interpretation of fluid movements during the first second of the filtration transient. However, in a capillary perfused via a micropipette we demonstrated that diameter changes are negligible after 1 s of occlusion. The same result has been described in frog mesenteric capillaries (Curry, Huxley & Sarelius, 1983). We can therefore separate compliance artifacts from true filtration in isolated perfused capillaries. It appears that the large values of L_p reported by Gore (1982) are measured from filtration transients lasting less than 1 s. Compliance artifacts may therefore account for some of the large discrepancy between our estimates and those of Gore (1982). Other differences associated with species and organ differences may also be involved.

Measurement of reflexion coefficient

The method used to measure the osmotic reflexion coefficient has been critically evaluated in a number of recent publications (Crone & Christensen, 1979; Gore & McDonagh, 1980; Michel, 1983). The principal assumption is that the concentration difference of the sucrose across the capillary wall is equal to the concentration of the sucrose in the superfusate. If there are significant barriers to extravascular solute diffusion, or significant accumulation of solute in the perfusate during the transit of perfusate through the capillary, the actual concentration difference will be smaller than expected from the superfusate concentration, and the true osmotic reflexion coefficient is underestimated. Two lines of evidence suggest that external barriers are small. First, the underside of the cutaneous pectoris muscle is free from restricting barriers such as the mesothelium in the mesentery: capillaries on the surface of the muscle are exposed directly to the superfusate. Secondly, measurements with ion-

selective electrodes indicated that potassium ions diffused rapidly away from the capillary (Frøkjaer-Jensen, 1982).

We estimated the possible error in transcapillary concentration difference from the expression for the solute gradient along a perfused capillary:

$$\Delta C_x = \Delta C_0 \exp(-PSx/Q), \quad (3)$$

ΔC_0 is the concentration difference at the cannulation site (equal to 100 mM). ΔC_x is the concentration difference at any fractional distance x along a capillary with

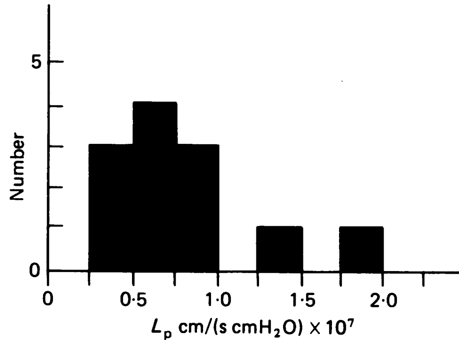

Fig. 5. Histogram of values of hydraulic conductivity for twelve arteriolar and mid-capillaries in frog muscle. The perfusate contained bovine serum albumin at a concentration of 1 g/dl. All determinations were made at 22–24 °C.

permeability P, surface area S, and perfusion rate Q. If $P_{sucrose}$ is set equal to $P_K . (D_S/D_K)$ where D_S and D_K are the free diffusion coefficients, $\Delta C_{sucrose}$ at the end of a 500 μm length of capillary, 10 μm in radius perfused at a mean velocity > 1000 μm/s is 98 mM. The mean ΔC_x is therefore close to 99 mM and the error in the estimate of σ, when its value is close to 0·1, is 1 %.

There is somewhat more uncertainty in our estimate of initial filtration rates during sucrose osmotic transients because the higher filtration rates make clearer separation of true filtration and compliance artifacts more difficult. However, the filtration rate was elevated during cell transients lasting for more than 5 s. The initial tangent was drawn to a smooth curve fitted to the whole measured transient to avoid undue weighting of the first second of the transient.

Structure of the transcapillary pathways

The analysis of errors, given above, indicates that the measured differences in L_p reflect properties of water pathways and are not due to experimental error. As shown in Fig. 5, the distribution of L_p in muscle capillaries is skewed, similar to the distribution of L_p in frog muscle (Michel *et al.* 1974; Curry, 1979) and rabbit omentum (Zweifach & Intaglietta, 1968). In the following section we extend our analysis of the permeability properties of muscle capillaries by taking into account the variation of L_p from capillary to capillary over a 6-fold range.

The simplest interpretation of the proportional changes in mean values of L_p and P_K is that variation in permeablity properties is due to changes in the *area* available for exchange both from capillary to capillary as well as from one capillary type to

another. Support for the latter idea has been presented from experiments in individually perfused mesenteric capillaries. For example, Michel (1980) found that the osmotic reflexion coefficients to both albumin and myoglobin were relatively constant from capillary to capillary even though L_p varied over a 10-fold range. The result indicates that structures within each pathway determining selectivity to hydrophilic solutes are similar in each vessel. It is the total area for exchange of water which varies. Curry (1979) reached a similar conclusion on the basis of proportional changes in measurements of sucrose permeability coefficient and hydraulic conductivity in frog mesenteric capillaries. The problem presented by these observations is to understand the factors which determine the area available for exchange and the permeability and selectivity of the pathway available for exchange.

Ultrathin serial sectioning of segments of frog mesenteric and frog muscle capillaries show that transendothelial channels (Simionescu, Simionescu & Palade, 1975*a*) are far too rare to play any role in transcapillary exchange of small solutes and water in these capillaries (Frøkjaer-Jensen, 1980; Frøkjaer-Jensen, 1983). The intercellular clefts therefore present the most likely extracellular route for water and small solutes.

A slit model of transcapillary exchange helps to illustrate the ideas developed above. The mean P_K for mesenteric capillaries (67×10^{-5} cm/s) can be described if diffusion occurs through greater than 90 % of the contact region (length between adjacent cells of intercellular junctions, 1800 cm/cm^2), open to a width of 17 nm. The cleft depth is 0·7 μm. The values for cleft length and depth are mean values measured from conventional transmission electron micrographs (Bundgaard & Frøkjaer-Jensen, 1982). The diffusion coefficient in the open cleft is 95 % of its free value. Unpublished measurements (J. Frøkjaer-Jensen) on muscle capillaries give similar values for slit geometry. The mean cleft length is 2480 cm/cm^2 and the mean cleft depth is 0·83 μm. The mean P_K of the muscle capillaries is accounted for if only 10 % of the cleft is open to the same extent, the remaining 90 % being so tightly closed as to be impermeable to potassium ions. The hydraulic conductivity is predicted to be 13×10^{-7} cm/(s cmH_2O) if the whole cleft is open in muscle capillaries. It will be reduced to a value only two times larger than the measured mean if 10 % of the cleft is open. A similar cleft model therefore provides a good approximation to the published data on water and small solute flow through muscle capillaries.

It was suggested (Frøkjaer-Jensen, 1982) that the difference in 'effectively' open cleft area in different types of capillaries merely reflects a difference in the junctional complex (appearing as strands) in the intercellular clefts between endothelial cells. Freeze-fracture studies of mammalian muscle capillaries (Wissig, 1979) have shown that these strands are discontinuous. The discontinuous pattern of the junctional complexes has also recently been demonstrated in transmission electron microscopy by serial sectioning of rat heart capillaries (Bundgaard, 1983). The idea may be developed to explain our results.

In brain capillaries junctional complexes form a continuous and complicated network (Møllgård & Saunders, 1975), which effectively seals the cleft area to small ions like lanthanum (Bundgaard, 1982). In muscle capillaries the junctional complexes form an almost continuous network where few discontinuities may reduce the 'effectively' open cleft area to about 10 %), while in mesenteric capillaries loose

junctional complexes (Simionescu, Simionescu & Palade, 1975*b*) may allow almost 100% of the cleft area to be 'effectively' open. This interpretation is illustrated in Fig. 6. When studied in conventional transmission electron microscopy all three types of arrangements may give rise to rather similar projections with a few zones of cell contacts ('tight junctions') in the intercellular clefts, as also shown in Fig. 6. In spite

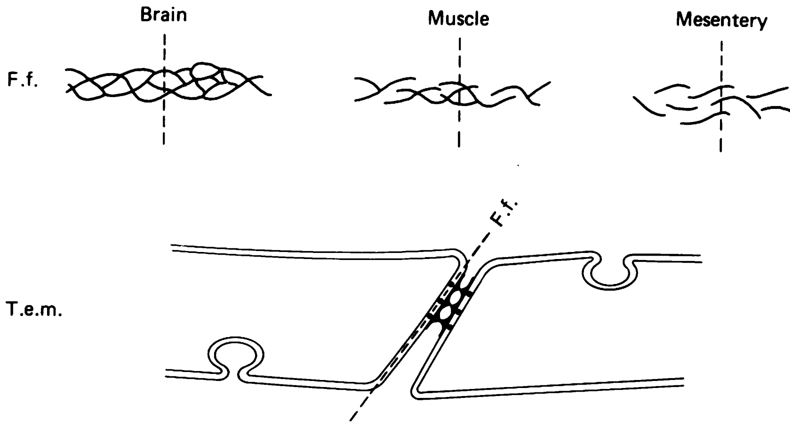

Fig. 6. Schematic drawing illustrating how similar transmission electron microscopic pictures (T.e.m.) of the intercellular clefts with a few points of fusions (tight junctions) may be obtained by sectioning of junctional complexes of vastly different organization (as revealed by freeze-fracture (F.f.)). In brain capillaries the junctional complexes may seal the clefts. In muscle the few discontinuities in the junctional complexes may act as if 10–15% of the cleft is 'effectively open' while in mesentery junctional complexes act as if 90% of the cleft is 'effectively open'.

of the similarity of appearance, the different labyrinthic complexes may result in large differences in small solutes and water exchange. A fibrous network associated with the junction complex may further modulate permeability as suggested by calculations using the fibre matrix model of capillary permeability (Michel, 1978; Curry & Michel, 1980; Curry, Huxley & Adamson, 1983).

Parallel pathways for water flow

It is assumed in the above analysis that most water flow crosses the capillary wall through extracellular pathways shared with hydrophilic solutes. This would not be the case if there were a significant water flow across channels from which solute was excluded: across the plasma membranes of the endothelial cell or through the tight region of the junction. For example, Curry *et al.* (1976) estimated that an exclusive water pathway across the capillary wall of frog mesenteric had an L_p between 0·1 and $0{\cdot}2 \times 10^{-7}$ cm/(s cmH_2O). If a pathway with the same conductivity was present in muscle cells, between 10 and 50% of the water would cross the capillary wall via a pathway inaccessible to small hydrophilic solutes. Our measured osmotic reflexion coefficients enable this idea to be evaluated.

The expression for the osmotic reflexion coefficient of the capillary membrane ($\bar{\sigma}$) consisting of an exclusive water pathway ($\sigma = 1$) of hydraulic conductivity $L_{p,w}$

in parallel with a porous pathway with a reflexion coefficient σ_p is:

$$\bar{\sigma} = \frac{L_{p,w}}{\bar{L}_p}(1-\sigma_p)+\sigma_p, \quad (4);$$

where $\bar{L}_p = L_{p,w}+L_{p,p}$ and $L_{p,p}$ is the hydraulic conductivity of the porous pathway (Curry *et al.* 1976). If the value of $L_{p,w}$ in eqn. (4) were equal to or greater than 0.1×10^{-7} cm/(s cmH_2O) as measured in mesenteric capillaries, then $\sigma_{sucrose}$ should vary from close to 0·05 to greater than 0·3 over the range of L_p measured. Our results

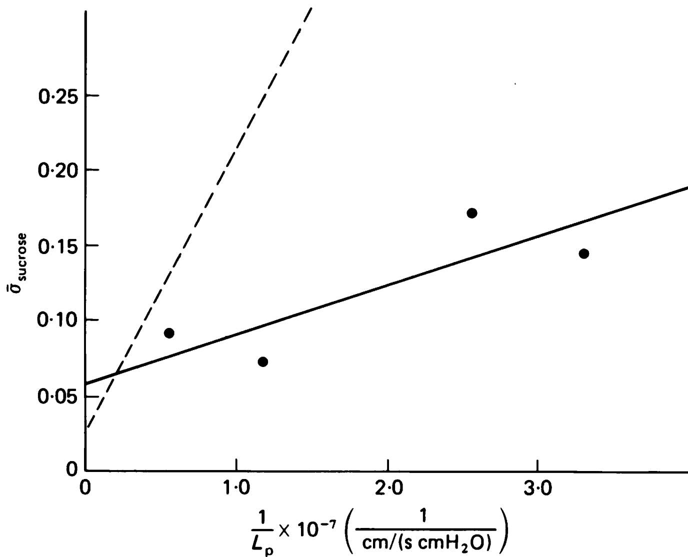

Fig. 7. The relationship between sucrose reflexion coefficient and the reciprocal of the hydraulic conductivity. Each point represents a separate capillary. The continuous line is the regression line for values measured in frog muscle capillaries. The dashed line is the regression line for frog mesenteric capillaries (Fig. 6, Curry *et al.* 1976). The hydraulic conductivity of the exclusive water pathway for muscle, 0.031×10^{-7} cm/(s cmH_2O), is one-seventh that in mesenteric capillaries, 0.19×10^{-7} cm/(s cmH_2O).

are inconsistent with this prediction. Measured values of sucrose vary over a smaller range. This observation indicates that $L_{p,w}$ is significantly smaller in muscle capillaries than in mesenteric capillaries.

Although our sample is small, we have attempted to obtain a better estimate of $L_{p,w}$ using an analysis introduced by Curry *et al.* (1976). If $L_{p,w}$ is assumed to be a constant for all capillaries, then according to eqn. (4), the regression of $\sigma_{sucrose}$ on $1/\bar{L}_p$ will have a slope of $L_{p,w}(1-\sigma_p)$ and an intercept of σ_p.

The continuous line in Fig. 7 is the relation $\sigma_{sucrose} = 0.029 \times 10^{-7}/\bar{L}_p + 0.06$. The new estimate of $L_{p,w}$, 0.031×10^{-7} cm/(s cmH_2O) is 10% of the smallest value of $\bar{L}_p$ and indicates that greater than 90% of transcapillary water flow is via an extracellular pathway. The result indicates that the distribution of water flow between extracellular and exclusive pathways is very similar in both muscle and mesenteric capillaries. It implies that the hydraulic conductivity of the exclusive water pathway is reduced in almost the same proportion as the mean values for the whole membrane.

Estimates of the hydraulic conductivities of the exclusive water pathway in muscle and mesenteric capillaries are compared directly in Fig. 7. The dashed line is the relation $\sigma_{\text{sucrose}} = 0{\cdot}191 \times 10^{-7}/\bar{L}_p + 0{\cdot}06$ re-plotted from Fig. 6 of Curry *et al.* 1976. The value of $L_{p,w}$ in muscle capillaries is close to one-seventh the value of $L_{p,w}$ in mesenteric capillaries when measured as the slope of the regression of σ on $1/\bar{L}_p$. The mechanism responsible for almost equal reductions in the hydraulic conductivities of both the exclusive water pathway and the principal water pathway are not known. Parallel changes in the osmotic water permeability coefficient (transcellular) and the permeability of the paracellalar pathway for ions have been noted in several epithelia (see for example, Burg, 1982).

Although our results do not rule out a transcellular route for water flow, it is possible that the exclusive water pathway may reside in the closed portion of the intercellular junctions (Curry *et al.* 1976; Renkin & Curry, 1978). If this is the case, then another important difference between muscle and mesenteric capillaries is the resistance of the closed portion of the junction to water flow. Further research is required to characterize the exclusive water pathway in both muscle and mesenteric capillaries.

In summary, our results conform to the hypothesis that a fraction (as small as 4 % and up to 25 %) of the intercellular clefts is 'effectively' open to form the principal pathway for water and hydrophilic solutes across the continuous capillaries of frog muscle. The resistance to water flow and solute diffusion per unit length of open junction is very similar in frog muscle and frog mesenteric capillaries.

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