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MICROVASCULAR PRESSURES AND FILTRATION COEFFICIENTS IN THE CAT MESENTERY

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SUMMARY

1. Filtration coefficient and hydrostatic pressure have been measured in single capillaries and venules in the cat mesentery using a modification of the Landis (1927) single vessel occlusion technique.

2. Venules were found to be filtering fluid, not absorbing it as is often supposed.

3. The mean filtration coefficient in capillaries was 0·018 $\mu m.s^{-1}.mmHg^{-1}$ ($1·35 \times 10^{-10} m.s^{-1}.Pa^{-1}$) while that in venules, was 0·027 $\mu m.s^{-1}.mmHg^{-1}$ ($2·02 \times 10^{-10} m.s^{-1}.Pa^{-1}$).

4. In both capillaries and venules, filtration coefficient increased with decreasing pressure.

5. The difference between directly measured venular pressure and that calculated from the occlusion data was used to determine the contribution of the interstitium to fluid exchange. In the mesentery superfused with Krebs solution the tissue pressure so determined was found to be zero or subatmospheric initially but became increasingly positive with lengthening exposure of the mesentery.

INTRODUCTION

In recent years quantitative techniques have been applied to individual segments of the microvascular bed, but although it is now apparent that venules may play an important role in fluid exchange, studies on venular function lag behind those on capillaries. Measurements of capillary filtration coefficient using modifications of the single vessel occlusion technique (Landis, 1927) have been obtained in the vascular beds of the amphibian mesentery (Intaglietta, 1967; Michel, Mason, Curry & Tooke, 1974), the mammalian mesentery and omentum (Lee, Smaje & Zweifach, 1971; Zweifach & Intaglietta, 1968) and in the cremaster muscle (Smaje, Zweifach & Intaglietta, 1970). These studies have provided detailed support for Starling's hypothesis of tissue fluid formation (Starling, 1896) but have introduced important qualifications. Among these are that the capillary filtration coefficient is not a fixed value in a particular bed but varies from one vessel to another and the value for any one capillary increases towards its venular end. Whether this trend continues

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into the venules is not certain. In order to answer this question a method for measurement of filtration coefficient in venules is required.

The single vessel occlusion technique of Landis (1927) affords this possibility but both the original method and the development introduced by Michel *et al.* (1974) require cannulation of the vessel, which is difficult in mammalian capillaries and may itself cause changes. The osmotic transient method of Zweifach & Intaglietta (1968) has the disadvantage that two independent occlusions, separated by the injection of a concentrated albumin solution, are required. The technique introduced by Smaje, Lee & Zweifach (1971), however, enables both pressure and filtration coefficient to be determined following a single occlusion lasting a few seconds and this method has been used in the present experiments. Theoretical and experimental support for this method have been published (Lee *et al.* 1971; Blake & Schneyer, 1974).

The present paper describes an alternative approach to the analysis of data obtained from capillaries using this micro-occlusion method and justifies its extension to venules. Measurements of filtration coefficient in capillaries and venules show that the gradient of increasing permeability does continue into the venules and a development of the occlusion technique has allowed estimates of interstitial fluid pressure in the exteriorized mesenteric preparation.

Preliminary accounts of part of this work have been presented previously (Smaje & Verrinder, 1972).

METHODS

Animals and anaesthesia. Experiments were performed on the mesentery of cats of either sex weighing between 1·2 and 2·5 kg. Anaesthesia was induced by ethyl chloride and maintained by ether until pentobarbitone (up to 35 mg/kg) could be given via a cannula placed in the femoral vein. Supplementary doses were given as required. The trachea and femoral vessels were cannulated.

Blood pressure was measured in the abdominal aorta via a Portex (Hythe, Kent) nylon I.V. cannula connected to a pressure transducer (Bell & Howell type 4-326-L212) and registered on a Devices M4 pen recorder. The cannula and transducer were filled with 0·9 % NaCl containing 10 i.u. heparin/ml. The temperature was maintained at 37 °C using a heating blanket placed beneath the cat, controlled via a rectal thermistor using the circuit described by Diete-Spiff, Ikeson & Read (1962).

Preparation of mesentery. A longitudinal incision 2–3 cm long was made in the shaved skin over the umbilicus and the animal laid on its side on a Perspex tray which had a heated side chamber to take the mesenteric preparation. A loop of colon was drawn out of the incision using cotton-wool buds soaked in Krebs solution and its mesentery was placed over a glass-topped pillar in the side chamber positioned above the microscope sub-stage condenser. The intestine rested on cotton-wool soaked in Krebs solution placed around the base of the pillar, and the whole of the exposed tissue was then covered in plastic film (Snappies Cling Film Wrap, Empress Products Ltd.) except for the region of the mesentery under investigation which was irrigated with the Krebs solution. This was composed as follows: NaCl, 118 mM; KCl, 4·7 mM; $CaCl_2$, 2·5 mM; KH_2PO_4, 1·1 mM; $MgSO_4.7H_2O$, 1·25 mM; $NaHCO_3$, 25 mM; glucose, 11 mM bubbled with 95 % O_2, 5 % CO_2 and heated to 37 °C in the final conduit by a stainless-steel heater. This was heated by a resistance wire and controlled by an incorporated thermistor using the circuit of Diete-Spiff *et al.* (1962), and further checked by a thermistor cemented to the stage beneath the mesentery. The mesentery was irrigated at 4 ml/min using a roller pump and the tube was positioned using a small micromanipulator attached to the animal tray.

Microscopy and filming. The tray containing the cat was clamped to a modified stage of a Leitz Intravital microscope equipped with standard Orthoplan optics. The tissue was trans-illuminated with a Leitz high pressure 150 W xenon lamp using neutral density filters to adjust the amount of light and a green filter to enhance the contrast of the red cells and to provide

monochromatic light for photography. A long working distance condenser (22 mm) and long working distance objectives (UM series) were used. The image was split so that light passed through to a binocular viewing head and to an Arriflex H16 16 mm cine camera mounted vertically above the microscope. Ilford Pan F film was used at 25 frames/s. Vessels were occluded by glass probes (micropipettes fused at the tip), held in a Leitz micromanipulator. The probes were pulled from Leitz Pyrex tubing (o.d. 1·2 mm) to give a flexible shank about 15–20 μm in diameter and the tips were fused in a small gas flame to provide a ball 10–50 μm in diameter. Pressure was measured in selected small vessels by insertion of sharpened glass micropipettes attached to a resistance null-balance servo system of the type described by Wiederhielm, Woodbury, Kirk & Rushmer (1964) and modified by Intaglietta, Pawula & Tompkins (1970). Film exposure was determined using a Leitz Microsix exposure meter. Exposed film was developed commercially (Humphrey's Laboratories Ltd.) and frame by frame analysis undertaken using a PCD XY data reader fitted with a Vanguard 16 mm Motion Analyser cine head. Microscope magnification was 160 and the final magnification on the analyser screen $\times 1000$. The output from the data reader was digitized by a Farnell DCV 100 voltmeter and fed via an 8-channel interface to a Data Dynamics model 390 teletype and paper tape punch.

Vessel radius was measured on the film analyser, and the volume of red cells between the marker cells was estimated by counting the number of cells and multiplying this by the individual red cell volume (57 μm^3: Altman & Dittmer, 1966).

Data analysis

Principle of method

When a capillary is occluded with a micropipette fused at its tip, red cells move from the open end of the vessel towards the occluding probe. Under these circumstances the rate of red cell movement reflects the rate of fluid movement out of the vessel (Landis, 1927; Zweifach & Intaglietta, 1968; Michel *et al.* 1974). Fluid movement is rapid at first, slowing later until a point is reached when no more movement takes place (Fig. 1*A* and *B*). As ultrafiltrate is lost from the capillary, the plasma colloid osmotic pressure rises until it balances the hydrostatic pressure tending to produce filtration. Thus fluid movement slows and at equilibrium ceases altogether. Since the capillary membrane is essentially impermeable to protein (Pappenheimer & Soto Rivera, 1948; Levick & Michel, 1973), the total quantity of protein in the trapped segment of capillary remains constant during the occlusion. The initial plasma colloid osmotic pressure is determined from a plasma sample and as the total volume change is known at every stage after the occlusion, the changing plasma volume and colloid osmotic pressure can be determined.

Red cell motion in the occluded capillary is governed by (*a*) the gain or loss of fluid across the endothelial wall due to the difference in transmural colloid osmotic and hydrostatic pressures (Starling's hypothesis) and (*b*) changes in vessel diameter in response to the pulsatile component of the hydrostatic pressure (see Clough, Fraser & Smaje, 1974).

Starling's hypothesis of tissue fluid formation may be expressed mathematically:

$$-J_v/A = L_p((P_c-\pi_c)-(P_t-\pi_t)), \tag{1}$$

where J_v is the fluid flux, A is the area of membrane, L_p is the filtration coefficient, P is the hydrostatic pressure and π is the colloid osmotic pressure. The subscripts c and t refer to the capillary and interstitial tissue respectively. The 'effective' driving pressure P_e is defined as

$$P_e = P_c-P_t+\pi_t, \tag{2}$$

so eqn. (1) above can be re-written as

$$-J_v/A = L_p(P_e-\pi_c). \tag{3}$$

The rate of fluid flux (J_v/A) and the colloid osmotic pressure in the occluded segment (π_c) may be determined during the occlusion from the following relationships (see Lee *et al.* 1971).

$$-\frac{J_v}{A} = \frac{r}{2l}\cdot\frac{dl}{dt}, \tag{4}$$

$$\pi_c = F\,C_0\frac{(V_0-V_c)}{V-V_c}, \tag{5}$$

where l is the distance between marker red cells, r is the vessel radius, dl/dt is the rate of relative motion of marker cells, F is the function relating plasma protein concentration c (g/100 ml) to colloid osmotic pressure, π (mmHg) = $2{\cdot}1c + 0{\cdot}16c^2 + 0{\cdot}009c^3$ (Landis & Pappenheimer, 1963), V_c is the volume of red cells between marker cells, V_0 is the volume between marker cells at the beginning of the occlusion, V is the volume between marker cells at any time during the occlusion, C_0 is the initial plasma protein concentration.

In order to use these equations, certain assumptions have to be made. These are as follows.

(1) Red cells are accurate markers of fluid movement.

(2) The capillary is circular in cross-section.

(3) Plasma proteins are retained within the capillary endothelium.

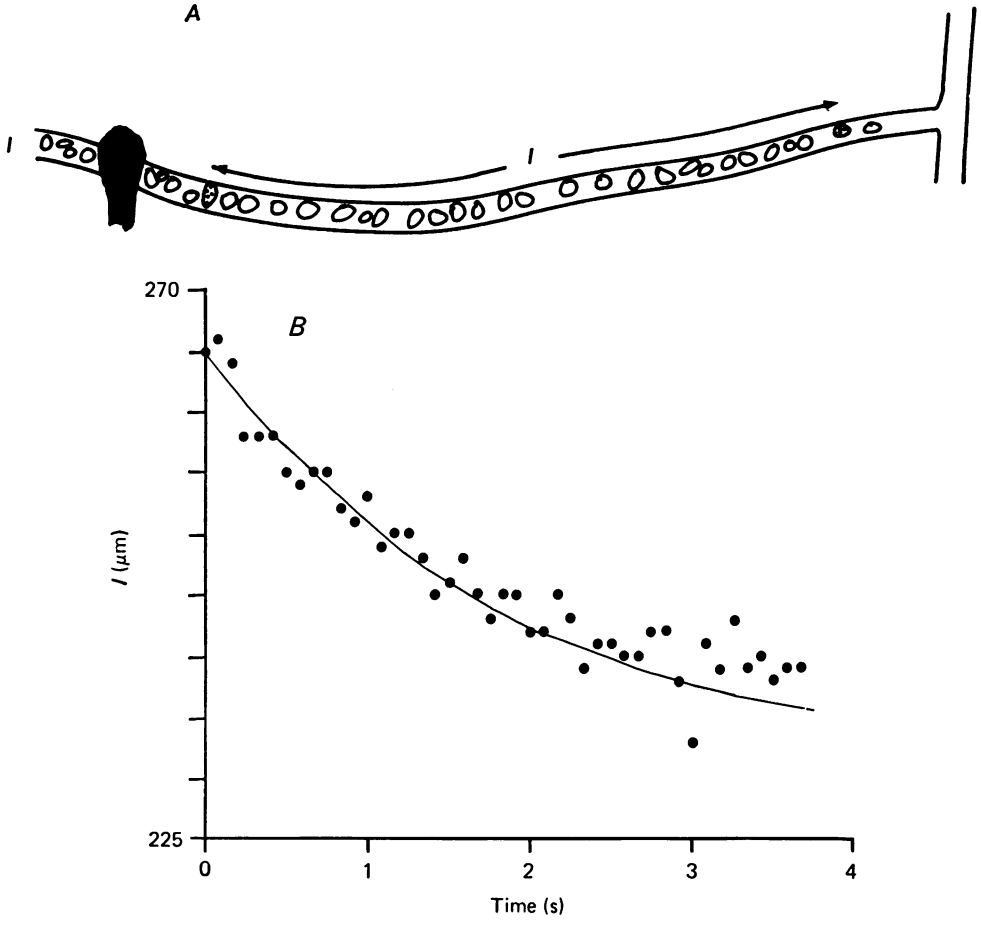

Fig. 1. *A*, a diagram of an occluded capillary. Shaded cells are arbitrarily chosen marker cells for determination of l with respect to time. *B*, distance between marker red cells in an occluded capillary plotted against time. The curve through the points is a fitted monoexponential.

(4) The distribution of plasma protein remains uniform within the capillary during the occlusion, and

(5) There is no net change in capillary hydrostatic pressure or in the contribution of the interstitium ($P_t - \pi_t$) during the occlusion and there is no longitudinal pressure gradient along the vessel.

If Starling's hypothesis is true and the above assumptions are valid, the plot of the calculated changing plasma colloid osmotic pressure (π_c) against the rate of fluid flux (J_v/A) will give a

linear relationship whose slope is the filtration coefficient (L_p), and whose intercept at zero fluid movement is the effective driving pressure (P_e).

The quantities l and dl/dt were estimated from a plot of distance between red cells against time during the occlusion (Fig. 1*A* and *B*). The oscillations in cell motion due to the pulsatile component of the hydrostatic pressure made the measurement of cell motion (dl/dt) due to net transendothelial fluid flux difficult. Two methods of minimizing the effects of the oscillations were tried: one was to draw successive tangents to the data, the other was to fit the data with a mono-exponential curve. Both procedures were performed on a Linc-8 computer, the programs being written in Calcplot (Bostock, 1972).

(a) Tangents

Tangents were drawn to the plot of cell distance against time (Fig. 1*B*) by performing linear regression on a number of neighbouring data points, the precise number depending on the frequency of the oscillations. The gradient of this regression gave an estimate of the mean cell motion (dl/dt) at the mean of those points. Several estimates of l and dl/dt were made on any set of data by moving the set of points chosen for the regression progressively down the curve.

(b) Curve fitting

A simple mono-exponential was fitted to the plot of l against time, and from this fitted curve successive values of dl/dt were obtained by differentiation. These, together with the other constants required (radius (r), protein concentration (c_0), initial distance between marker red cells (l_0), number and volume of red cells), were substituted in eqns. (4) and (5). From these values a plot of J_v/A against π_c was obtained.

Extension of method to venules

In the present study capillaries are defined as the final branching point of the arteriolar network in which flow is divergent and venules begin when flow starts to become confluent. In general this follows the nomenclature of Chambers & Zweifach (1944). Although it was possible to count the number of red cells in the occluded venule segment at the beginning of the occlusion it was not possible to use red cells close to the probe as marker cells during the course of the occlusion. This is to be expected in vessels wide enough to take two or three cells side by side. The changing volume of the red cell column was therefore determined by measuring the distance between the probe and a cell close to the open end of the venule. In order to ensure that this movement did not merely reflect leakage of plasma past the occlusion probe, the probe was always applied so that a segment of venule remained beyond the probe and the next junction. If the cells trapped in this short segment moved away from the probe at a significant rate it was assumed that the occlusion was imperfect and leakage had occurred and the data were discarded.

RESULTS

Filtration coefficient and effective pressure in capillaries

Occlusions were performed at one or other end of twenty-nine capillaries from ten cats and the flux data were analysed by both tangent and exponential fitting methods. Fig. 2 is a typical plot of J_v/A against π_c. The individual points were obtained using the 'tangents' method of minimizing the oscillations in the original data. L_p and P_e were estimated from a linear regression through these points. The scatter in the plot indicates the residual effect of the oscillations. On the other hand using the 'exponential' method all the variation due to oscillation was removed. The slight difference in the resulting estimates of L_p and P_e is probably due to the original data not being a simple mono-exponential. In this occlusion, and in the series as a whole, there was no significant difference between the two methods in their estimates for L_p and P_e.

The L_p value obtained was $0{\cdot}018 \pm 0{\cdot}01$ $\mu m^3/\mu m^2.s.mmHg$ ($1{\cdot}35 \pm 0{\cdot}75 \times 10^{-10}$ $m.s^{-1}.Pa^{-1}$) (mean $\pm$ S.D. for this and all subsequent values quoted) and P_e was $23{\cdot}9 \pm 8{\cdot}2$ mmHg ($3{\cdot}18 \pm 1{\cdot}09$ kPa). The colloid osmotic pressure was $19{\cdot}3 \pm 3{\cdot}7$ mmHg ($2{\cdot}57 \pm 0{\cdot}49$ kPa). When ln L_p is plotted against P_e a significant negative correlation is found. That is, the higher the transmural pressure, the lower the filtration coefficient (Fig. 3).

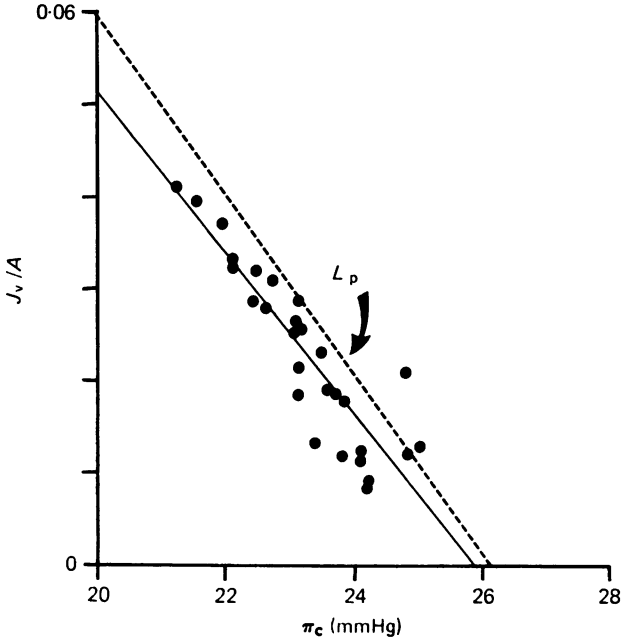

Fig. 2. Comparison of the two methods of data analysis used. The points were derived from the data in Fig. 1*B* by using the tangents method. The continuous line is the linear regression on those points, its gradient giving L_p and the intercept with the abscissa giving P_e. The interrupted line was constructed from the values of dl/dt derived from the fitted mono-exponential. See Methods for further details.

Filtration coefficient and effective pressure in venules

Venules up to three junctions beyond the capillary (up to 43 μm in diameter) were occluded near the venous end of the segment and in most cases red cell movement was towards the occlusion probe. In some vessels there was no net fluid movement, but red cell movement away from the occlusion probe, indicating reabsorption, was never seen.

Movement of cells at the same longitudinal position relative to one another was not observed in venules although considerable difference in velocity would have been expected had a parabolic velocity profile developed. The column of red cells thus moved as a plug and any suitable cell could be used as a marker for fluid flux measurements; its radial position in the vessel was unimportant. Cine film taken of vessels before and during an occlusion using stroboscopic illumination (Chadwick-Helmuth Inc. U.S.A., flash duration < 50 μs) revealed an abrupt change in erythrocyte shape. During free flow (*ca.* 1000 $\mu m.s^{-1}$) the cells tend to be deformed into 'parachute' shapes much as previously described (Bloch, 1962; Skalak & Branemark,

1969), but during the much lower flow of an occlusion (*ca.* 10 μm.s^{-1}) the shapes more nearly resembled the familiar biconcave disk.

The form of the relationship between cell position and time was similar to that in capillaries and could be readily analysed. As in the capillary, movement was more rapid initially, slowing later to reach a point at which no further net movement took place. Using the same analyses as described for the capillaries, the filtration coefficient in twenty-one venules from a further nine cats was found to be $0{\cdot}027 \pm 0{\cdot}015$ μm.s^{-1}.mmHg^{-1} ($2{\cdot}02 \pm 1{\cdot}5\ 10^{-10}$ m.s^{-1}.Pa^{-1}). This is significantly greater than that found in capillaries ($P < 0{\cdot}025$). Fig. 4 summarizes these findings.

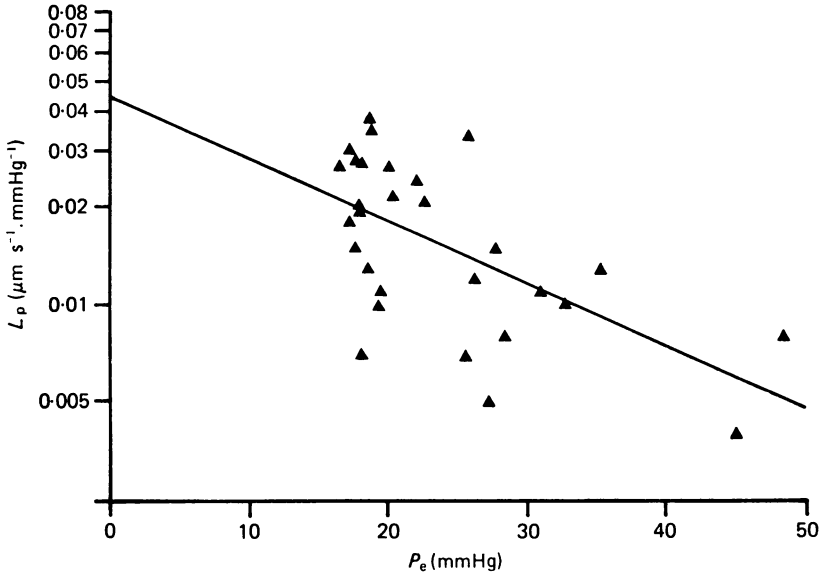

Fig. 3. The relationship between ln L_p and P_e for capillaries. The regression ($\ln L_p = -3{\cdot}12 - 0{\cdot}045P_e$) is statistically significant ($P < 0{\cdot}001$).

The P_e in venules was $28{\cdot}7 \pm 8{\cdot}5$ mmHg ($3{\cdot}82 \pm 1{\cdot}1$ kPa) while the colloid osmotic pressure in these animals was $19{\cdot}2 \pm 4{\cdot}1$ mmHg ($2{\cdot}55 \pm 0{\cdot}54$ kPa). Not only was the mean P_e greater than the mean π_c but in no individual venule was P_e smaller than π_c in that animal.

The gradient of increasing capillary permeability suggested by Fig. 3 thus appears to extend to venules, but within venules there seems to be no relationship between diameter and filtration coefficient (Fig. 5). There is, however, a similar negative relationship between filtration coefficient and effective pressure as found in Fig. 3 for capillaries; Fig. 6 shows this relationship for venules. The gradients for capillaries and venules are similar but their intercepts on the L_p axis are significantly different (analysis of co-variance $P < 0{\cdot}005$).

Comparison of directly and indirectly measured pressure

The assumptions underlying the single vessel occlusion technique were tested by comparing the directly measured hydrostatic pressure (P_c) with the effective pressure (P_e) in the same vessels. Five capillaries and five venules in seven cats were occluded during simultaneous pressure measurements. Capillary P_e measured by the occlusion

technique is the pressure in the nearest flowing supplying vessel, and it was this vessel that was punctured for the P_c measurement. Since single capillaries were occluded, there was no change in pressure in the supplying arteriole and the mean capillary pressure, P_c, was found to be 27·6 mmHg (3·68 kPa). Venules were large enough to cannulate directly so that on occlusion the change in pressure could be noted: the mean P_c in venules was 27·8 mmHg (3·70 kPa) which increased to a mean of 28·9 mmHg (3·84 kPa) during occlusion, the maximum individual rise being

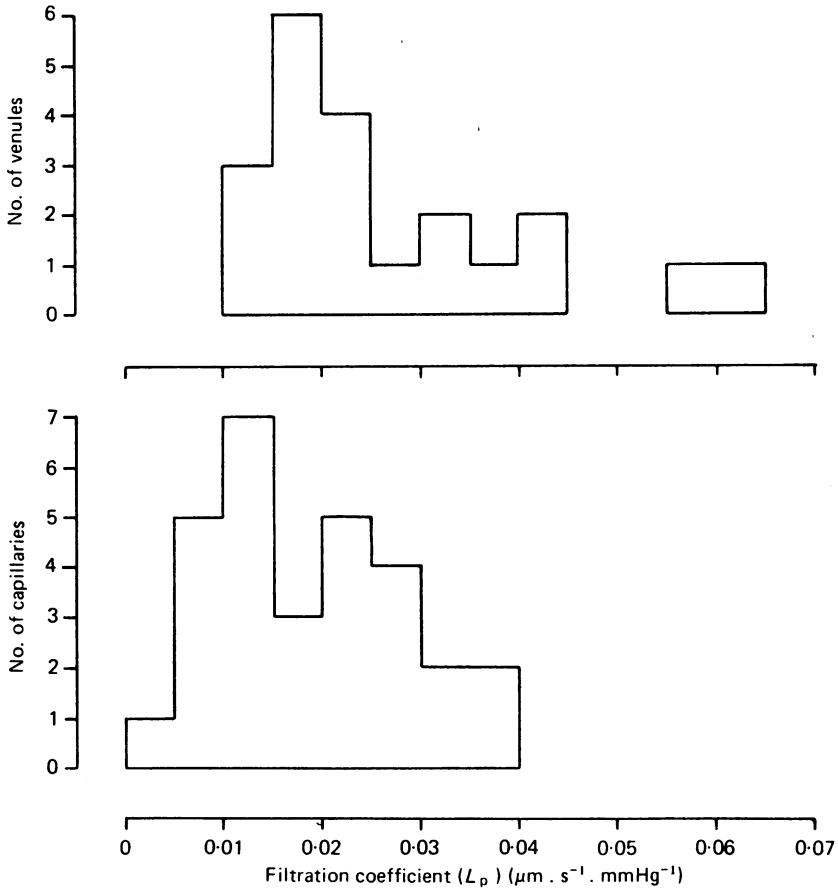

Fig. 4. Histograms illustrating the frequency distributions of filtration coefficients in capillaries and venules. The mean venular L_p (0·027 $\mu m \cdot s^{-1} \cdot mmHg^{-1}$) was significantly greater than the mean capillary L_p (0·018 $\mu m \cdot s^{-1} \cdot mmHg^{-1}$; t test, $P < 0·025$).

3 mmHg. The hydrostatic pressure in venules in free flowing conditions was in each case greater than the plasma colloid osmotic pressure of the animal in which it was measured. The differences ranged from 4 to 15 mmHg (0·53–2·0 kPa).

When the pressures were compared during the first hour of exposure of the mesentery there was no statistically significant difference between them (paired t test, see Fig. 7). A different picture emerges when P_e and P_c are compared at later times. Since $P_e = P_c - P_t + \pi_t$ (see eqn. (2), Methods), the difference between P_c and P_e allows an estimate of the contribution of interstitial pressures to be made.

In Fig. 8, $P_c - P_e$ has been plotted against time after exteriorization of the mesentery. It can be seen that the regression of $P_c - P_e$ against time suggests a slightly sub-atmospheric pressure at zero time. As time of exteriorization increases, the pressure difference becomes positive, reaching a value of about 2·5 mmHg at 2 hr.

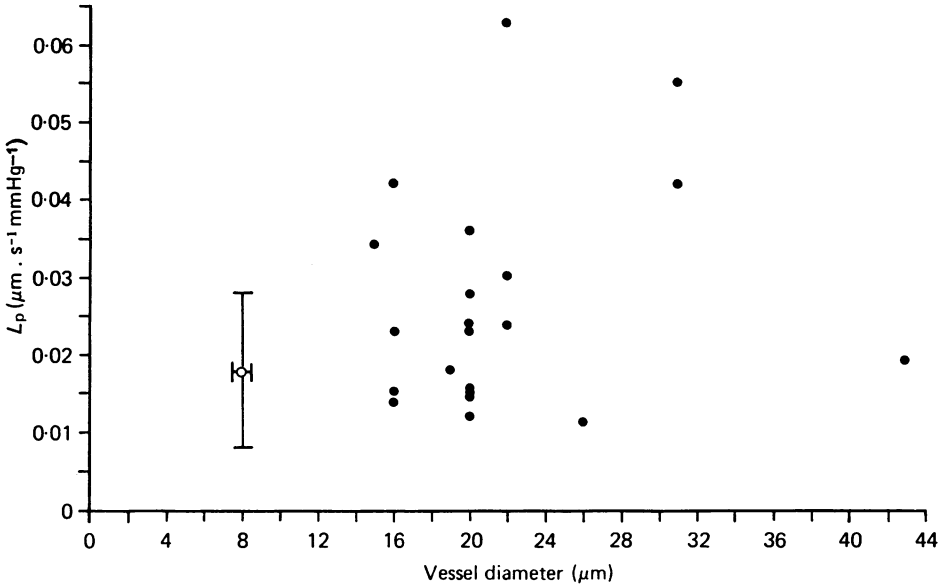

Fig. 5. Filtration coefficient plotted against vessel diameter for individual venules (●). The mean value for capillaries is also shown (○); the bars denote ±1 S.D.

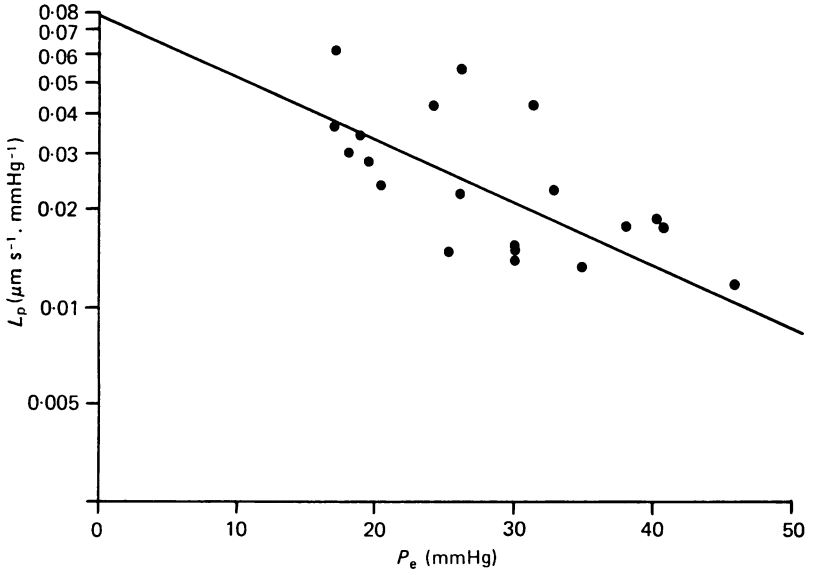

Fig. 6. The relationship between ln L_p and P_e for venules. The capillary regression line (Fig. 3) ($\ln L_p = -3{\cdot}12 - 0{\cdot}045P_e$) has a similar gradient to the venular regression ($\ln L_p = -2{\cdot}35 - 0{\cdot}043P_e$) but the difference in elevations is significant (analysis of co-variance $P < 0{\cdot}005$).

DISCUSSION

The present findings provide further confirmation, if confirmation be needed, of the general validity of Starling's hypothesis of tissue fluid formation; they also serve to emphasize certain qualifications. These are that the gradient of increasing permeability continues into the venules and that in the exteriorized cat mesentery

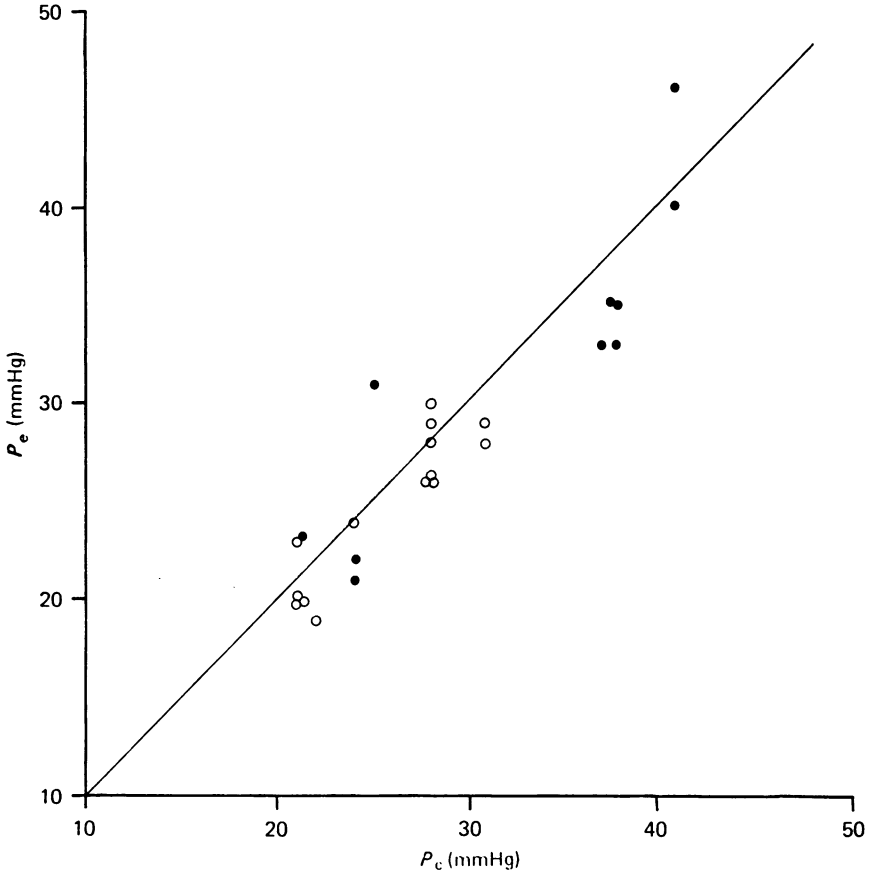

Fig. 7. Indirectly measured 'effective pressure' (P_e) compared with directly measured hydrostatic pressure (P_c) in capillaries (○) and venules (●). Each point represents an individual occlusion. The line of identity shown was not significantly different from the regression line.

reabsorption does not occur even in the venules. Before considering these points further it is appropriate to justify the single vessel occlusion technique and in particular to justify its application to venules.

Validity of single vessel occlusion technique

One of the most important assumptions is that the mean red cell movement accurately reflects net transmural fluid flux. This problem has been considered previously for capillaries (Zweifach & Intaglietta, 1968) and for situations in which the red cells are considerably smaller than the vessel diameter (Michel *et al.* 1974).

New points added to the argument from the present work are the close correspondence between pressure measured by direct puncture (P_c) and by the occlusion technique (P_e) early after exteriorization (see Fig. 7), the recovery of a biconcave disk shape by red cells after occlusion and the fact that the radial position of red cells in venules did not appear to influence their longitudinal velocity. This implies that the forces on the cells are not sufficient to distort them and that the flow profile in the vessel is unlikely to be parabolic.

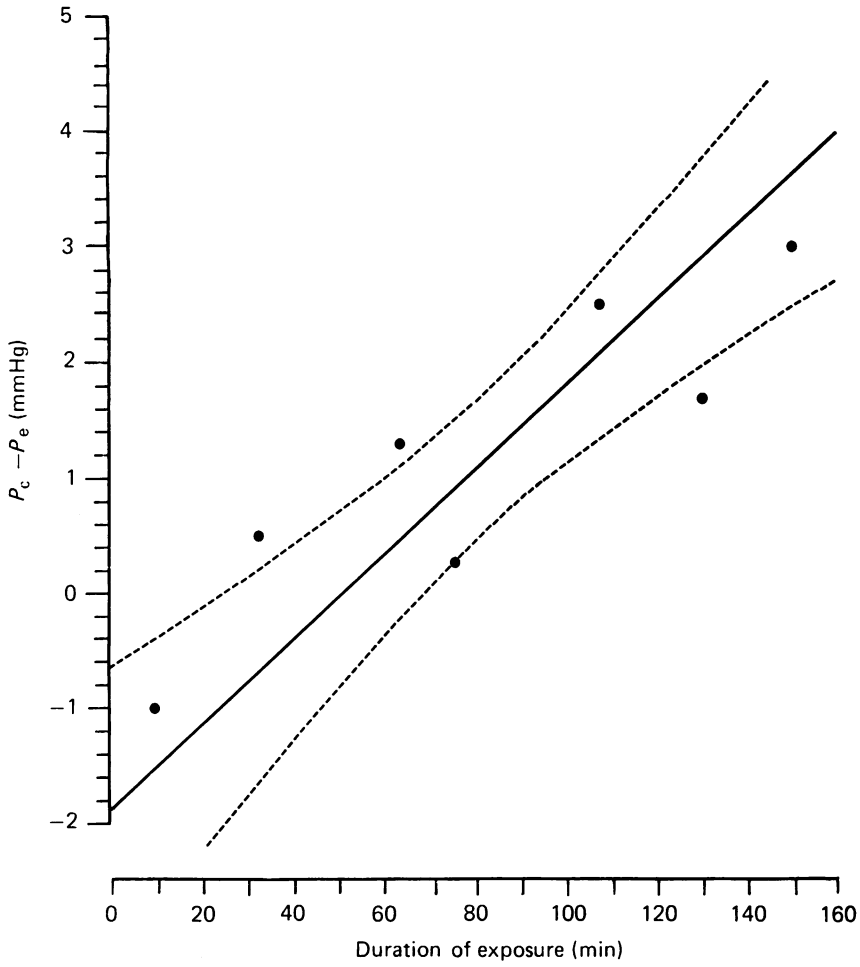

Fig. 8. Tissue pressure ($P_c - P_e$) plotted against time of exteriorization of the mesentery. In order to reduce the errors due to measurement each point is the mean of up to six individual measurements in one mesentery carried out over a period of 2–20 min (the resulting increase in variance in the abscissa was accounted for in the regression). The continuous line is the regression line and the interrupted lines are the 95% confidence limits.

If the latter observation were in error and a parabolic flow profile were to occur, then red cells in the centre of the vessel would be expected to move more rapidly than the surrounding plasma (see Skalak, 1972) so fluid flux would be overestimated.

The ratio of the cell and plasma velocities depends on the relative diameter of red cell and vessel:

$$\frac{V_c}{V_p} = \frac{2}{1+R^2}, \tag{6}$$

where V_c is the cell velocity, V_p is the mean plasma velocity, R is the ratio of cell : vessel diameter. According to this, a considerable correction would need to be applied to the red cell velocity in order to obtain the correct plasma motion. Fig. 9 shows

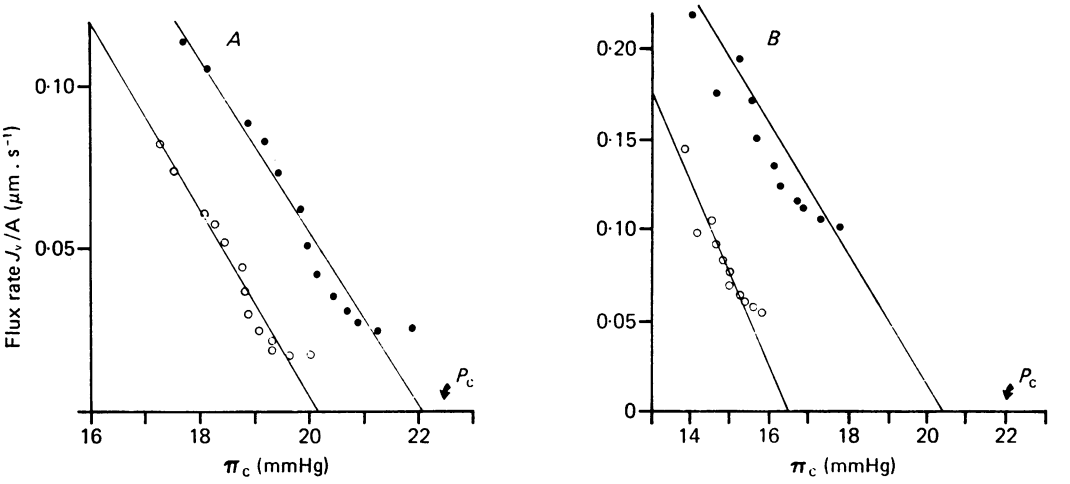

Fig. 9. The effect of adjusting mean plasma flow to those predicted by theoretical considerations in an occluded (*A*) capillary, (*B*) venule. Flux rate (J_v/A) is plotted in the uncorrected form (●–●) and corrected according to Skalak (1972) (○–○) – see text. The predicted L_p is not greatly affected but P_e is. Simultaneously recorded P_c indicates that the adjustment is probably inappropriate.

plots of J_v/A against π_c for (*a*) a capillary and (*b*) a venule, comparing the estimates of L_p and P_e obtained when red cell motion is used uncorrected as a marker of fluid flux and when the theoretical correction is applied. In both situations the simultaneously measured hydrostatic pressure is more closely approximated by the uncorrected data and since the net contribution of tissue colloid osmotic and hydrostatic pressures is small in mesentery (Clough & Smaje, 1978), it appears that such a correction is unnecessary. Furthermore, as can be seen from eqn. (6), the effects of parabolic flow would be expected to be greater in larger diameter vessels. As this is not evident it may be regarded as confirmation that in this situation parabolic flow does not occur.

P_e measurements

It will be noted that P_e for capillaries (23·9 ± 8·2 mmHg, 3·18 ± 1·09 kPa) is less than P_e for venules (28·7 ± 8·5 mmHg, 3·82 ± 1·1 kPa). This is a curious finding at first sight and may represent a sampling error. While venules were always open at the 'arterial' end, capillaries were often occluded at the arterial end, the pressure then being that prevailing at the 'venous' end of the capillary. Moreover, the two sets of data were obtained in different animals. The technique itself may be respon-

sible for another form of sampling problem. Red cell motion is difficult to detect reliably at velocities of less than 2 μm s^{-1}. For the same pressure difference ($P_e - \pi_c$) and L_p, red cell velocity will be inversely proportional to the diameter. Thus low pressure venules will be less readily sampled than low pressure capillaries.

Filtration in the microvasculature

The observation that filtration is occurring in the venules of the exteriorized mesentery does not accord with the general view of Starling's hypothesis. In his classical studies, Landis (1927) shows that reabsorption generally took place at the venous end of capillaries of the frog mesentery and this observation is the main basis of the general consensus view. In the mammalian mesentery and omentum, however, filtration occurs throughout the capillaries (Zweifach & Intaglietta, 1968; Lee *et al.* 1971) and we have now shown that this continues into the venules.

Net filtration will occur when P_e exceeds π_c. In the present experiments the mean values for P_e in venules (28·7 ± 8·5 mmHg, 3·82 ± 1·1 kPa) was 9·5 mmHg (1·26 kPa) greater than that for π_c and in no case was an individual P_e less than the π_c in the same cat. It is thus not surprising that reabsorption was not observed. The main criticism that can be levelled at this assertion is that filtration was observed in *occluded* vessels whose pressure would be that of the next open vessel upstream. In the cat mesentery there are frequent interconnexions and the greatest rise in pressure observed on occlusion was 3 mmHg (0·4 kPa). These values compare very well with the more extensive series of Zweifach (1974) who found a pressure of 28·3 ± 7·4 mmHg (3·76 ± 0·98 kPa) and a pressure drop of 3–4 mmHg (0·4–0·53 kPa) per segment in venules of the cat mesentery 7–40 μm in diameter. Furthermore, the values for π_c obtained in the present investigation (19·2 ± 4·1 mmHg, 2·55 ± 0·54 kPa) correspond with those of Zweifach & Intaglietta (1971) who found π_c in the cat to be 19·4 ± 2·4 mmHg (2·58 ± 0·32 kPa).

If reabsorption does not occur in vessels of 30 and 40 μm in diameter then it is unlikely to occur in larger ones as smooth muscle and pericytes make the walls too thick. Where then does the filtered fluid go? It could pass into the mesenteric lymphatics or possibly into the peritoneal cavity. In either case the Starling 'balance' is clearly not observed and the questions arise as to whether this is observed normally *in vivo* and if so does this occur elsewhere too? There is no simple answer.

If exposure produced a modest vasodilatation then under normal conditions it might be expected that the 'zero movement' venules would in fact be resorbing fluid. It is unlikely that exteriorization is the whole explanation as in an exteriorized preparation of the rat cremaster muscle, although filtration occurred in capillaries there appeared to be resorption in venules (Smaje *et al.* 1970). Splanchnic venules drain into the portal vein and thus have a higher pressure than systemic venules and this may explain the difference between the two preparations. On the other hand the tissue pressure of the mesentery *in vivo* appears to be subatmospheric and the mesentery tends to imbibe fluid, indicating an unsaturated interstitium (Clough & Smaje, 1978).

In order to explain the *in vivo* situation, either the vasculature must absorb fluid or the lymphatics may normally be responsible for preventing oedema. If the vessels have the prime responsibility it could be that during flowing conditions filtration is

observed throughout the length of the exchange bed, as observed in the present experiments, but during periods of stasis, reabsorption takes place. The recent description of arteriolar constriction in response to raising the P_{O_2} such that there was no capillary flow in the bed supplied by the arteriole (Lindbom & Arfors, 1978) supports this suggestion. Whilst our preparations exhibited vasomotion, arteriolar constriction was not observed and the filtration mode predominated. If, however, *in vivo*, arteriolar constriction did occur sufficient to prevent flow through the capillaries, the vascular pressures could well fall below the colloid osmotic pressure and reabsorption would occur. More work needs to be undertaken on this intriguing possibility.

TABLE 1. Filtration coefficients in single vessels of various tissues

Tissue	Filtration coefficient ($m.s^{-1}.Pa^{-1}.10^{10}$)		Authors
Mesentery			
Frog (20–26 °C)			
Capillaries	0·57	(mean)	Landis (1927)
Capillaries	0·5	(mode)	Michel *et al.* (1974)
	0·2–3	(range)	Mason *et al.* (1977)
Capillaries (no protein leakage)	0·91 ± 0·3	(mean ± S.D.)	Levick & Michel (1977)
Capillaries (protein leakage)	2·1–3·3	(range)	
Cat (37 °C)			
Capillaries	1·35 ± 0·75	(mean ± S.D.)	Present study
Venules	2·02–1·75	(mean ± S.D.)	Present study
Guinea-pig (37 °C)			
Capillaries	1·7 ± 0·75	(mean ± S.D.)	Clough & Smaje (1977)
Omentum			
Rabbit (37 °C)			
Capillaries	0·5	(mode)	Zweifach & Intaglietta (1968)
	0·2–2·5	(range)	
Capillaries (arterial end)	3·1 ± 1·6	(mean ± S.D.)	Lee *et al.* (1971)
Capillaries (venous end)	4·5 ± 1·2	(mean ± S.D.)	Lee *et al.* (1971)
Muscle (Cremaster)			
Rat (37 °C)			
Capillaries	0·1 ± 0·01	(mean ± S.D.)	Smaje *et al.* (1970)

L_p values in capillaries and venules

Table 1 summarizes L_p values obtained from the literature. It can be seen that within a single microvascular bed L_p varies by a factor of 10 or more. Nevertheless, it would appear that mesentery and omentum have roughly comparable L_ps in different species but muscle seems to have a lower value. A similar discrepancy is seen in whole organs, as capillary filtration coefficient for rat hind limb (0·033 ml. $min^{-1}.mmHg^{-1}$ 100 g^{-1}, Renkin & Zaun, 1955) is about 6% that of isolated rat mesentery (0·55 ml.$min^{-1}.mmHg^{-1}$ 100 g^{-1}, Davies & Gamble, 1976). The other point of interest is that L_p increases towards the venous end of the microvasculature and this point will now be considered in further detail.

Gradient of permeability

The continuing increase in filtration coefficient into the venules found in the present investigation confirms older observations using dyes (Rous, Gilding & Smith, 1930) but contrasts with Wiederhielm's finding (1968) of a lower filtration coefficient in the venules of the frog mesentery than in the venous capillaries. The present work does correlate well with recent morphological findings in the rat. Simionescu, Simionescu & Palade (1975) have shown that whereas the capillaries have tight interendothelial cell junctions, the venular junctions are more loosely arranged and continuous pathways between lumen and interstitium may be traced.

The negative correlation between L_p and P_e in both capillaries and venules could merely reflect the anatomical situation in the vasculature as pressure falls progressively. This interpretation has been suggested by Michel (1972) and was the explanation favoured by Zweifach & Intaglietta (1968) when they observed a similar trend in capillaries of the omentum but it is not consistent with the present finding of a lack of correlation between L_p and vessel diameter (Fig. 6). Even if it were partly responsible, it cannot be the whole explanation as the significantly raised regression line for the venules on the $\ln L_p - P_e$ plot (Figs. 4 and 7) argues for a real difference between venules and capillaries. The anatomical studies of Simionescu *et al.* (1975) would also support such an interpretation.

Before discussing the relationship further, the possibility of an experimental artifact should be considered. First, P_e is not the hydrostatic pressure normally within the occluded vessel but the pressure within the supplying vessel. However, for the purposes of this correlation it is likely that P_e is a good estimate for the 'normal hydrostatic pressure' as the mean pressure rise on occlusion is only 1·1 mmHg. Secondly the negative correlation between L_p and P_e could be explained if a greater hydrostatic pressure increased protein concentration near the endothelial wall by causing a greater transmural fluid flux. This would lead to an apparent lowering of the measured L_p. However, experiments on individually perfused capillaries of the frog mesentery (Michel *et al.* 1974) show that over a large range of pressures, including those at which resorption occurs, L_p remains constant, so this explanation would not appear to account for the relationship.

Thus the significant negative correlation between capillary and venular pressure and filtration coefficient in the absence of effects due to vessel diameter, gives rise to speculation that pressure itself may be an important determinant of L_p. This is consistent with an observation of Zweifach (1940) in which injected marker dyes were seen to be retained by arterioles of the frog mesentery but not by venous capillaries or venules. Flow relationships were then altered by appropriate occlusion of vessels such that flow now passed from venule to arteriole and under these circumstances dye now leaked from the arteriole. Although Michel *et al.* (1974) found no significant change in filtration coefficient on altering the hydrostatic pressure this does not rule out the possibility of pressure influencing filtration coefficient over a longer time scale. On the other hand, if the relationship were a dynamic one, the low rate of filtration in the legs of the erect subject could be partly explained. Mellander, Oberg & Odelram (1963) observed a fall in capillary filtration capacity of the human foot and cat calf on moving to an upright posture, which they inter-

preted as a reduction in capillary surface area due to closure of pre-capillary sphincters. The authors obtained evidence for an increase in pre-capillary resistance but at least part of the effect could be explained by a reduction in filtration coefficient. Recently Levick & Michel (1978) have shown a similar increase in pre-capillary resistance in the human subject on standing, but no change in capillary surface area was observed. It would be worth designing experiments specifically to test the possibility that pressure itself influences filtration coefficient.

Interstitial pressure

The method of assessing the contribution of the interstitium to fluid exchange by determining the difference between direct and indirect hydrostatic pressures in capillaries and venules is interesting as it provides similar values to those obtained by wicks in contact with the mesentery (Clough & Smaje, 1978), or by lymphatic puncture (Clough & Smaje, 1978; Zweifach & Prather, 1975). On the basis of wick measurements *in situ* it seems that tissue pressures in the mesentery are not as low as those in subcutaneous tissue but they do appear to be subatmospheric. Exteriorization and immobilization leads to oedema, presumably because lymph drainage is inadequate for the rate of tissue fluid production. Most venules are filtering in the exteriorized preparation but whether this is responsible for the oedema or whether a relatively reduced lymph flow is the prime cause cannot be answered directly but is likely to be a combination of both (see Clough & Smaje, 1978).

One interesting feature of the measurements of tissue pressure obtained from the difference between P_c and P_e is that the contribution of the tissue around the venules and around capillaries can be separately determined (Fig. 8). The data are somewhat scanty so far but no obvious differences are apparent, suggesting that there are no significant longitudinal hydrostatic pressure gradients in the tissue.

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