Fluid uptake from the renal medulla into the ascending vasa recta in anaesthetized rats

P. J. MacPhee and C. C. Michel*

Department of Physiology & Biophysics, St Mary's Hospital Medical School, Imperial College of Science, Technology and Medicine, Norfolk Place, London W2 1PG, UK

1. We have investigated fluid movements between superficial ascending vasa recta (AVR) and the interstitium of exposed papillae of the renal medullae in 15-day-old Sprague–Dawley rats anaesthetized with Hypnorm and Hypnovel.

2. Using a development of the red cell micro-occlusion technique, fluid filtration and reabsorption rates per unit area of vessel wall (J_v/A) were determined in 54 single perfused AVR at known microvascular pressures (P_c). The relation between J_v/A and P_c was non-linear suggesting hydraulic permeabilities (L_p) of 50–100 $\times 10^{-7}$ cm s^{-1} cmH_2O^{-1} when P_c was between 0–10 cmH_2O and 150–200 $\times 10^{-7}$ cm s^{-1} cmH_2O^{-1} when P_c was 10–15 cmH_2O.

3. Rates of fluid reabsorption into the AVR estimated by a densitometric technique in a further fourteen vessels were consistent with L_p values of 50–100 $\times 10^{-7}$ cm s^{-1} cmH_2O^{-1} when P_c was −2 to 0 cmH_2O.

4. The effective oncotic pressures of perfusates containing bovine serum albumin (BSA) were consistent with minimum values for the reflection coefficients of the walls of the AVR to BSA of between 0·59 and 0·72.

5. The concentration of native serum albumin in the papillary interstitial fluid was 9·1 ± 0·6 mg ml^{-1} (mean ± S.E.M., $n = 16$, from 9 rats), which is approximately 25% of the plasma level.

6. After their microinjection into the medullary interstitium, Patent Blue V and Evans Blue–albumin cleared within 1 min. There was no evidence of preferential movement of either dye towards the base of the exposed renal medulla.

7. Because L_p of the AVR is high, mean pressures of only ~3 cmH_2O are necessary to account for the total clearance of fluid from the medullary interstitium into the AVR. From published data and from our own observations, it appears that differences in hydrostatic and oncotic pressure across the walls of the AVR are more than sufficient to provide this driving force. The possibility of the clearance of protein from the interstitium into the AVR is discussed.

The reabsorption of fluid from the renal interstitium into the ascending vasa recta (AVR) is the final stage in the conservation of water by the formation of a concentrated urine. The details of this process are unclear. In most microvascular beds, net fluid uptake from tissues to blood occurs when the oncotic pressure of the plasma exceeds the sum of the oncotic pressure of the pericapillary fluid and the hydrostatic pressure difference across the microvascular walls (Starling, 1896). In these tissues there are lymphatics, which act both as an overflow and as a route for clearing plasma protein from the interstitial fluid. It has been argued that in tissues, such as the intestinal mucosae and the renal cortex, the steady uptake of fluid into the blood depends on a parallel but much smaller flow of lymph which, by clearing interstitial proteins, maintains the oncotic pressure differences across the capillary walls (Michel, 1984; Levick, 1991). In the inner zone of the renal medulla, however, lymphatics are difficult to demonstrate and it is concluded that they are either sparse (Cuttino, Jennette, Clark & Kwock, 1985; Cuttino, Clark & Jennette, 1989) or absent (e.g. Ulfendahl & Wolgast, 1992). Furthermore, plasma proteins are known to enter the medullary interstitium from the circulating blood (e.g. Lassen, Longley & Lillienfield, 1958). Thus, if oncotic pressure differences across the walls

* To whom correspondence should be addressed.

of the AVR are responsible for the clearance of fluid from the inner medulla, some mechanism equivalent to lymphatic drainage must simultaneously clear protein from the interstitium in order to keep its oncotic pressure low.

The experiments described in this paper were designed against this background of thinking. They have been carried out on young anaesthetized Sprague–Dawley rats where it is possible to make direct microscopic observations of the medullary microcirculation after the removal of the pelvis of the ureter. In a preliminary set of experiments, we observed the extent to which Evans blue–albumin was cleared from the medullary interstitium, for it has been suggested that spaces surrounding the collecting ducts may act as prelymphatic channels (Schmidt-Nielsen, Lacy & Patel, 1976). We then estimated the hydraulic permeability (L_p) of individually perfused vasa recta located between 0·2 and 1·2 mm from the tip of the papilla. From the magnitude of L_p we were able to estimate the pressure (or effective oncotic pressure) necessary to maintain a given rate of fluid uptake into the AVR. By measuring L_p during perfusion with different concentrations of serum albumin, it was also possible to estimate the reflection coefficient of the walls of the AVR to albumin (σ_{Alb}). Since our estimates of L_p were based on changes in the outward filtration of fluid through the walls of the AVR into the tissues with changes in microvascular pressure, we have additionally demonstrated fluid uptake into the AVR using a densitometric technique. These measurements allowed us to make minimal estimates of L_p under conditions of fluid reabsorption.

Since we embarked on these experiments, estimates for the L_p of AVR and for σ_{Alb} in the AVR have been reported in a series of papers by Pallone (1991*a*, *b*, 1992). Because the methods that we have employed are different from those used by Pallone (1991*a*, 1992) and since he worked on Munich Wistar rats (as opposed to Sprague–Dawley rats), it seemed worthwhile to continue our investigation.

Preliminary communications of our results have been made to the British Microcirculation Society (MacPhee & Michel, 1993*a*) and to the Physiological Society (MacPhee & Michel, 1993*b*).

METHODS

Preparation

The experiments were carried out on 15-day-old Sprague–Dawley rats which we have found are able to concentrate the urine to at least 650 mosmol (kg H_2O)$^{-1}$. The animals were anaesthetized with an intraperitoneal injection of 0·1–0·2 ml of a mixture of Hypnorm (0·315 mg ml^{-1} fentanyl citrate and 10 mg ml^{-1} fluanisone), Hypnovel (5 mg ml^{-1} midazolam hydrochloride) and water made up in the proportions 1 : 1 : 2 by volume. The kidney was exposed through a left lateral incision in the abdominal wall.

The pelvis of the ureter was carefully dissected away to expose the inner medulla. The kidney itself was supported in a cup to minimize respiratory movements. The papilla, which projected through an opening in the wall of the cup, was supported by a quartz rod which was also used to transilluminate it. The exposed tissue was continuously washed with a physiological saline solution and viewed through a Wild M10 stereomicroscope fitted with a camera attachment on which a Panasonic BL200 CCD video camera was mounted. The camera was linked to a monitor and a JVC videorecorder. Individual AVR were cannulated with sharpened glass micropipettes (10–15 μm tip diameter) and perfused with buffered solutions containing bovine serum albumin (BSA).

Solutions

The tissues were washed with a phosphate-buffered saline solution which had the following composition: 0·122 M NaCl, 0·03 M KH_2PO_4, 0·03 M Na_2HPO_4, 2 mg ml^{-1} glucose, adjusted to pH 7·44 with 0·1 M NaOH and an osmolality of 300 mosmol (kg H_2O)$^{-1}$ with distilled water.

Single vasa recta were perfused with the same solution containing BSA (crystallized, lyophylized, globulin-free; Sigma) at concentrations of either 10 or 30 mg ml^{-1}. For the determination of L_p, the perfusates contained human red cell ghosts filled with Evans Blue. The red cell ghosts were prepared by the method of Nash & Meiselman (1985) except that the lysing medium also contained 4·89 g (100 ml)$^{-1}$ Evans Blue (Sigma) and was filtered through a 0·22 μm filter before use.

In the densitometric measurements and in the preliminary experiment on protein clearances from tissues, solutions containing T1824 (Evans Blue) and bovine serum albumin (30–50 mg ml^{-1}) were used. Reference to the binding curves of Levick & Michel (1973) revealed that in these mixtures 99·5% of the dye was bound to the albumin.

Estimation of fluid filtration and L_p using the red cell micro-occlusion technique

The method used was a modification of that originally developed to determine the L_p of single frog mesenteric capillaries (Michel, Mason, Curry & Tooke, 1974; Michel, 1980). A single superficial AVR was cannulated with a sharpened glass micropipette and perfused with a solution containing BSA and a suspension of resealed human red cell ghosts filled with Evans Blue (T1824). These Evans Blue-containing ghosts acted as flow markers and it was essential to use them rather than normal red cells: (i) in order that they could be distinguished from the rat's own red cells flowing through adjacent and underlying vessels; (ii) so that they could be clearly visualized against the pink background of the transilluminated medullary tissue. The resealed ghosts were very variable in size. While the larger cells were used as flow markers, the smaller ones served as edge markers enabling the observer to visualize the walls of the perfused vessel more easily. After a short period of perfusion, the vessel was occluded 400 μm or more downstream from the micropipette tip and at a point before the vessel received its next tributary. The movement of cells within the occluded segment indicated flow from the micropipette into the vessel. In principle these cell movements reflect: (i) the expansion of the vessel as the pressure within it rises to equal that applied from the micropipette (compliance effect), and (ii) the replacement of fluid which is being lost by filtration from the AVR into the surrounding tissues. The compliance effect was difficult to observe following the standard micro-occlusion procedure in the vasa recta: (i) because micro-occlusion on the relatively soft renal medulla took the tissues below the plane of focus of the microscope and the compliance effect was over by the time the microscope had been refocused; (ii) because the volume change was relatively small

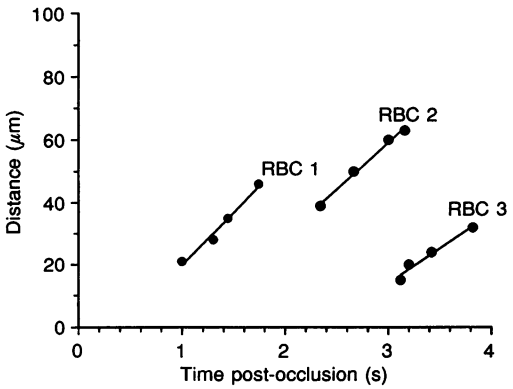

Figure 1. The movements of three red blood cells in a microperfused AVR after it had been occluded (at zero time) at a site 259 μm from the pipette tip

The diameter of the vessel was 20 μm and the pressure applied to the micropipette was 10 cmH_2O. The velocity of the three cells was $28{\cdot}7 \pm 5{\cdot}9$ $\mu m\ s^{-1}$ (mean ± S.D.) corresponding to a value of J_v/A of $0{\cdot}55$ $\mu m\ s^{-1}$, i.e. 11% of the total volume of the occluded segment per second.

and no changes in vessel diameter were seen even when the vessel remained in focus. Thus cell movements 2–3 s after micro-occlusion were interpreted to reflect filtration rates through the vessel walls. In support of this interpretation was the steady rate at which cells entered the vessel from the micropipette during the first 10 s or so of observation. Figure 1 shows the trajectories of three cells over the same stretch of vessel after entering an occluded segment from the micropipette at different times during a single occlusion.

It is seen that the velocities of the cells are similar at 1 and 3 s. These velocities are relatively high and if they were due to compliance changes, they would represent a 33% increase in volume of the vessel between 1 and 4 s, i.e. an increase in vessel radius of approximately 15%. While a 5% change in vessel diameter could be resolved, no change was observed.

Cell velocities could therefore be used to estimate fluid filtration per unit area of vessel wall (J_v/A) and measurements were made in duplicate or triplicate at two or three levels of pressure (P_c) in each vessel (Michel *et al.* 1974). In some experiments, the pressure applied to the micropipette was raised and lowered in steps during occlusions when J_v/A was determined from the cell movements following the initial occlusion and the step change in pressure (Michel, 1980). In all cases J_v/A was plotted against P_c and the slope of this relation was taken to be L_p.

Densitometric observation of reabsorption and filtration

A densitometric method was used to obtain unambiguous evidence of fluid absorption from the medullary tissue into the AVR. A single AVR was perfused with a Ringer solution containing 0·005 M Evans Blue and BSA (50 mg ml^{-1}) such that 99% of the dye would be bound to the BSA (Levick & Michel, 1973). After a short perfusion, the vessel was occluded with a glass microrod to form a closed-off segment of vessel, 400 μm or more in length, between the occlusion site and the micropipette tip. The intensity of the light passing through the solution in the pipette close to the tip was monitored over a small rectangular window (see Fig. 2) using image analysis software (HOOO TPL VI) and a monochrome image analysis system (A010) both from Seescan PLC (Cambridge, UK). During and immediately after micro-occlusion, the pressure applied to the pipette was adjusted to balance oncotic pressure differences across the vessel wall and was determined to be about 15 cmH_2O. Between 3 and 5 s after micro-occlusion, the pressure in the micropipette was dropped to 0 or −2 cmH_2O and the light intensity was measured at 1 s intervals over the next 30 s. Over this period the fluid within the occluded segment of AVR flowed up into the micropipette past the observation window as the Evans Blue–albumin solution was diluted by fluid entering the vessel from the surrounding tissues. Since the optical path length through the micropipette remained constant, the increase in the light intensity was proportional to the dilution of Evans Blue by fluid entering the occluded section of AVR from the surrounding tissues. During these periods of fluid reabsorption, the vessel beyond the micro-occlusion site was observed to check that the optical density in these vessels remained greater than that in the occluded segment, so eliminating the possibility that dye-free plasma was being drawn into the vessel from below the occlusion site.

Interpretation of the densitometric measurements

If I is the mean intensity of light passing through the sampling window, the output from the Seescan videodensitometer gave a signal proportional to $\log_{10} I$. Usually a signal such as this is subtracted from the logarithm of a background intensity, $\log_{10} I_0$, to yield a value for the optical density of the solution of interest. In our experiments it proved difficult to obtain values for the background intensity. This would represent the intensity of light passing through the same micropipette filled with a colourless

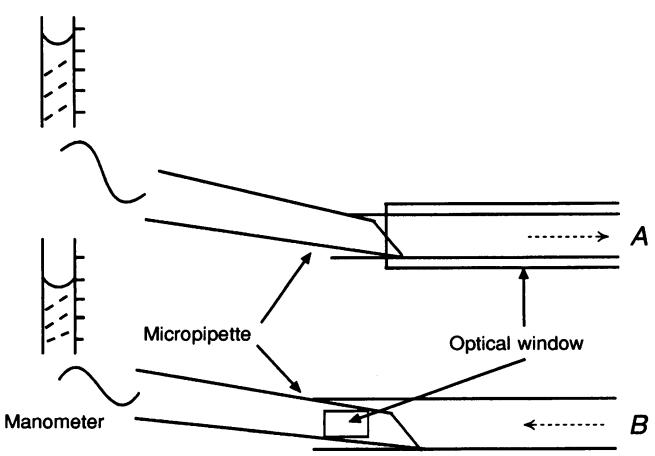

Figure 2. Diagram to show the arrangement of the optical window with respect to the perfused occluded microvessel and the micropipette for measurements of (*A*) fluid filtration and (*B*) fluid reabsorption

In *A*, the optical window overlaps the microvessel walls from the middle of the micropipette tip to the occlusion site as described by Levick & Michel (1977). In *B*, the optical window is confined to a region over the glass micropipette close to its tip.

solution and held in exactly the same position with respect to the tissue. To have determined it would have required refilling the micropipette and ensuring that its position was precisely the same over the two periods of measurement. Because of this limitation, our initial aim was merely to obtain objective but only semi-quantitative evidence of fluid reabsorption. We did, however, analyse the data from experiments such as that shown in Fig. 5*A*, by two different methods which allowed us to estimate the magnitude of L_p for the AVR.

Both methods assumed that the output from the video-densitometer, D, was proportional to the concentration of dye (C) in the region of the micropipette beneath the sampling window:

$$D = aC + B, \tag{1}$$

where a represents a constant combined function of the path length through the dye-filled vessel and the extinction coefficient for the dye and B, the background. It was also assumed that, after the micropipette pressure was lowered, fluid entered the occluded segment of AVR by reabsorption and uniformly diluted the Evans Blue dye, causing the diluted fluid in the AVR to expand into the micropipette. Thus, the fall in D measured over the micropipette was interpreted to mirror the changes in dye concentration which had occurred in the occluded segment of AVR shortly beforehand. The diffusion of dye in the front of the column of fluid advancing up the micropipette was neglected, an assumption we recognize as a major simplification. We note, however, that this assumption should lead to an underestimate of the rate of change of D and hence to an underestimate of the reabsorption rate. The delay between the changes in the vessel and those observed in the micropipette were estimated from the volume of micropipette between the proximal edge of the window and the midpoint of the bevel of the tip divided by the fluid reabsorption rate, J_{V_R}. It was argued that the rate of fall in concentration of albumin-bound dye, dC/dt, within the occluded segment of AVR was related to the rate of fluid uptake into the AVR from the surrounding tissues, J_{V_R}, and the volume of the occluded segment, V_c. Thus, rate of loss of albumin-bound dye from occluded segment at time, t is:

$$J_{V_R} C = -V_c \frac{dC}{dt},$$

or

$$\frac{dC}{dt} = -\frac{J_{V_R}}{V_c} C. \tag{2}$$

J_{V_R}/V_c is the fractional clearance of fluid out of the AVR into the micropipette. This is determined by the Starling principle:

$$\frac{J_{V_R}}{V_c} = \frac{2L_p}{r}\{\sigma(\pi_p - \pi_i) - \Delta P\}, \tag{3}$$

where r is the radius of the occluded segment of AVR; $2V_c/r$, the surface area of the occluded segment of AVR; σ, the osmotic reflection coefficient of the walls of the AVR to albumin; π_p, the oncotic pressure of the perfusate in the occluded segment of the AVR; π_i, the oncotic pressure of fluid surrounding AVR; and ΔP, the hydrostatic pressure difference across walls of AVR.

For albumin, π_p (in cmH_2O) at 37 °C is related to C (in g (100 ml)$^{-1}$) through a non-linear expression (Moyses, 1986):

$$\pi_p = 1{\cdot}424\,(2{\cdot}5C + 0{\cdot}13C^2 + 0{\cdot}012C^3). \tag{4}$$

The first method we used to analyse the data involved a simple iterative programme which calculated the changes in fluid volume (δV), and albumin–dye concentration (δC) for assumed values of L_p and σ, constant values of ΔP and π_i and the known initial values of V_c, C and hence by eqn (4), of π_p. Equation (3) was written as a difference equation to establish the increase in fractional volume over a short period of time, δt:

$$\frac{\delta V}{V_c} = \frac{2L_p}{r}\{\sigma(\pi_p - \pi_i) - \Delta P\}\delta t. \tag{5}$$

The volume of fluid, originally contained in V_c was expanded to a value V (i.e. $V = V_c + \delta V$) and consequently C fell to $C_{(t+\delta t)}$:

$$C_{(t+\delta t)} = \frac{C_t}{1 + \delta V/V_c}. \tag{6}$$

The value $C_{t+\delta t}$ was displayed and used as C to calculate π_p in the next iterative cycle.

For these purposes it was assumed that after the pressure was lowered to either -2 cmH_2O or 0, ΔP lay in the range of -2 to $+2$ cmH_2O, i.e. that the interstitial fluid pressure in our preparation lay in the range of 0–2 cmH_2O. The concentration of albumin in the fluid surrounding the AVR (C_i) was assumed to be constant throughout a run; values of C_i in the range between 20 and 30% of the initial perfusate concentration (C_o) were used to estimate π_i (see Results). The program was run first with assumed values for σ of 0·7 and L_p of 100×10^{-7} cm s^{-1} cmH_2O^{-1}. The value of L_p was then adjusted until the calculated results matched the data. The sensitivity of the fit to variations of σ and ΔP were then examined. In making the comparison between the calculated results and the data, C_t, the concentration of protein in the perfusate at time t, was expressed in terms of its initial value, C_o, and its final value, C_∞, in the form of the ratio: $\{C_t - C_\infty\}/\{C_o - C_\infty\}$. This ratio was then compared with the similar ratio of equivalent values of D, i.e. $\{D_t - D_\infty/\{D_o - D_\infty\}$ (see eqn (1)).

The method assumes that the ratio of surface area to volume of the occluded microvessel is equal to $2/r$ and that r remains constant. A significant reduction in V_c during the course of the experiment or an eliptical cross-section of the vessel would increase the area to volume ratio above $2/r$ and this would mean that the chosen value of L_p which fits the data is an overestimate of its true value. This possible source of error acts in the opposite direction to the assumption that diffusion of dye is negligible in the tip of the micropipette (which leads to an underestimate of L_p).

The second method of analysis made three additional assumptions: (i) that the perfusate oncotic pressure was directly proportional to its perfusate albumin concentration, i.e. $\pi_p = KC$, where K is a constant; (ii) that $(P_i - P_c)$ was zero; and (iii) that π_i was negligible. Assumption (i) becomes reasonable introducing an error of less than $\pm 15\%$ when the concentration of albumin falls below 50 mg ml^{-1}. Assumptions (ii) and (iii) are likely to influence the results in opposite directions so that only a minor difference to J_{V_R} will result from assuming that both $(P_i - P_c)$ and π_i are zero (i.e. P_i may be greater than P_c favouring reabsorption into the AVR and $\pi_i > 0$ favouring filtration from the AVR into the tissue). Combining eqns (2) and (3) gives:

$$\frac{dC}{dt} = -\frac{2L_p \sigma K C^2}{r},$$

which can be integrated to yield:

$$\frac{1}{C} = \left(\frac{2L_p \sigma K}{r}\right) t + \frac{1}{C_o}. \tag{7}$$

It was noted earlier that values of D cannot be converted to values of C as the background intensity could not be measured satisfactorily. In the reabsorption experiments, however, the

asymptotic value of D, D_∞, is the background value for it represents the magnitude of D when all dye bound to albumin has been washed out of the vessel by fluid absorption. Thus, by plotting the reciprocal of $D_t - D_\infty$ against time, one should obtain a linear relation with a slope proportional to σL_p and an intercept equal to the reciprocal of $D_o - D_\infty$, enabling us to estimate L_p for assumed values of σ. As with the first method of analysis, it is assumed that the surface area to volume ratio for the occluded segment is equal to $2/r$, so that a reduction of V_c during the course of the experiment could lead to an overestimate of L_p.

Estimation of σ to serum albumin from densitometric measurements

The reflection coefficient, σ, of the walls of the AVR to serum albumin was estimated from differences in the effective oncotic pressure exerted by perfusates containing different concentrations of BSA. When the BSA concentration exceeded 30 mg ml^{-1}, however, it was not possible to use the cell occlusion technique to measure fluid filtration because the Evans Blue-containing cells aggregated within the micropipette, blocking its tip. We therefore adapted the densitometric technique of Levick & Michel (1977) to measure the effective oncotic pressure of perfusates containing Evans Blue–albumin at a concentration of 50 mg ml^{-1}. Levick & Michel (1977) substituted optical density for C in eqn (2) to estimate absolute values of fluid filtration from microperfused occluded segments of capillaries. Because of the difficulties of estimating the background density, this was impossible in our experiments. It was, however, possible to measure the rate of change of optical density (which was proportional to the filtration rate) immediately before and after a step change in pressure within an occluded segment of AVR (see Levick & Michel, 1977; Method 1). Data of this kind could be extrapolated to a value of pressure at which $dD/dt = 0$ and this pressure was interpreted as the effective oncotic pressure opposing filtration, i.e. $\sigma(\pi_p - \pi_i)$; see Fig. 7*B*. A minimal value of σ was calculated from this effective oncotic pressure and the oncotic pressure of the perfusate (π_p) by using the suggestion of Michel (1984) that $\sigma(\pi_p - \pi_i)$ has a minimal value at high filtration rates of $\sigma^2\pi_p$ (see also Michel & Phillips, 1987). Measurements of light intensity through the entire length of the occluded segment of a microvessel were made using the Seescan videodensitometer.

Estimation of protein content of medullary interstitial fluid

Interstitial fluid from the excised papilla of the renal medulla was isolated according to the method of Shirley, Walter & Sampson (1992). Briefly, isolated papillae were blotted dry on absorbent paper, centrifuged at 200 *g* for 10 min under oil and the fluid discarded. This fluid is believed to represent that from the terminal-collecting ducts, the loops of Henle and the vasa recta. Fluid separated out by a second centrifugation of the papillae at 2000 *g* for 10 min is believed to be derived from the interstitial space. Samples of interstitial fluid were then analysed using radial immunodiffusion with rat albumin and rat plasma as standards (Serotec, Oxford, UK).

RESULTS

Clearance of dyes from the renal medulla

Patent Blue V (PBV) and Evans Blue–albumin complex (T1824–BSA) were injected at constant pressure through a micropipette (tip diameter < 5 μm) into the tissue of the exposed papilla. The injection pressure was set a few centimetres of water above the local microvascular pressure (6–7 cmH_2O). In four animals where Patent Blue was used, the injected dye slowly spread in all directions through what appeared to be a fine network of channels. When the pressure in the micropipette was lowered sufficiently to halt the flow of dye into the tissue, or when the micropipette was removed, the dye cleared from the interstitium. There was no preferential movement of the dye towards the base of the medulla. In a further eleven animals where T1824–BSA was used, the injected dye was more localized and spread less rapidly. Once again there was no evidence for preferential movement of dye towards the base of the exposed medulla. After the pipette was removed the dye usually cleared from the tissue within 1 min.

When collecting ducts were perfused, no leakage of either PBV or T1824–BSA could be observed even after 10 min at 20 cmH_2O. Perfusion of the ducts was accompanied by the development of translucent spaces alongside and parallel to them. These spaces appeared to swell and shrink as the perfusion pressure was raised and lowered and reached a maximum width of 2–3 μm. Attempts to cannulate and perfuse the spaces with dye were unsuccessful.

By contrast, T1824–BSA leaked through the walls of perfused AVR within 1–2 min appearing to fill a fine ladder-like network in the interstitium alongside the vessel. This cleared within minutes when the perfusion was stopped.

Estimation of L_p in single perfused AVR

Measurements of fluid filtration and reabsorption rates were made at two or more perfusion pressures in thirty-two single AVR perfused with solutions containing BSA (10 mg ml^{-1}) and red cell ghosts filled with Evans Blue. The data from one of these experiments is shown in Fig. 3 as the relation between fluid filtration per unit area of vessel wall (J_v/A) and microvascular pressure (P_c). Two features, typical of the series of experiments are illustrated here.

The first and most obvious feature is the curvilinear nature of the relation between J_v/A and P_c. This was seen in most but not all the vessels which we investigated. The second feature is the high level of filtration with relatively modest increments in hydrostatic pressure. Because of the non-linearity of the relation, it is not possible to give a single value of L_p but in the region 13–15 cmH_2O, L_p would appear to be 200×10^{-7} cm s^{-1} cmH_2O^{-1} whereas in the region 9–11 cmH_2O it appears to be approximately 50×10^{-7} cm s^{-1} cmH_2O^{-1}. It is worth noting that the mean value of L_p for frog mesenteric capillaries is 5×10^{-7} cm s^{-1} cmH_2O^{-1}, i.e. an order of magnitude below the L_p of the AVR in the low pressure range.

The non-linearity of the J_v/A *vs.* P_c relation is present but less obvious when the data from all thirty-two experiments are pooled (Fig. 4). It approximates to a linear relation over the range of P_c from 0 to 12 cmH_2O.

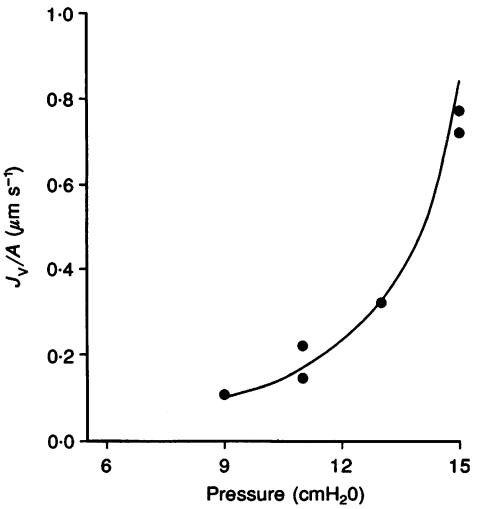

Figure 3. The relation between fluid filtration per unit area of vessel wall (J_v/A) and intraluminal hydrostatic pressure in a single AVR perfused with a solution containing BSA (10 mg ml^{-1})

The continuous curve has been fitted to the points by eye.

While J_v/A shows large variation between different vessels, the mean values of J_v/A in the range 9–15 cmH_2O yield an average L_p of 152×10^{-7} cm s^{-1} cmH_2O^{-1} whereas between 0 and 8 cmH_2O, L_p appears to be 80×10^{-7} cm s^{-1} cmH_2O^{-1}. It is seen that during perfusion with 1% BSA, fluid movements across the microvascular walls were always reversed when the intravascular pressure was reduced to atmospheric (0 cmH_2O). These results suggest that $J_v/A = 0$ when $P_c = 4{\cdot}0$ cmH_2O.

A further twenty-two experiments were carried out in which single AVR were perfused with solutions containing BSA (30 mg ml^{-1}) and Evans Blue-filled ghosts. Non-linear relations between J_v/A and P_c were found in these experiments also. The apparent L_p had a mean value of 148×10^{-7} cm s^{-1} cmH_2O^{-1} when P_c lay between 13 and 20 cmH_2O and 33×10^{-7} cm s^{-1} cmH_2O^{-1} when P_c was in the range 9–13 cmH_2O. The impression from individual experiments that the relation was shifted by a few centimetres of water to a higher pressure was confirmed by a statistical evaluation of the pooled data. Measurements were not made at P_c lower than 9 cmH_2O in these experiments but the value of P_c at which $J_v/A = 0$ was determined by linear extrapolation and found to be 8·64 cmH_2O (Fig. 6). This is significantly higher than the value found for 10 mg ml^{-1} BSA at $P < 0{\cdot}02$. The slopes of the regression lines describing the relationship between J_v/A and P_c are significantly different at $P < 0{\cdot}001$ for 10 and 30 mg ml^{-1} BSA.

Estimates of L_p by densitometry during fluid reabsorption

Figure 5 shows results from an experiment where a single AVR was perfused initially for 1 min with a solution containing Evans Blue–albumin (50 mg ml^{-1} BSA). At zero time the vessel was occluded 400 μm downstream from the pipette tip with the pressure in the pipette maintained at 20 cmH_2O. At 5 s, the pressure was lowered to −2 cmH_2O and the dye in the capillary and in the pipette tip became progressively paler as the perfusate was diluted by fluid reabsorbed through the wall of the vessel.

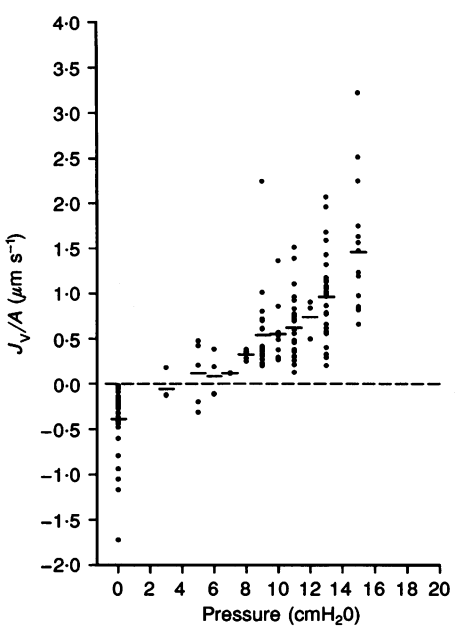

Figure 4. Values for fluid filtration and reabsorption per unit area (J_v/A) and microvascular pressure (P_c) from 32 AVR perfused at two or more pressures

All vessels were perfused with solutions containing BSA at a concentration of 10 mg ml^{-1}. Over the range of pressures between 0 and 10 cmH_2O, the relation approximates to a straight line, with reabsorption occurring when P_c is less than 4 cmH_2O. Note that the horizontal bars represent the mean of J_v/A at each pressure and that not all 32 points are visible on the graph due to superimposition of points.

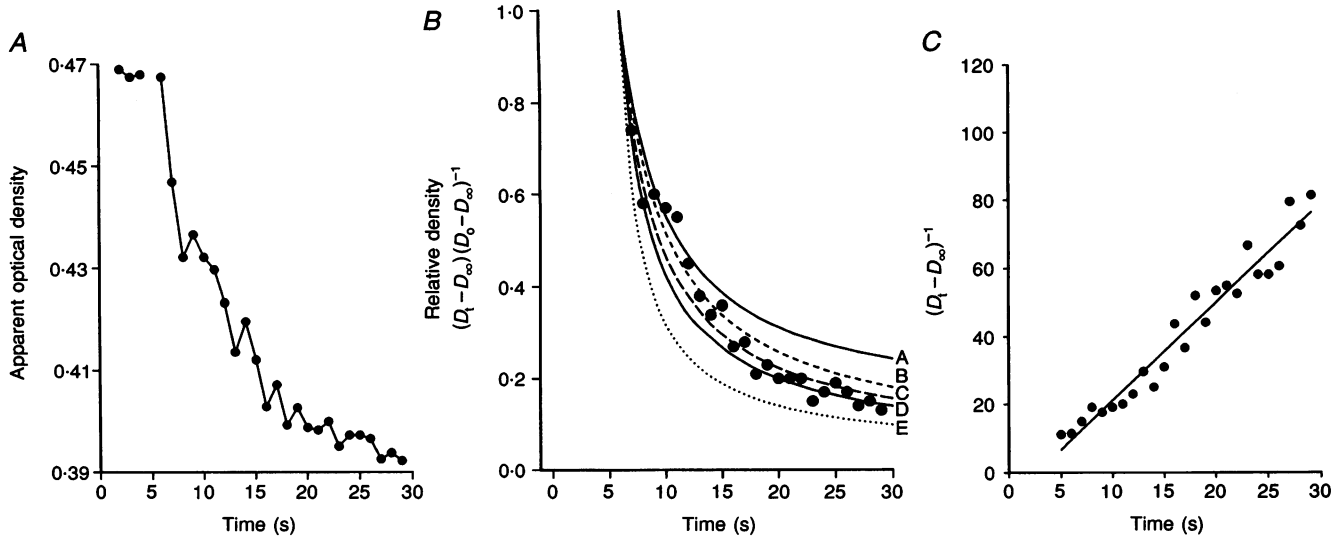

Figure 5. Densitometric method for estimating fluid reabsorption rates and σL_p

A, changes in apparent optical density of Evans Blue–albumin solution entering a micropipette as a result of fluid absorption into an occluded segment of AVR. Initially a pressure of 15 cmH_2O was applied to the micropipette but this was lowered abruptly to −2 cmH_2O at 5 s. *B*, same data as in *A* re-expressed as the relative changes in optical density and compared with curves predicted for different values of L_p, σ and π_i. Thus, with L_p in units of 10^{-7} cm s^{-1} cmH_2O^{-1} and π_i in cmH_2O, for curve A, $L_p = 80$, $\sigma = 0{\cdot}7$, $\pi_i = 5{\cdot}8$; for curve B, $L_p = 80$, $\sigma = 0{\cdot}7$, $\pi = 3{\cdot}76$; for curve C, $L_p = 100$, $\sigma = 0{\cdot}7$, $\pi_i = 3{\cdot}76$; for curve D, $L_p = 120$, $\sigma = 0{\cdot}7$, $\pi_i = 3.76$; for curve E, $L_p = 200$, $\sigma = 1{\cdot}0$, $\pi = 3{\cdot}76$. *C*, same data as in *A* plotted as the reciprocal relation given in eqn (7), the slope of which is L_p.

The ordinate in Fig. 5*A* is the negative logarithm of the light intensity passing through a small region of the micropipette close to its tip and varies with the concentration of dye in the pipette. It is seen that the concentration of dye in the fluid entering the micropipette fell rapidly at first and then after 5 s slowed to approach an asymptote, equivalent to a density between 0·38 and 0·39 (D_∞).

Figure 5*B* and *C* shows the same data replotted to estimate values for L_p and σ of the vessel which are consistent with the time course of the fall in *D*.

In Fig. 5*B*, the ratio $(D_t - D_\infty)/(D_0 - D_\infty)$ has been plotted against time and compared with that calculated from eqns (5) and (6) using different values for σ, L_p and π_i. It is seen that values of 0·7, 80–100 × 10^{-7} cm s^{-1} cmH_2O^{-1}

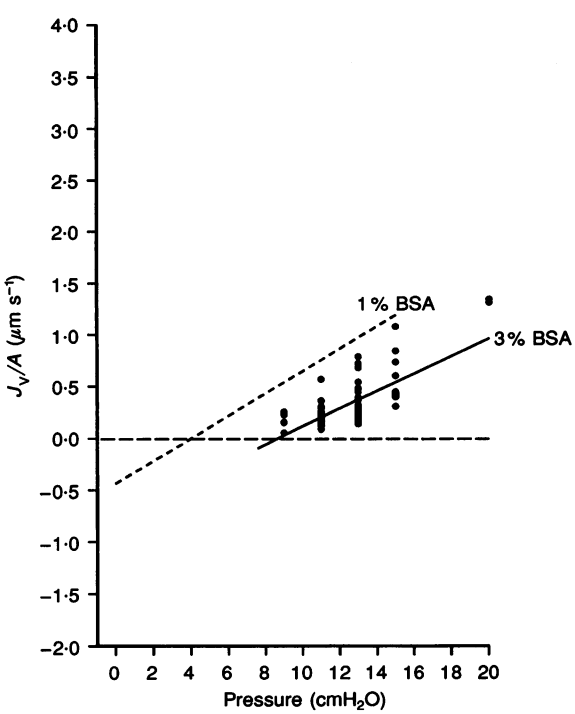

Figure 6. Measurements of fluid filtration per unit area of vessel wall (J_v/A) and the microvascular pressure pooled from 22 AVR perfused with solutions containing BSA (30 mg ml^{-1})

The regression line through the data (continuous line) may be compared with the regression line (dashed line) which fitted the relation for the lower microvascular pressures used when vessels were perfused with solution containing 10 mg ml^{-1} (as in Fig. 4). The regression lines intersect the zero flux line ($J_v/A = 0$) at 4·01 cmH_2O (10 mg ml^{-1}) and 8·64 cmH_2O (30 mg ml^{-1}). Note that not all data points are visible owing to superimposition.

and 3·75 cmH_2O for σ, L_p and π_i, respectively, provide a reasonable fit for the data. Figure 5*C* demonstrates the alternative analysis in which the reciprocal of $D_t - D_\infty$ is plotted against time. The slope of this relation yields a value for $L_p\sigma$ of 52×10^{-7} cm s^{-1} cmH_2O^{-1}. Thus if $\sigma = 1$, $L_p = 52 \times 10^{-7}$ cm s^{-1} cmH_2O^{-1} whereas if $\sigma = 0{\cdot}7$ (see below), L_p has a minimum value of 74×10^{-7} cm s^{-1} cmH_2O^{-1}. The value of $L_p\sigma$ estimated from changes in optical density during fluid reabsorption is $62 \pm 2 \times 10^{-7}$ cm s^{-1} cmH_2O^{-1} (mean ± S.E.M.; $n = 14$).

Estimation of σ to serum albumin

Figure 6 summarizes the measurements of J_v/A and P_c in AVR perfused with solutions containing BSA at concentrations of 10 and 30 mg ml^{-1}. Although there is considerable scatter, the regression line for the data from 30 mg BSA ml^{-1} lies significantly to the right of that made during perfusion with 10 mg BSA ml^{-1}. The regression line intersects the P_c axis at values of 8·64 cmH_2O (P_{30}) during perfusion with 30 mg BSA ml^{-1} and 4·01 cmH_2O (P_{10}) during perfusion with 10 mg BSA ml^{-1}. Each of these intercepts (P_{30} and P_{10}) represents the sum of the interstitial hydrostatic pressure (P_i) and the effective oncotic pressure difference across the vessel walls, $(\sigma\pi_p - \sigma\pi_i)$. If we assume that P_i is not affected by the different BSA concentrations in the perfusate, the difference between the intercepts $(P_{30} - P_{10})$ is the mean difference in effective oncotic pressure exerted across the vessel walls by the two perfusates. It has been argued that the effective oncotic pressure difference across microvascular walls has a maximum value of $\sigma^2\pi_p$ (Michel, 1984; Michel & Phillips, 1987). On this basis the ratio of the effective oncotic pressure to the perfusate oncotic pressure yields a minimum value for σ^2. Thus from the data in Fig. 6, σ to BSA may be estimated as:

$$\sigma = \sqrt{\left(\frac{P_{30} - P_{10}}{\pi_p - \pi_i}\right)} = \sqrt{\left(\frac{8{\cdot}64 - 4{\cdot}01}{12{\cdot}81 - 3{\cdot}76}\right)} = 0{\cdot}715, \quad (8)$$

where π_{30} (12·81 cmH_2O) and π_{10} (3·76 cmH_2O) are the oncotic pressures of perfusates containing 30 mg BSA ml^{-1} and 10 mg BSA ml^{-1}, respectively, calculated from Moyses's (1986) modification of the equation of Landis & Pappenheimer (1963).

In a separate set of experiments, $\sigma\Delta\pi$ was estimated from densitometric measurements made during fluid filtration from an AVR perfused with a solution containing 5% BSA complexed with Evans Blue. Figure 7 shows the changes in density from one of these experiments before and after a step increase in pressure from 15 to 25 cmH_2O. The dashed lines S_1 and S_2 are the rates of changes of D when P_c is at 15 and 25 cmH_2O, respectively. S_1 and S_2 are proportional to J_v/A at these pressures. By extrapolating the values of dD/dt to the pressure at which $dD/dt = 0$, one obtains an estimate of P_c at $J_v/A = 0$ and hence of $\sigma(\pi_p - \pi_i) + P_i$. The value of P_c at $J_v/A = 0$ was $12{\cdot}7 \pm 0{\cdot}8$ cmH_2O (mean ± S.E.M.) during perfusion with 50 mg ml^{-1} BSA. Estimates of σ were then made by the method outlined above and yielded a mean value of 0·718.

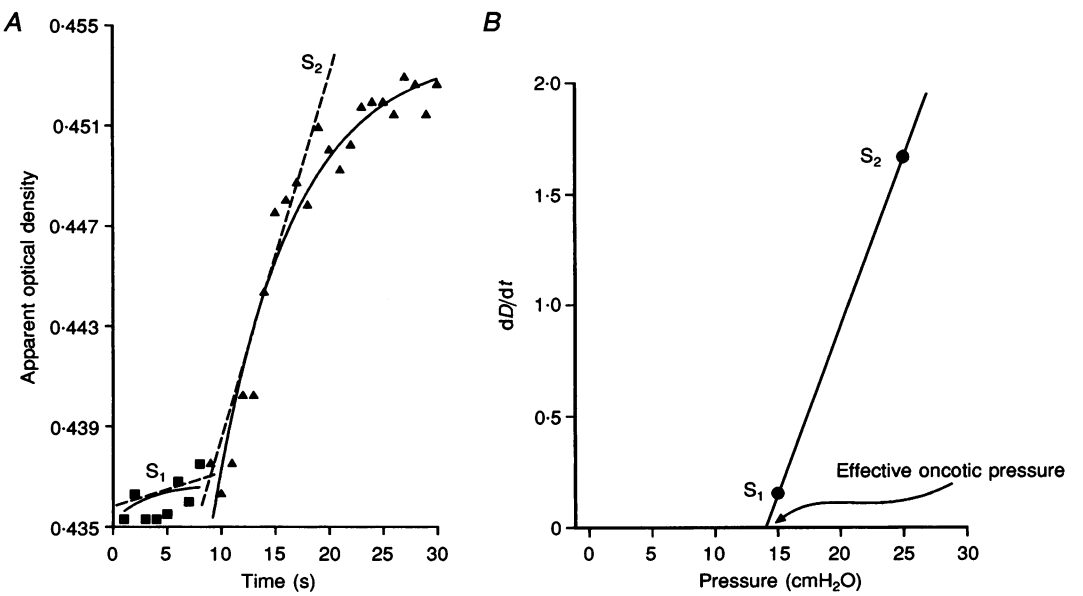

Figure 7. Estimation of the effective oncotic pressure of a perfusate containing Evans Blue–albumin (BSA, 50 mg ml^{-1}) across the walls of a single perfused AVR

A, changes in apparent optical density before and after a step increase in microvascular pressure from 15 to 25 cmH_2O. S_1 and S_2 have slopes which are proportional to fluid filtration before and after the pressure change. *B*, the values of S_1 and S_2 plotted against microvascular pressure to yield a value of effective oncotic pressure of 14 cmH_2O.

By comparing this intercept (P_{50}) with those obtained by the red cell technique (P_{30} and P_{10}), two further estimates of σ to BSA were made using equivalent forms of eqn (8). With π for perfusates containing BSA (50 mg ml^{-1}) as 24·6 cmH_2O, the difference between P_{50} and P_{30} yielded a value for σ of 0·59 while that between P_{50} and P_{10} gave a value of 0·64.

Overall these calculations reveal that the walls of the AVR have a σ to BSA which has a minimal value of 0·6.

Concentration of serum albumin in the medullary interstitial fluid

A total of sixteen papilla from nine rats were studied. The albumin concentration was 0·91 ± 0·06 g (100 ml)$^{-1}$ (mean ± S.E.M.) in the fluid from the second centrifugation. The range of values were 0·58–1·28 g. The concentration of albumin in rat plasma was 3·5 ± 0·36 g (100 ml)$^{-1}$ (mean ± S.E.M.). Thus, the interstitial concentration of albumin is approximately a quarter of the albumin concentration in the plasma.

DISCUSSION

Observations of the clearance of Evans Blue–albumin complex

The clearance of both Patent Blue V and Evans Blue–albumin after their injection into the exposed medulla suggests that there is a mechanism for rapidly diluting the dyes or transporting them out of the interstitial fluid (ISF), even in the absence of the normal rhythmic contractions of the pelvis of the ureter. We cannot eliminate the possibility of the dye–albumin complex leaking across the papillary interstitium. This could occur either through the hole made by the micropipette in those experiments where this was removed or directly through the epithelium. The first of these possibilities would not account for the disappearance of dye in those experiments when the micropipette was left in place and the second seems unlikely as it would imply a steady loss of protein into the urine *in vivo*. There was, however, no suggestion of net movement of these dyes towards the base of the medulla such as would be consistent with drainage via lymphatics or pre-lymphatic channels. Our observations on the exposed surface of the inner medulla are consistent, therefore, with reports that lymphatics are absent from the papilla. It is possible, however, that the clearance of fluid from the medulla may be different in the intact kidney. Schmidt-Nielsen (1987) has proposed that contractions of the renal pelvis around the papilla may propel interstitial fluid through prelymphatic channels towards the cortex. We have observed translucent regions around the collecting ducts which enlarge under pressure. While these translucent zones are consistent with pre-lymphatic channels, another interpretation would be that these zones represent enlarged and hypodense collecting-duct epithelium resulting from the rapid accumulation of fluid within these cells.

Estimations of L_p of the AVR

The most important finding reported in this paper is that the L_p of the AVR is high. This means that the forces responsible for clearing fluid from the renal medulla may be quite small. While we have observed that, in individual vessels, the increase in fluid filtration with microvascular pressure is greater at higher than at lower pressures, our values for L_p of between 30×10^{-7} and 180×10^{-7} cm s^{-1} cmH_2O^{-1} comfortably encompass the range of L_p (92×10^{-7}–138×10^{-7} cm s^{-1} cmH_2O^{-1}) reported for perfused AVR in Munich Wistar rats by Pallone (1991*a*). Furthermore, the mean value for the slope of the regression line through our pooled data for perfusion with 1% BSA over the P_c range of 0–12 cmH_2O is 92×10^{-7} cm s^{-1} cmH_2O^{-1} (Fig. 4) and this is identical to the slope of the regression line through Pallone's (1991*a*) data when these are expressed in the same units. Pallone (1991*a*) perfused AVR with 0·1% BSA over a range of pressures from 0 to 27 cmH_2O. He estimated fluid filtration from the rise in concentration of an impermeant fluorescent dextran which was present in his perfusate and collected in a second micropipette at a known pressure. To obtain an adequate sample of the solution leaving the vessel, it was necessary to perfuse for several minutes. Pallone (1991*a*) suggested that the high rates of filtration maintained throughout a perfusion would raise P_i and so reduce the pressure drop across the vessel wall. On this basis, he regarded his measured value of L_p as a minimum and the range of values of L_p which he cites is calculated on the assumption that P_i may rise in proportion to P_c, with mean P_i having a maximum value of one-third of P_c. It is also worth noting that he pretreated his rats with furosemide so as to minimize gradients of small solutes in the medulla. In spite of these differences in techniques, the agreement between Pallone's (1991*a*) observations and our own is good.

There were some differences, however, between our observations and those of Pallone (1991*a*). We observed a non-linear relation between fluid filtration and P_c, and even our pooled data from perfusions with 1% BSA and 3% BSA show that J_v/A increases more with P_c when P_c is high. Non-linearity between J_v/A and P_c is not indicated in the pooled data of Pallone (1991*a*) even though he investigated J_v/A at values of P_c as high as 27 cmH_2O (20 mmHg).

We have considered two possible hypotheses to account for the non-linear relation between J_v/A and P_c. Our first hypothesis is that it represents true non-linearity of hydraulic conductance of the walls of the AVR. The failure of Pallone (1991*a*) to observe an increase of L_p with increases in P_c could be accounted for if, in his experiments, the rise in P_i with filtration was relatively higher at higher filtration rates. This would attenuate the high filtration rates and tend to 'linearize' the relation between J_v/A and P_c in the steady state. Our second hypothesis is that the non-linearity between J_v/A and P_c reflects adjustments of

the interstitial oncotic pressure towards a new steady-state value (Michel, 1984; Michel & Phillips, 1987). For this to be so, it must be argued that the adjustments occur more rapidly around AVR than around continuous capillaries (such as those of the mesentery) because of the high L_p of the AVR.

This second hypothesis can be examined more closely because a theoretical analysis of steady-state filtration (Michel, 1984; Michel & Phillips, 1987) makes three predictions which are relevant to the interpretation of our data. The first is that the L_p is represented by the slope of the relation between J_v/A and P_c, only when J_v/A is high. This would imply that L_p of the AVR has a value in the range of 200×10^{-7} rather than 100×10^{-7} cm s^{-1} cmH_2O^{-1}.

The second prediction is that, at low J_v/A, changes in P_c lead to transient changes in J_v/A which are consistent with values of L_p much greater than suggested by steady-state relations between J_v/A and P_c. The experiments, in which fluid reabsorption into AVR was detected densitometrically, yielded values for J_v/A which were greater than those extrapolated from the steady-state relations and are consistent with a moderately high L_p (62×10^{-7} cm s^{-1} cmH_2O^{-1}).

A third prediction of the steady-state theory is that, over a given range of P_c, the slope of the relation between J_v/A and P_c falls as the oncotic pressure of the perfusate is increased. Our observations are consistent with this prediction also; the slope of the relation between J_v/A and P_c was lower during perfusions with 30 mg BSA ml^{-1} than with 10 mg BSA ml^{-1} (Fig. 6).

Taken together these arguments suggest that the non-linearity of the relations between J_v/A and P_c are the result, at least in part, of rapid changes in the interstitial oncotic pressures surrounding the AVR as they adjust to new steady-state values of filtration and reabsorption.

Estimates of σ to serum albumin

According to eqn (8), our estimates of the effective oncotic pressure exerted by BSA solutions across the walls of the AVR are consistent with a value of σ to BSA of between 0·59 and 0·72. Equation (8) assumes the interstitial concentration of BSA can be calculated from the perfusate concentration (C_p) through the relation $(1 - \sigma)\ C_p$ under conditions of steady-state filtration. The assumption is reasonable when J_v/A is high and the protein concentration of the pericapillary space is determined primarily by the composition of the filtrate from the perfused vessel. The assumption is likely to underestimate the interstitial concentration of BSA particularly when J_v/A is low and diffusion makes a larger contribution to transport of BSA through the vessel wall. This will be particularly true where many vasa recta run close to each other. Under these conditions the microenvironment of the perfused vessel, in which J_v/A is low, will be dominated by diffusion of plasma protein from its neighbours. Thus, values in the range 0·6–0·7 are probably minimal for σ to serum albumin.

It is worth noting that Pallone (1992) arrived at a figure of 0·78 for σ to albumin from measurements of the net transport of radioactively labelled albumin from perfused AVR at different filtration rates from the perfusate into the medullary tissue. Just as our figure is a minimal value for σ, the same could be argued for Pallone's value since he neglected diffusion in his analysis of net albumin transport.

Estimates of renal interstitial albumin concentration

Our evidence from analysis of papillary interstitial fluid suggests that the albumin concentration is approximately one-quarter that of the plasma. We are not aware of other measurements with which we can make a direct comparison, though it is interesting that Boberg, Müller-Suur & Persson (1985) found the concentration of total protein in renal hilar lymph was 0·9–1·0 g $(100\ ml)^{-1}$ in Sprague–Dawley rats (i.e. 15–20% of the plasma levels). This is in spite of the fact that hilar lymphatics receive contributions from renal cortical vessels and have roots extending as far as the outer medulla (Cuttino *et al.* 1985, 1987).

In addition to albumin and small amounts of other plasma proteins, the medullary interstitial space is rich in the glycosaminoglycan hyaluronan (HA) (Pitcock, Lyons, Brown, Rightsel & Muirhead, 1988). Laurent & Ogston (1963) have shown that albumin in the presence of HA has an osmotic pressure greater than the sum of the two macromolecules alone (exclusion amplification). One implication of this is that the mean protein concentration may underestimate the oncotic pressure in the free fluid spaces of the interstitium.

The maintenance of low levels of protein suggests that albumin, which enters the interstitium from the vasa recta, must be cleared efficiently enough to maintain substantial gradients of oncotic pressure across the walls of the vasa recta. Clearance must be either by percolation through the interstitial space to the lymphatics near the cortico-medullary junction or by fluid entering the vasa recta during reabsorption. Possible mechanisms are considered in the next section.

Possible mechanisms for clearing (a) fluid and (b) protein from the renal medullary interstitium

Fluid

In addition to the conventional Starling forces (the hydrostatic and oncotic pressures), osmotic pressure differences set up by small solutes (e.g. NaCl and urea) have a potential role in determining fluid movements between the ISF and the microvessels of the renal medulla. Jamison and his colleagues (Pallone, Robertson & Jamison, 1990*a*)

Table 1. Starling forces and coefficients in the AVR of rats

Force or coefficient	Chosen value	Reported values (pressures in cmH_2O)	References
σ	0·65	0·59–0·71	Present paper
		0·78	Pallone (1992)
π_c	25	25	Sanjana *et al.* (1975); Brenner, Ueki & Daugharty (1972)
π_i*	5	†	Present paper
P_i	6	6	Garcia-Estañ & Roman (1989)
P_c	6	10·6	Sanjana *et al.* (1975)
		5–7	MacPhee & Michel (1992)

All pressures are expressed in cmH_2O. * π_i has been calculated here for a total protein concentration based on the proportion of albumin in plasma and so might be an overestimate since albumin represents a higher fraction of the ISF proteins. † Based on value for albumin concentration.

have drawn attention to the potential importance of the small solute gradients in concentrating the plasma flowing through the descending vasa recta (DVR). In the AVR, however, there is good reason for believing their influence will be small. Since the L_p of the AVR is four to five times greater than that of the DVR (Pallone, Work & Jamison, 1990*b*), it is reasonable to conclude that the permeability of AVR to small hydrophilic solutes is similarly greater (Michel, 1985) so promoting the rapid dissipation of differences of concentration of small solutes across their walls. Thus, it is more likely that fluid uptake into the AVR is dominated by conventional Starling forces. Two calculations suggest they are sufficient to account for clearance of fluid from the interstitium.

(i) To assess the direction and magnitude of the net Starling force (SF), it is convenient to express it as:

$$SF = \sigma(\pi_c - \pi_i) + P_i - P_c. \quad (9)$$

When evaluated in this form, positive values of SF indicate that it is directed from the ISF to AVR, i.e. favouring fluid uptake.

Table 1 lists the range of values for the component Starling forces and for the reflection coefficient to albumin. It also gives the values which have been chosen to be substituted in eqn (9). The resulting value of SF is +13 cmH_2O.

While there is uncertainty about some of the values chosen (e.g. π_i could be greater due to exclusion amplification by interstitial macromolecules), it is unlikely that they will differ sufficiently to reverse the sign of SF. Our observation, that a pressure of approximately 15 cmH_2O was necessary to prevent fluid uptake into a closed off section of AVR filled with Evans Blue–albumin solution, suggests that when P_c is in the physiological range of 5–10 cmH_2O, SF favours fluid uptake with a pressure of 5–10 cmH_2O.

It is worth noting that, even in the absence of an oncotic pressure difference across the walls of the AVR, fluid could be driven into the vessels by a hydrostatic pressure difference. Values for P_i reported in the literature are similar to those for P_c (Table 1). If fluid were to accumulate in the medullary interstitium, P_i would be expected to increase and it could rise to at least 3 cmH_2O above P_c without the vessel collapsing (MacPhee & Michel, 1992). With the high value of L_p for the AVR, a relatively small hydrostatic pressure difference such as this could be responsible for a substantial flux of fluid from the ISF into the vessels.

(ii) If we take the minimal value for SF of 5 cmH_2O, a second calculation reveals that this is more than enough to account for fluid uptake by the medullary capillaries and AVR in the medulla. Since fluid uptake per unit area of AVR surface is the product of L_p and SF:

$$SF = \frac{(\text{Rate of fluid uptake from all the medullary AVR})}{(\text{Surface area of AVR}) \times L_p \text{ of AVR}}.$$

For the Munich Wistar rat, Holliger, Lemley, Schmitt, Thomas, Robertson & Jamison (1983) have estimated that fluid is cleared from the entire papilla via 2400 AVR at a rate of 4·9 μl min^{-1} ($\equiv 8{\cdot}2 \times 10^{-5}$ cm^3 s^{-1}). If the AVR are assumed to have a mean diameter of 20 μm and a minimal mean length of 2 mm, their total surface area is 3 cm^2. When these figures are combined with a value for L_p of 100×10^{-7} cm s^{-1} cmH_2O^{-1}, the value of SF necessary to account for fluid uptake is only 2·7 cmH_2O. Thus the calculated net Starling force of 13 cmH_2O across the walls of the AVR is nearly five times greater and our minimal estimate of 5 cmH_2O is twice as great as the value of SF required to account for fluid uptake from the medullary interstitium under conditions of antidiuresis.

Protein

Many observations over the past 35 years have established that proteins circulating in the plasma may enter the medullary interstitium (e.g. Lassen *et al.* 1958). Our findings that Evans Blue–albumin leaks through the walls of perfused AVR and that σ to albumin in these vessels lies in the range of 0·6–0·7 are consistent with this view. Furthermore, we have obtained evidence for protein clearance from the medulla. Not only have we observed the diffuse clearance of Evans Blue–albumin from medullary

tissue but our finding of a mean albumin concentration in the medullary ISF, which was one-quarter of that in the systemic plasma, reveals that leakage of albumin from the microcirculation is balanced by its continuous removal from the interstitium. In the absence of lymphatics from the inner medulla, the clearance of protein from the medullary ISF would seem to be restricted to three possible mechanisms: (i) proteolysis either by ectoenzymes or after uptake into cells (Földi & Casley-Smith, 1978); (ii) clearance to the outer medulla and cortex by percolation through the interstitium or via prelymphatic channels; (iii) clearance directly into the microcirculation.

(i) We know of no quantitative evidence to suggest that proteins are broken down or taken up at relatively high rates by the cells of the medulla. Therefore, we will consider it no further. There is a possibility that Evans Blue dissociates from the albumin and is secreted into the nephron, so giving the appearance of a decreasing protein concentration in the interstitium. This mechanism, however, would not account for the low concentration of protein measured in the ISF

(ii) Similarly, although we do not discount the possibility of drainage of medullary ISF via prelymphatic channels, we have not been able to obtain direct evidence for clearance by this route in the exposed medulla. We are ready to accept that if the presence of these channels can be confirmed, movement of fluid through them or through the interstitial space could be aided considerably by contractions of the pelvis of the ureter which rhythmically compress the medullary tissue in the intact kidney (Schmidt-Nielsen, 1987).

(iii) The third possibility is of protein clearance directly into the AVR or the medullary capillaries. Since this involves transporting protein from a lower concentration in the ISF to a higher concentration in the AVR (or capillaries), the rate of transport into the AVR has to exceed the rate of diffusion of protein out of the AVR. It is possible that this might be achieved by convective transport which could be driven by either hydrostatic pressure differences or oncotic pressure differences across the walls of the AVR.

Crucial for the potential role of hydrostatic pressure differences is our finding that superficial AVR can remain open when the pressure within them (P_c) is up to 4 cmH_2O below that of their surroundings (MacPhee & Michel, 1992). The presence of fine microvilli attaching the walls of deeper AVR to neighbouring DVR and loops of Henle (Takahashi-Iwanaga, 1991) suggests that, with support on all sides, these vessels may be able to remain open when P_i is even greater than 3 cmH_2O above P_c. Whereas there are some reports of medullary P_i being comparable to expected values of P_c even in the decapsulated kidney (Garcia-Estañ & Roman, 1989), there is no evidence at present that P_i exceeds P_c in the intact kidney. It is possible that P_i exceeds P_c transiently during contractions of the pelvis of the ureter (Schmidt-Nielsen, 1987) or as a result of expansion of the medullary ISF volume. Even if these periods of raised P_i are relatively brief, the high L_p of the AVR could ensure that significant quantities of fluid-containing protein are shifted from the ISF into these vessels.

A more continuous flow of fluid from the medullary ISF into the AVR is driven by differences in oncotic pressure across the vessel walls. If protein clearance were coupled to this osmotic flow, it would mean that protein was carried up its concentration gradient by the osmotic flow resulting from the same concentration gradient. A hypothesis of this kind has previously been suggested to account for fluid and protein uptake in the intestinal villi and the terminal lymphatics (Casley-Smith, 1971). With certain important restraints, the hypothesis does not contravene the principles of thermodynamics (Casley-Smith, 1975; Ogston & Michel, 1978) and it is considered in the Appendix to this paper. In most capillary beds, a mechanism of this kind is unlikely to be of quantitative significance for it would lead to the concentration of plasma proteins in the pericapillary spaces around sites of fluid uptake so diminishing the very oncotic pressure difference responsible for fluid uptake (Michel & Phillips, 1987; Levick, 1991). Special characteristics of the renal medulla, however, could favour this mechanism. First and perhaps most important, there is a continuous influx of protein-free fluid into the medullary ISF from the nephrons. This dilutes the interstitial proteins opposing the concentrating effect of fluid uptake into the AVR. A second important factor is that the AVR appear to have a lower σ to serum albumin (and presumably other plasma proteins) than the DVR (Pallone, 1992). As shown in the appendix, this difference in σ also reduces the interstitial protein concentration resulting from convective transport into the AVR.

Convective transport into the AVR is also favoured by the high L_p of these vessels. Not only does this minimize the oncotic pressure differences necessary to clear fluid and protein into the AVR but it means that small reversals of pressure across the AVR walls can contribute significantly to fluid uptake. If the compliance of the interstitial space of the medulla is low, it is possible that a relatively constant ISF volume is maintained by adaptation of P_i and π_i to favour fluid movements into the microcirculation. This conclusion is identical to that proposed by Aukland, Bogusky & Renkin (1994) in their recent analysis of fluid balance in the renal cortex.

Conclusion

Our values for the L_p and σ to serum albumin of perfused AVR are very similar to those obtained using a very different technique by Pallone (1991*a*, 1992). The high values for L_p of the AVR reveal that fluid uptake from the medullary ISF into these vessels can be maintained by conventional Starling forces of only 2–3 cmH_2O.

From our direct observations of dye–albumin clearance from the medullary tissue and our estimates of ISF albumin concentration, we deduce that proteins are continually cleared from the ISF of the inner medulla.

The high L_p of the AVR together with reflection coefficients to serum albumin of 0·7 favour high rates of reabsorption and could also promote the net transport of protein from the ISF into the blood. Further work is required to establish whether protein is cleared from the ISF of the inner medulla into the AVR by convection driven by oncotic or hydrostatic pressure differences. Once established it will be necessary to determine whether this mechanism can account for the total clearance of protein or whether a significant fraction of the protein reaches the outer medulla directly through the inner medullary interstitium.

APPENDIX

Convective transport of a solute by osmotic flow up its own concentration gradient

Three types of transport can occur spontaneously through a partially selective membrane ($1 > \sigma > 0$) which separates two solutions of the same solute at the same temperature and pressure but initially at different concentrations. Identifying the solutions as A and B and their concentrations as C_A and C_B, with initially $C_A < C_B$, the possible types of transport are: (a) net diffusion of solute from B to A; (b) a volume (osmotic) flow of water from A to B; and (c) convective transport of the solute coupled to the osmotic flow from A to B.

All three forms of transport will dissipate the concentration difference between C_A and C_B. While obvious in the cases of diffusion and osmotic flow, it is seen also to be the case for the convective transport of solute for, although the solute is carried from a region of low concentration, C_A, to a higher concentration, C_B, it travels in the osmotic flow at a concentration less than C_A. Thus, the osmotic flow still concentrates the more dilute solution, A, and dilutes the more concentrated solution, B. This is seen explicitly from the standard expression for convective transport. If $J_{V_{AB}}$ is the osmotic flow and σ_{AB} the reflection coefficient:

$$\text{Convective transport of solute} = J_V(1 - \sigma_{AB})C_A. \quad \text{(A1)}$$

Thus, solute is transported at a concentration of $(1 - \sigma_{AB})C_A$ which is less than C_A since $1 > \sigma_{AB} > 0$.

The net movement of solute through a particular membrane will be determined by the values of the membrane permeability coefficients, σ_{AB}, the hydraulic permeability ($L_{p_{AB}}$) and diffusional permeability to the solute (p_{AB}). Depending on the values of C_A and C_B and these coefficients it is possible for net movement of solute to occur either from A to B or from B to A.

Let us now consider an extended form of this system which is shown in Fig. A1. Here, solution A not only makes contact through a partially selective membrane with solution B (as before), but also receives a filtrate containing a low solute concentration through a second membrane from C which is maintained at a higher pressure than A and B. The concentration of solute in the filtrate entering A is $(1 - \sigma_{CA})C_C$ where σ_{CA} is the reflection coefficient of the membrane between C and A and C_C is the solute concentration in C. If C_C and C_B are held constant by continuous replenishment and if A is allowed to expand at constant pressure, a set of conditions is possible whereby the rate of entry of fluid into A from C, $J_{V_{CA}}$, is balanced by the osmotic flow from A to B:

$$J_{V_{CA}} = J_{V_{AB}} = L_{p_{AB}}\sigma_{AB}RT(C_B - C_A), \quad \text{(A2)}$$

where RT is the product of the gas constant and absolute temperature.

The balance of volume flows described by eqn (A2) will be a transient phenomenon unless solute influx into A from C can be balanced by an equal solute efflux from A to B. For simplicity, let us consider solute influx from C to A to be bought about by convection (filtration) alone while the convective efflux of solute from A to B has to exceed the diffusion of solute from B to A. Thus if influx of solute into A equals efflux of solute from A:

$$J_{V_{CA}}(1-\sigma_{CA})C_C = J_{V_{AB}}(1-\sigma_{AB})C_A - p_{AB}(C_B - C_A)\left(\frac{Pe}{e^{Pe}-1}\right), \quad \text{(A3)}$$

where p_{AB} is diffusional permeability of membrane AB to solute and Pe (Peclet number) is $J_{V_{AB}}(1 - \sigma_{AB})/p_{AB}$.

If C_C is expressed in terms of C_B, i.e. $C_C = \gamma C_B$ (where γ is a constant), eqn (A3) can be rearranged to yield:

$$\frac{C_A}{C_B} = \frac{J_{V_{CA}}}{J_{V_{AB}}}\frac{(1 - \sigma_{CA})\gamma + p_{AB}Y_{AB}}{(1 - \sigma_{AB}) + p_{AB}Y_{AB}}, \quad \text{(A4)}$$

where Y_{AB} is $Pe/(e^{Pe} - 1)$. Equation (A4) reveals that if $J_{V_{AB}} = J_{V_{CA}}$ and $C_B > C_A$, so as to maintain $J_{V_{AB}}$, γ must be less than the ratio of $(1 - \sigma_{AB})/(1 - \sigma_{CA})$.

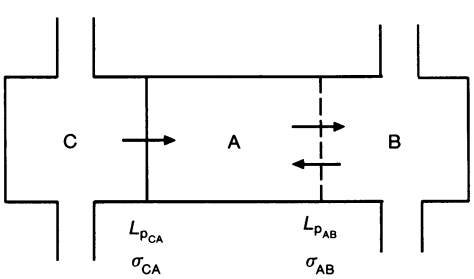

Figure A1. Diagram of a three compartment system in which a solution is ultrafiltered from C to A (through membrane with $L_{p_{CA}}$ and σ_{AB}) and cleared from A by an osmotic flow into B (through membrane with $L_{p_{AB}}$ and σ_{AB})

The concentrations in B and C are maintained at a constant level and initially the concentration in B is greater than the concentration in A.

This conclusion is relevant to fluid and protein transport across capillary walls. If C_C and C_B are kept constant, compartments C and B may be considered to be the arterial and venous ends, respectively, of a capillary while A is the intervening interstitium. Because the filtration fraction in most capillary beds is low, C_C should approximate to C_B ($\gamma \approx 1$). Thus, steady-state fluid absorption can balance net fluid filtration into the interstitium only if σ at the arterial end of the capillary (σ_{CA}) is sufficiently greater than σ at the venous end (σ_{AB}) to sustain the necessary difference in protein concentration across the walls of the venous capillaries ($C_B - C_A$). In the venules and capillaries, however, the osmotic pressure difference has to exceed the hydrostatic pressure difference between the plasma and the interstitium if fluid uptake is to proceed. Furthermore, unlike compartment A in Fig. A1, the interstitium is not a perfectly mixed compartment and the higher concentration of protein in the fluid surrounding venous capillaries is likely to bring fluid reabsorption here to a halt. Similar conclusions have resulted from different lines of inquiry where a single value of σ has been assumed (Michel, 1984; Michel & Phillips, 1987; Levick, 1991).

Let us now extend the system depicted in Fig. A1 by incorporating an input of pure water, J_{V_E}, into compartment A. Once again J_{V_E} and $J_{V_{CA}}$ can be adjusted so that the difference between C_A and C_B is sufficient for the resulting osmotic pressure difference to drive $J_{V_{AB}}$ (at least transiently) with a magnitude that balances total fluid input into A:

$$RT\sigma_{AB}L_{p_{AB}}(C_B - C_A) = J_{V_{AB}} = J_{V_{CA}} + J_{V_E}. \quad \text{(A5)}$$

Since solute is entering and leaving compartment A by the same routes as in Fig. A1, the conditions for an equality between solute influx and solute efflux are described by eqn (A3) and the relation between C_A and C_B is given by eqn (A4). The effect of J_{V_E} on solute exchange can be seen if eqn (A5) is used to substitute for $J_{V_{AB}}$ in eqn (A4):

$$\frac{C_A}{C_B} = \frac{J_{V_{CA}}(1-\sigma_{CA})\gamma + p_{AB}Y_{AB}}{(J_{V_{CA}} + J_{V_E})(1-\sigma_{AB}) + p_{AB}Y_{AB}}. \quad \text{(A6)}$$

If J_{V_E} is comparable in magnitude to $J_{V_{CA}}$, C_A will remain less than C_B even if $\sigma_{CA} = \sigma_{AB}$ and $\gamma = 1$. Thus, if the solute concentrations of compartments C and B are kept constant, it is possible for steady fluxes of fluid and solute to pass through A from E and C into B.

This extended system is analogous to the relations for fluid and protein transport through the interstitium of the inner medulla, between the DVR (compartment C), the AVR (compartment B), the medullary interstitium (compartment A) and the flux of protein-free fluid entering the interstitium from the loops of Henle and the collecting ducts, J_{V_E}. In the renal medulla, J_{V_E} has a minimal value which is equal to or more probably greater than $J_{V_{CA}}$ (the fluid flux from the DVR) and whereas σ_{AB} (AVR) has a value of 0·6–0·7 (Pallone, 1992 and this paper), σ_{CA} has a minimum value of 0·9 (Pallone, 1992). Even though the concentration of proteins in the DVR at the tip of the papilla may be 1·5 times greater than that in the AVR ($\gamma = 1{\cdot}5$; Pallone *et al.* 1990), C_A may be maintained well below C_B. Thus, in principle, fluid and protein may be cleared from the renal interstitium by osmotic flow into the AVR.

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