Albumin modulation of capillary permeability: test of an adsorption mechanism

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HUXLEY, V. H., AND F. E. CURRY. *Albumin modulation of capillary permeability: test of an adsorption mechanism.* Am. J. Physiol. 248 (Heart Circ. Physiol. 17): H264–H273, 1985.—We investigated further the mechanism whereby albumin interacts with the walls of capillary blood vessels to maintain normal permeability properties. In individually perfused capillaries of frog mesentery, hydraulic conductivity was measured as the albumin perfusate concentration was first reduced in three steps from 0.1 to 0 g/dl (intermediate concns 0.01 and 0.001 g/dl), then increased in up to four steps to 1 g/dl (intermediate concns 0.001, 0.01, and 0.1 g/dl). The albumin concentration required to return hydraulic conductivity toward control values, following Ringer perfusion, was at least an order of magnitude larger than that required to maintain permeability close to control values prior to Ringer perfusion. The experiments indicate that the affinity of albumin for binding sites on the capillary wall following Ringer perfusion is less than the albumin affinity for binding sites after the capillary has been perfused with solutions containing 0.1 g/dl albumin. Before the hysteresis of hydraulic conductivity on albumin concentration can be understood, the factors determining the transport of albumin into the membrane must be investigated further.

single capillary; hydraulic conductivity; albumin adsorption to endothelium

THE SERUM PROTEIN ALBUMIN plays an important role in the maintenance of normal permeability and selectivity properties of the walls of capillary blood vessels (7, 11, 14). Removal of albumin from the perfusate results in a fivefold increase in the hydraulic conductivity of the capillary wall and reduction in the effective osmotic pressure exerted by clinical dextrans used to replace albumin in the perfusate (6, 11). Factors that change the interaction of albumin with the capillary wall may play an important role in the modulation of capillary permeability. The purpose of this study was to investigate the hypothesis that albumin interaction with the capillary wall could be described in terms of a simple adsorption model.

Several investigators have noted that the removal of adsorbed albumin from the walls of a rectangular channel will result in an increase in hydraulic conductivity of the capillary similar in magnitude to that found experimentally (11, 14). Furthermore, it has been observed that plasma albumin concentration must be increased to greater than 0.1 g/dl to restore the permeability properties of the capillary following Ringer perfusion (9, 14). This observation suggests that there is a threshold of albumin concentration required to maintain normal permeability. In the adsorption model this threshold is taken as a measure of the affinity of albumin for the sites of adsorption on the wall.

To investigate further the interaction of albumin with the capillary wall, we have measured the hydraulic conductivity of capillary wall as the albumin concentration is first reduced from control levels to zero, then increased back to control levels. All experiments were carried out on individually perfused capillaries in frog mesentery. It has been possible to perfuse capillaries with up to five different perfusate compositions to carry out complete experiments on individual vessels. Our results demonstrate that the albumin concentration required to maintain normal hydraulic conductivity when albumin has always been present in the perfusate is much lower than that required to return hydraulic conductivity toward control values following Ringer perfusion. Analysis of this hysteresis phenomenon allows reevaluation of the adsorption model. A preliminary report of these experiments has been published (9).

METHODS

Measurement of hydraulic conductivity (L_p). The methods to perfuse single capillaries and measure the hydraulic conductivity have been described (2, 4, 17). All measurements were based on the modified Landis technique, which measures the volume flux of water across the capillary wall immediately following occlusion of the vessel. The method, assumptions concerning vessel wall compliance, red blood cells as flow markers, measurement of capillary pressure, cannulation patency, and other limitations of the measurement have been evaluated in detail by Curry et al. (3). The initial transcapillary water flow per unit area of the capillary wall $(J_v/S)_0$ was calculated from the initial cell velocity $(dl/dt)_0$, the capillary radius (r), and the distance of the marker cell from the point of occlusion (l)

$$(J_v/S)_0 = (dl/dt)_0\, r/(2l) \qquad (1)$$

$(dl/dt)_0$ was measured from the initial tangent of cell movement, drawn by eye to fit the first 1–2 s of the plot of l against t. Except where otherwise indicated (see *Experimental design* below), $(J_v/S)_0$ was measured at two or more pressures and L_p was determined from the slope of the relation between $(J_v/S)_0$ and pressure. The absolute value of L_p measured using red blood cells as flow

markers may overestimate the true value by 1.75-fold (3).

Preparation. All experiments were performed on the frog *Rana pipiens* (male 2.5–3 in.) supplied by J. M. Hazen, VT. The brain of the frog was destroyed by pithing while the spinal cord was left intact. The abdominal cavity was opened, the mesentery floated over a short Lucite pillar, and the gut supported on Ringer-soaked cotton. The tissue was continuously superfused with cooled frog-Ringer to maintain the temperature at the surface of the tissue at 15°C.

The vessels chosen for these studies were usually mid-capillaries (diam 15-30 μm) although occasionally arterial or venular capillaries (diam 25-40 μm) were used. In all cases the vessels were unbranched and long (700-1,400 μm), with brisk blood flow and free from white cell sticking or rolling along the vessel wall. Long vessels were chosen for these studies to accommodate repeated cannulations. The occlusion site was moved approximately 20 μm toward the micropipette after two or three occlusions (often more than 20 on each vessel). The majority of the experiments reported in this study were performed on frogs supplied in fall and winter.

Micropipettes. The experiments required multiple cannulations of a single vessel. The ability to perform repeated cannulations was facilitated by individual classification of each micropipette. The pipettes were drawn from 1.50-mm OD borosilicate glass (WPI) in a micropipette puller (David Kopf). Each pipette was beveled to a tip diameter of 10–14 μm using an air-driven grindstone fitted with Imperial lapping film (3M, 0.5 μm chromium oxide on Mylar backing). After this procedure a scale drawing of each pipette was made detailing the width, shape, and length of the beveled tip. At the time of the experiment it was then possible to select a matched set of pipettes that best fitted the bore of the vessel under study.

Solutions and red cell suspensions. A stock solution of frog Ringer was prepared daily and used for the dissection of the mesentery, superfusion of the tissue, and preparation of the perfusion solutions. The composition of the frog Ringer solution (in mM) was NaCl 111, KCl 2.4, $MgSO_4$ 1.0, $CaCl_2$ 1.1, $NaHCO_3$ 0.195, glucose 5.5, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and Na-HEPES 5.0. All chemicals were purchased from Mallinckrodt except for Na-HEPES and HEPES acid (Research Organics). The pH of the frog Ringer at 15°C was pH 7.4 and determined by the ratio of Na-HEPES to HEPES.

The protein solutions for perfusion were prepared by dilution of a freshly prepared stock solution of 1 g/dl bovine serum albumin (BSA, Sigma A4378) by frog Ringer to 0.1, 0.01, or 0.001 g/dl BSA. The pH of the solutions was adjusted to 7.4 ± 0.05 at 15°C. Perfusate solutions containing 1, 0.1, 0.01, 0.001 g/dl albumin or Ringer were filtered through 0.2-μm pore diameter Metricel membranes (Gelman) and then refrigerated until use.

A small number of frog Ringer washed, buffy-coat-free human red blood cells was suspended in each of the perfusion solutions as flow markers. The superfusate contained frog Ringer alone.

Experimental design. In the first set of six determinations of L_p, at differing perfusate albumin concentrations, $(J_v/S)_0$ was measured at least four times at each of the pressures above 20 cmH_2O. In an additional eight capillaries, during the course of this study we checked for linearity. After determining that $(J_v/S)_0$ was indeed a linear function of capillary pressure (ΔP), we measured $(J_v/S)_0$ for protein perfusion concentrations of 0.1 g/dl or less only at 30 cmH_2O. L_p was calculated as $(J_v/S)_0/\Delta P$ because the osmotic pressure of perfusates containing 0.1 g/dl albumin is less than 0.3 cmH_2O. This abbreviated procedure was instituted to shorten the total length of time of the experiment.

Care was taken, especially for the determination of L_p before removal and after replacement of perfusion albumin, to make measurements of $(J_v/S)_0$ at several points along the length of the vessel. The video camera (Dage-MTI) records cell movement over 240 μm of vessel at any one time, although the experimenter views the entire vessel length, pipette, and occluder. The whole microscope stage on our setup was attached to an an *X-Y* drive that enabled cell movement at different portions of the vessel to be recorded (3). L_p at each point along the capillary is reported as the value for the section of the capillary wall between the initial position of the marker cell and the site of occlusion (l_0). In this way, we could compare measurements from each experimental condition on a common length of capillary.

RESULTS

Figure 1 shows an experiment to determine the dependence of L_p on the perfusate albumin concentration. The capillary was perfused first from Ringer solution containing albumin at a concentration of 0.1 g/dl. L_p, measured from the mean slope of the relation between transcapillary water flux (J_v/S) and capillary pressure (ΔP), was 1.3×10^{-7} $cm \cdot s^{-1} \cdot cmH_2O^{-1}$. The capillary was recannulated with a Ringer perfusate containing 0.01 g/dl albumin and L_p redetermined as 1.8×10^{-7} $cm \cdot s^{-1} \cdot cmH_2O^{-1}$. Finally the capillary was perfused with frog Ringer containing no albumin. The L_p of the vessel increased to 9.2×10^{-7} $cm \cdot s^{-1} \cdot cmH_2O^{-1}$. The same sequence of albumin perfusate concentrations was tested in four additional capillaries with similar results. The filled circles joined by a solid line in Fig. 2 are the mean value of the ratio $L_p/L_{p\,Ringer}$ measured in each capillary. Measured L_p's for each capillary are given in Table 1.

In another five experiments the albumin concentration in the perfusate was reduced first from 0.1 to 0.001 g/dl and then to zero. The data are summarized in Table 2, and mean values are plotted as open circles joined by a broken line in Fig. 2. The results in Figs. 1 and 2 for albumin depletion experiments do not conform to the hypothesis that there is a threshold of albumin concentration between 0.01 and 0.1 g/dl below which albumin no longer interacts with the capillary wall to maintain L_p. Instead of a threshold of albumin concentration indicative of an "all-or-none" effect, there appears to be a graded increase in hydraulic conductivity as albumin

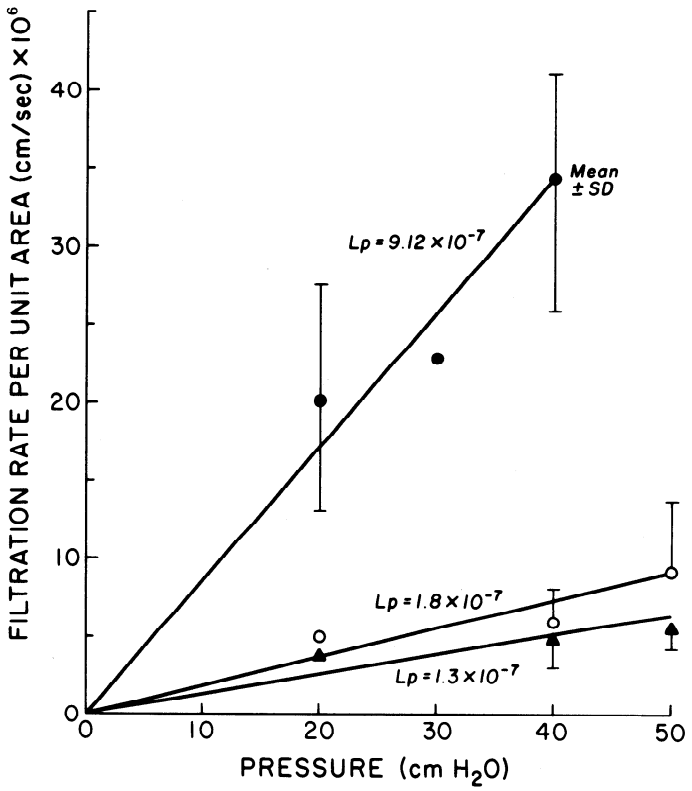

FIG. 1. Measurement of transcapillary water flux (J_v/S) as a function of pressure at 3 albumin concentrations on a single capillary. Vessel was first perfused with 0.1 g/dl bovine serum albumin (BSA) in frog Ringer (▲) and J_v/S was measured at each of 3 pressures. Mean slope of relation between J_v/S and capillary pressure is the filtration coefficient 1.3×10^{-7} cm·s^{-1}·cmH$_2$O^{-1} at 15°C. Vessel was perfused with 0.01 g/dl BSA (○); hydraulic conductivity (L_p) rose to 1.8×10^{-7} cm·s^{-1}·cmH$_2$O^{-1}. Finally vessel was perfused with Ringer solution alone (●); L_p was 9.12×10^{-7} cm·s^{-1}·cmH$_2$O^{-1}.

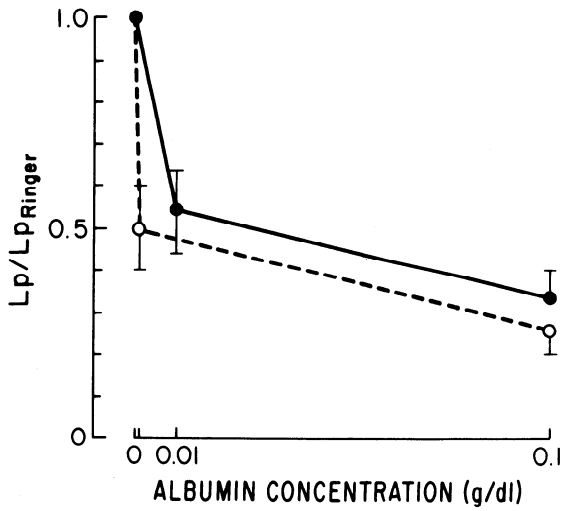

FIG. 2. L_p as a function of decreasing albumin concentration. Ratio $L_p/L_{p\,\text{Ringer}}$ is plotted against albumin concentration in perfusate. *Solid line* connects mean values (±SE) from the group of 5 vessels perfused successively with 0.1, 0.01, and 0 g/dl bovine serum albumin (BSA) (Table 1). *Broken line* connects mean values from a 2nd group of 5 vessels perfused successively with 0.1, 0.001, and 0 g/dl albumin (Table 2). Largest increase in L_p occurred when concentration of albumin was reduced below 0.001 g/dl BSA.

TABLE 1. *Measurement of L_p when perfusate albumin is decreased from 0.1 to 0.01 and finally 0 g/dl*

	Perfusate Albumin, g/dl		
	$L_p^{0.1} \times 10^7$	$L_p^{0.01} \times 10^7$	$L_p^{\text{Ringer}} \times 10^7$
3-25-81	1.85 ± 0.74	4.58 ± 0.71	7.82 ± 2.16
4-01-81	1.28 ± 0.55	1.80 ± 0.85	9.12 ± 2.94
	3.51 ± 0.87	8.88 ± 1.78	11.81 ± 2.18
7-03-81	6.29 ± 1.10	5.43 ± 3.30	11.30 ± 5.90
	3.07 ± 1.10	4.73 ± 2.50	6.78 ± 2.45

Values are means ± SD. Hydraulic conductivity (L_p) measured in cm·s^{-1}·cmH$_2$O^{-1}.

TABLE 2. *Measurement of L_p when perfusate albumin is decreased from 0.1 to 0.001 and finally 0 g/dl*

	Perfusate Albumin, g/dl		
	$L_p^{0.1} \times 10^7$	$L_p^{0.001} \times 10^7$	$L_p^{\text{Ringer}} \times 10^7$
4-01-81	1.28 ± 0.55	7.68 ± 2.64	9.12 ± 2.94
8-19-81	2.85 ± 0.51	3.17 ± 1.45	9.56 ± 5.49
8-26-81	25.74 ± 7.54	22.18 ± 8.17	68.90 ± 11.34
8-27-81	1.46 ± 0.42	5.82 ± 2.21	18.59 ± 1.92
9-01-81	0.87 ± 0.41	1.44 ± 0.15	2.13 ± 0.72

Values are means ± SD. Hydraulic conductivity (L_p) measured in cm·s^{-1}·cmH$_2$O^{-1}.

concentration is reduced from 0.1 g/dl. This result was unexpected because Mason et al. (14) found that albumin at a concentration of 0.01 g/dl failed to return L_p toward control values following perfusion of a single frog mesentery capillary with Ringer solution.

Taken together, the results in Fig. 2 and the experiments of Mason et al. (14) suggested that the dependence of L_p on albumin concentration as albumin was removed from the perfusate was different from that expected as albumin was replaced in the perfusate after Ringer perfusion. The experiment shown in Fig. 3 shows that this was indeed the case: there was hysteresis in the return of L_p toward control values after Ringer perfusion. In this experiment the albumin concentration in the perfusate was first reduced in two steps (0.1–0.001, 0.001–0 g/dl) and then increased in two steps (0–0.01, 0.01–0.1 g/dl). Five separate cannulations of the capillary were required for this experiment.

The results from 10 experiments in which L_p was measured using the same experimental protocol are given in Table 3. Figure 4 is the hysteresis curve formed by plotting mean values of the ratio $L_p/L_{p\,\text{Ringer}}$ at each concentration. In five additional vessels we measured capillary L_p when the albumin concentration was 0.001 g/dl following Ringer perfusion. L_p's were not significantly reduced from their Ringer controls (Table 4). The mean value of $L_p/L_{p\,\text{Ringer}}$, 1.034 ± 0.457 (SD), is plotted on Fig. 4 for comparison with values at the same albumin concentration during albumin depletion.

A hysteresis phenomenon may result from a specific restructuring of the resistance elements in the principal pathways for water flow after Ringer perfusion. An analysis of these results should provide new information about the physical and chemical nature of the water

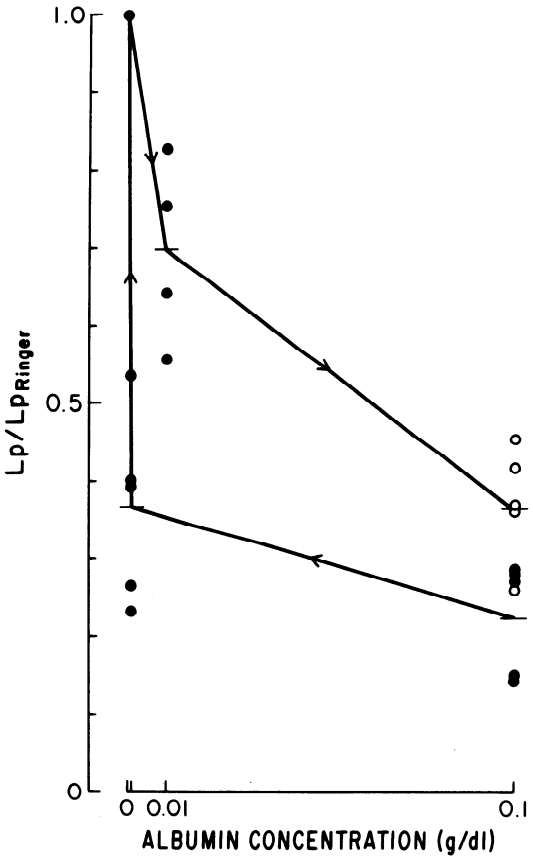

FIG. 3. Five separate cannulations of a single capillary to demonstrate hysteresis. Individual values of hydraulic conductivity (L_p) at each albumin concentration are expressed relative to mean value of L_p with Ringer perfusion. *Solid line* connects mean value of each group of determinations. *Arrows* on connecting lines show sequence of the experiment. *Open circles* represent perfusion with 0.1 g/dl bovine serum albumin following Ringer perfusion.

pathway. However, it is possible that L_p's measured following Ringer perfusion were high due to factors other than a change in the principal water pathway.

One possibility was the development of a small number of very leaky sites not previously present in the capillary wall. Another possibility was that the return of L_p to control values was delayed in time. The following experiments were designed to investigate these possibilities.

If additional low-resistance pathways for water were formed in parallel to the protein-modulated pathway, the L_p would remain elevated as albumin concentration was increased to 1 g/dl. Figure 5 shows that this was not the case. L_p returned to control levels when the albumin concentration in the perfusate was increased to 1 g/dl after Ringer perfusion. The increase in L_p following Ringer perfusion was fully reversible: the hysteresis phenomenon is not due to irreversible modification of existing pathways or information of new pathways. The same result was found in two additional capillaries. Reversibility of the protein effect has been described by several other investigators (11, 14).

During each experiment, the length of capillary used to measure L_p became successively shorter as the occlusion site was moved toward the cannulation site. In addition, with several cannulations, the cannulation site

TABLE 3. *Hysteresis of L_p on perfusate albumin concentration*

	Perfusate Albumin, g/dl				
	$L_p^{0.1} \times 10^7$	$L_p^{0.001} \times 10^7$	$L_p^{Ringer} \times 10^7$	$L_p^{0.01} \times 10^7$	$L_p^{0.1} \times 10^7$
10-15-81	21.47	34.70	94.85	65.82	34.52
	±6.21	±10.25	±23.84	±9.87	±5.20
10-20-81	9.86	29.40	87.83	36.38	
	±2.62	±11.46	±4.22	±17.20	
10-21-81	2.93	12.31	12.80	9.53	6.79
	±1.21	±1.62	±6.42	±4.20	±1.81
10-27-81	7.84	16.57	20.16	5.21	4.96
	±1.52	±2.69	±1.02	±2.37	±3.00
10-28-81	6.34	19.50	36.74		15.16
	±2.52	±3.71	±9.36		±4.32
10-29-81	2.60	6.25	12.62	6.11	4.76
	±0.35	±0.76	±3.12	±1.47	±2.49
	1.78	6.55	14.52	7.56	3.55
	±0.27	±1.26	±2.93	±2.17	±0.70
12-15-81	2.40	2.90	9.22	9.41	1.02
	±1.31	±1.44	±3.79	±3.16	±0.63
12-16-81	10.74	9.20	14.87	17.71	11.47
	±1.54	±2.43	±2.20	±3.19	±3.59
12-17-81	3.41	3.44	13.56	10.54	6.08
	±1.25	±0.82	±4.42	±5.34	±2.20

Values are means ± SD. Hydraulic conductivity (L_p) measured in $cm \cdot s^{-1} \cdot cmH_2O^{-1}$.

was advanced along the vessel. Figure 6 shows two experiments to measure the capillary L_p along the whole capillary length during each perfusion. Each value of L_p was calculated as $(J_v/S)_0/\Delta P$ where $(J_v/S)_0$ is given by *Eq. 1*. The lower line is the control L_p value measured with 0.1 g/dl albumin. The upper lines join L_p's measured with Ringer perfusion (open circles) and Ringer plus an albumin concentration of 0.01 g/dl (triangles). The L_p along the length of the capillary increases by a constant fraction relative to control values. The vertical scale is logarithmic. Our conclusions are valid at every position along the capillary.

Figure 7*A* shows $L_p/L_{p\,Ringer}$ plotted as a function of time after the beginning of reperfusion of the capillary with perfusate containing 0.1 g/dl albumin. In these experiments L_p was calculated as $(J_v/S)_0/\Delta P$ from a single measurement of $(J_v/S)_0$ over a period of 3–8 s. The time reported is the time following reperfusion at which the measurement of $(J_v/S)_0$ was begun. In all six vessels there was a substantial reduction in hydraulic conductivity from the Ringer-perfused value at the time the first measurement was made (20–57 s after the perfusate filled the capillary lumen). In the capillary that initially showed the least reduction in L_p, hydraulic conductivity fell during the first 2 min of perfusion but remained constant thereafter. In the other five vessels there was very little tendency for L_p to fall with time.

The values of $L_p/L_{p\,Ringer}$ plotted as a function of time after the beginning of perfusion with 0.01 g/dl are shown in Fig. 7*B*. In contrast to the behavior in vessels perfused with 0.1 g/dl after Ringer perfusion, L_p may be either increased or decreased relative to Ringer values by the time of the first measurement is made. Furthermore, there was variability in the time course of L_p changes with continued perfusion. In all but one vessel, L_p was reduced to below the Ringer perfusion level after more

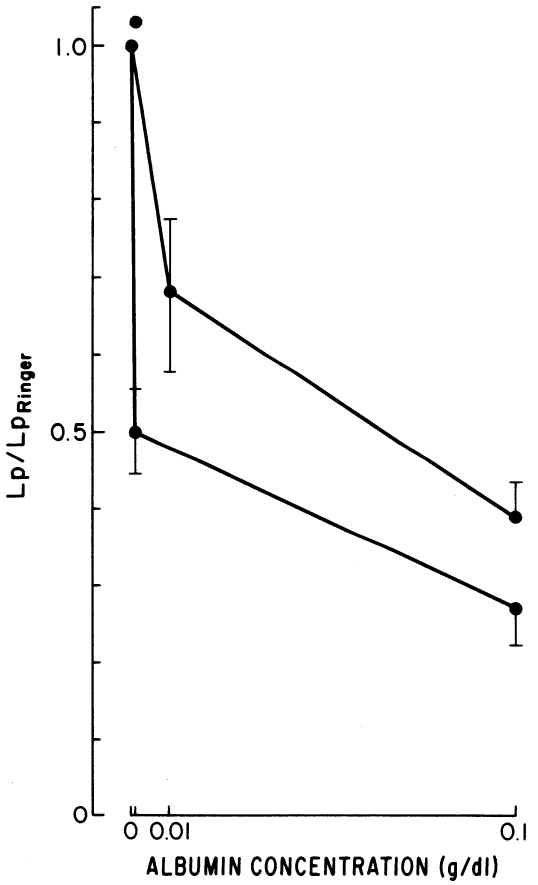

FIG. 4. Mean ratios (±SE) of $L_p/L_{p\ Ringer}$ are plotted as a function of albumin concentration. In 10 vessels protocol was the same as in Fig. 3. In an additional 5 vessels L_p at 0.001 g/dl albumin following Ringer perfusion was measured. Mean value of $L_p/L_{p\ Ringer}$ for these vessels is 1.034 (*filled circle*). The significance of this point is that, in contrast to result shown in Fig. 2, perfusion with 0.001 g/dl bovine serum albumin following Ringer perfusion was not significantly different from Ringer perfusion values. At all concentrations of albumin following Ringer perfusion L_p was elevated relative to corresponding value of L_p measured prior to Ringer perfusion.

TABLE 4. *L_p measured as albumin is replaced in perfusion solution*

	Perfusate Albumin, g/dl			
	$L_p^{Ringer} \times 10^7$	$L_p^{0.001} \times 10^7$	$L_p^{0.01} \times 10^7$	$L_p^{0.1} \times 10^7$
4-01-81	9.12 ± 2.94			9.19 ± 2.61
7-03-81	2.48 ± 0.69		2.40 ± 0.69	1.52 ± 0.86
9-01-81	2.13 ± 0.72		1.71 ± 0.41	
9-02-81	8.33 ± 2.56			2.24 ± 2.16
	7.65 ± 1.14	4.08 ± 2.34		
	7.26 ± 1.28	5.10 ± 2.20		
9-09-81	34.17 ± 6.47	38.62 ± 15.50		19.39 ± 6.54
10-09-81	5.57 ± 1.31	10.31 ± 4.78		4.08 ± 1.13
10-14-81	6.28 ± 2.64	6.00 ± 3.05	4.73	

Values are means ± SD. Hydraulic conductivity (L_p) measured in $cm \cdot s^{-1} \cdot cmH_2O^{-1}$.

than 100 s of perfusion. However, within the first 100 s, the hydraulic conductivity may transiently overshoot the value measured during Ringer perfusion, remain constant at a value close to 70% of the Ringer value, or fall steadily. Variation of the value of L_p, up or down, showed

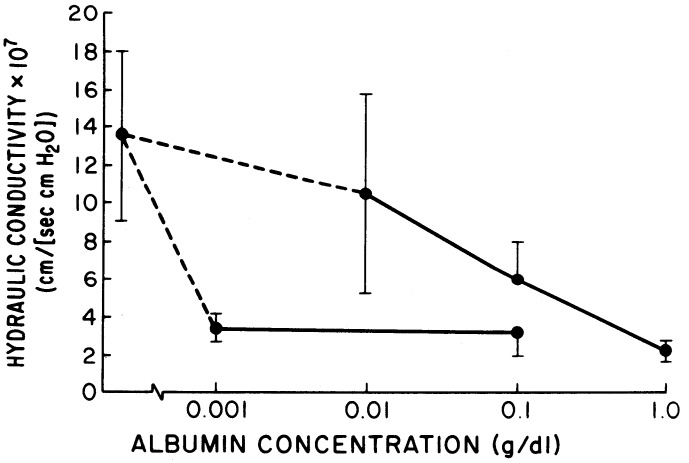

FIG. 5. Reversibility of albumin interaction with capillary wall. An extended form of experiment described by Fig. 3 is shown. *Ordinate*: mean (±SD) hydraulic conductivity; *abscissa*: log of albumin concentration plotted. *Abscissa* is broken in an arbitrary position below 0.001 g/dl bovine serum albumin (BSA) to include a Ringer (0 g/dl BSA) value. When albumin concentration was raised to 1 g/dl BSA following Ringer perfusion, hydraulic conductivity returned to control levels.

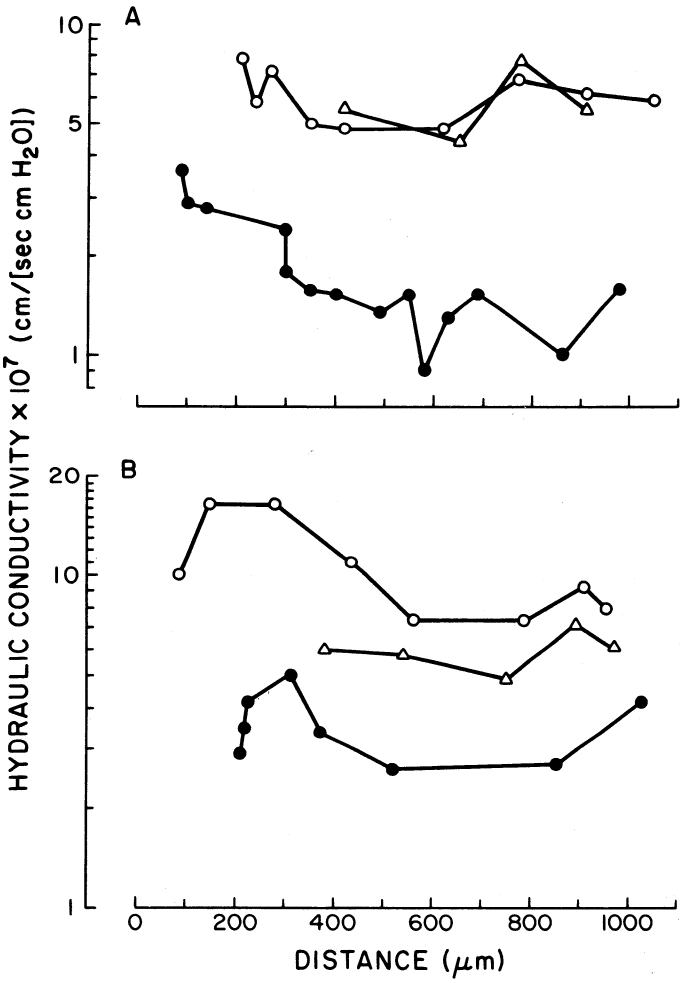

FIG. 6. Measurement of hydraulic conductivity (L_p) along length of 2 perfused capillaries. *Ordinate*: L_p for 2 vessels is plotted (logarithmic scale) prior to Ringer perfusion (●), with Ringer perfusion (○), and with 0.01 g/dl bovine serum albumin (△) at different points along length of capillary. The *0 point on abscissa* represents initial site of cannulation. There was a proportional change in L_p along length of capillary when albumin was removed.

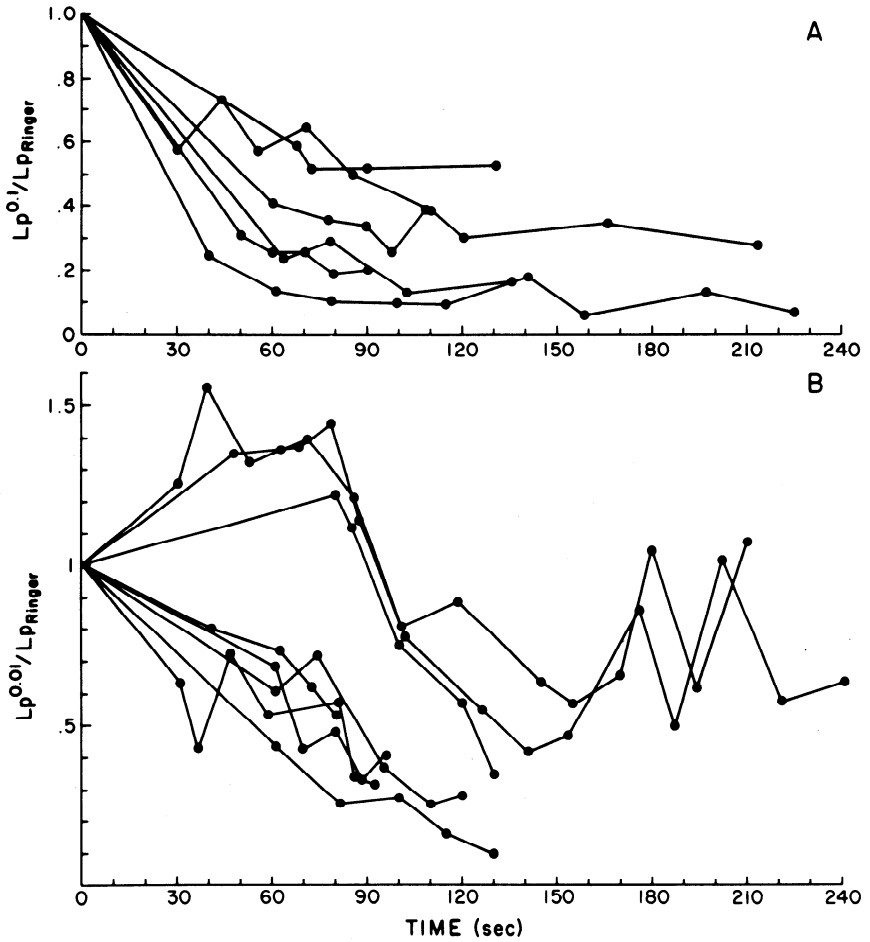

FIG. 7. Dependence of hydraulic conductivity (L_p) on time following commencement of reperfusion with albumin. Individual values of L_p are expressed relative to mean value of L_p when capillary was perfused with Ringer. *Abscissa*: time following recannulation. In all experiments 30 s elapsed before measurements were made. Perfusion pressure was 30 cmH_2O for each determination; capillary pressure only equaled this pressure when vessel was occluded. *A*: perfusate contained 0.1 g/dl bovine serum albumin (BSA; 6 capillaries). *B*: perfusate contained 0.01 g/dl BSA (8 capillaries). Tables 3 and 4 contain data from 8 capillaries perfused with 0.1 g/dl BSA and 3 capillaries perfused with 0.01 g/dl BSA following Ringer perfusion where time of cannulation was not recorded.

no correlation with changes in the site of occlusion and was therefore not the result of changes in the fragility of the vessels at the site of occlusion.

We did not design experiments to measure a time course for the onset of the protein effect when the capillary was perfused with albumin concentrations less than 0.1 g/dl prior to Ringer perfusion. For the data in Tables 1 and 2, we took three to four samples as soon as possible after establishing a good perfusion, then moved on to Ringer perfusion. However, in three capillaries reported in Table 3 (12/15, 12/16, 12/17) we perfused capillaries for an initial period lasting from 90 to 244 s. There was no increase in L_p with time during this period. We then extended the period of perfusion to measure the variation of L_p with position along the vessel. At the end of a total period of perfusion lasting from 195 to 400 s we could find no evidence for an increase of L_p with time. The variation of L_p with distance was similar to that found during later Ringer perfusion. It appears that the effect of depleted protein on hydraulic conductivity is fully developed before our first measurement is made. An alternative explanation is that the perfusion period required to measure any effect of time on protein depletion prior to perfusion with Ringer solution is much longer than the 100 s.

DISCUSSION

Our experiments demonstrate two new properties of the interaction of albumin with the transcapillary water pathway, *1*) hysteresis of L_p dependent on perfusate albumin concentration and *2*) a time dependence of albumin accumulation at low perfusate concentrations. These two properties are not necessarily independent phenomena. We appear to be dealing with an albumin adsorption process that may be limited by the rate of albumin transport to or from the principal adsorption sites. To evaluate this idea further we have extended the model, described in the introduction, of albumin binding to the walls of a rectangular slit to include transport of albumin into the slit.

Evaluation of methods. Before we analyze the data we shall briefly comment on some new aspects of our methods relevant to the continued development of single capillary methods. The mean hydraulic conductivity of capillaries perfused with 0.1 g/dl albumin (control) was 5.9×10^{-7} $cm \cdot s^{-1} \cdot cmH_2O^{-1}$, and the mean hydraulic conductivity of Ringer-perfused capillaries was 23.6×10^{-7}. These values are consistent with other published values on frog mesentery (3, 14, 17). The mean and range of our control values is also consistent with values obtained on capillaries in mammalian mesentery and omentum. We have found no significant gradient of hydraulic conductivity along lengths of individual capillaries 400–1,200 μm long. This result enables us to compare results from repeated cannulations on segments of capillary wall with the same permeability properties. Gore (8) reported a significant gradient of hydraulic conductivity along

lengths of capillaries in rat intestinal muscle. Some of the differences in results are undoubtedly due to differences in the microcirculatory beds, but important differences between our methods and the microocclusion technique of Gore should be noted. Gore reports large increases in hydraulic conductivity in the distal third of the capillary. A puzzling aspect of the result is that the gradient appears to be independent of the original length of the capillary being studied. The largest hydraulic conductivities appear to be measured on segments that may be as short as 16 μm and located within 50 μm of the occlusion site (see Fig. 4 of Ref. 8). We avoid measurements within 100 μm of the occlusion site because artifacts due to compliance, distortion of the vessel geometry, and nonideal behavior by the marker cells are accentuated on such short lengths. These artifacts may account for some of the large gradients reported by Gore. We note, however, that our method measures an integrated mean hydraulic conductivity over longer segments of the capillary wall. Thus local variations of L_p over the length of one endothelial cell (30 μm) will tend to be smoothed out. We deduce that hydraulic conductivity remains constant because our mean L_p remains constant. A detailed evaluation of the methods for measuring single capillary hydraulic conductivity is given in a recent review of techniques in the microcirculation (3).

Simple adsorption model. A simple model to describe how the adsorption of albumin might modify the hydraulic conductivity of a rectangular channel is shown in Fig. 8. A monolayer of albumin on one wall is assumed to reduce the width of a rectangular channel from 14 to 8 nm. A slit, 14- to 20-nm wide, may be formed by the space between endothelial cells at a junction.

We call N_{max} the maximum number of albumin molecules which can adsorb to the channel and N the number of molecules adsorbed when the concentration, C, is less than that required to saturate the wall. The Langmuir adsorption model (12) predicts the relation

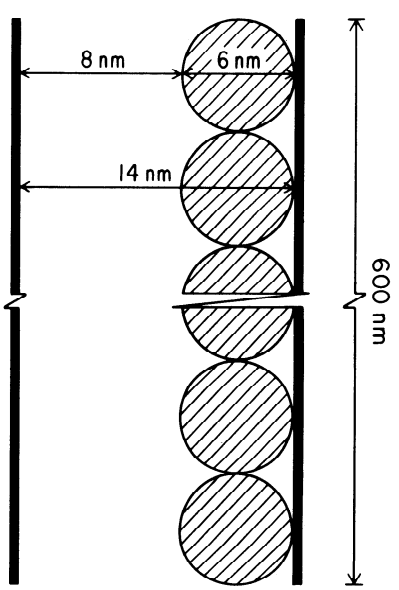

FIG. 8. Model of albumin adsorption on transendothelial channel. In this simple case albumin molecules of 6-nm diameter are assumed to adsorb to one wall of a transendothelial channel, reducing effective channel width from 14 to 8 nm and also reducing hydraulic conductivity of the channel.

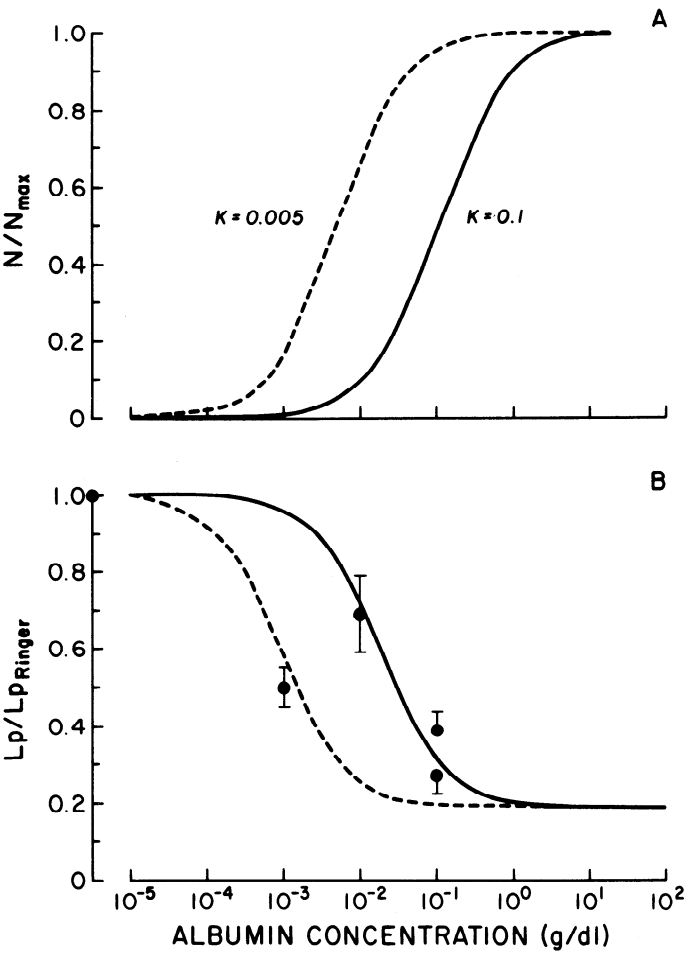

FIG. 9. Langmuir adsorption model for albumin association with transendothelial channels. *A*: fraction of albumin binding sites (N/N_{max}) occupied by protein in transendothelial pathway is plotted against concentration of protein in bulk perfusion solution at affinity constants (K) of 0.1 g/dl (*solid line*) and 0.005 g/dl (*broken line*) (*Eq. 2*). *B*: resulting change in $L_p/L_{p\ Ringer}$ at K = 0.005 (*broken line*) and at 0.1 (*solid line*) is plotted against perfusate protein concentration (*Eq. 3*). For comparison, data in Fig. 4 are shown.

$$N/N_{max} = C/(K + C) \quad (2)$$

K is an affinity constant with units of concentration. K is analogous to the Michaelis constant, i.e., when $K = C$, $N/N_{max} = 0.5$. Figure 9*A*, also known as a Bjerrum or "titration plot" (13), shows N/N_{max} as a function of C for Ks of 0.005 and 0.1. The concentration scale is logarithmic; the result is an S-shaped curve characteristic of many kinetic processes (20). We can interpret the variation of L_p relative to the Ringer-perfused value ($L_{p\ Ringer}$) as a function of concentration as follows.

When albumin is removed from the channel, L_p of the channel increases by the amount $(14\ nm/8\ nm)^3$, 5.4-fold. The calculation assumes Poiseuille flow through the slit. We interpret the quantity N/N_{max} in terms of the amount of the channel lined with adsorbed protein by assuming N/N_{max} equals the fraction of the cleft depth that has a width of 8 nm. The L_p is calculated as

$$\frac{1}{L_p} = \frac{N/N_{max}}{L_{p\ protein}} + \frac{(1 - N/N_{max})}{L_{p\ Ringer}} \quad (3)$$

where $L_{p\ protein}$ is the hydraulic conductivity of the cleft

when one wall is fully lined with albumin. *Equation 3* is the standard description of a series resistor where the total resistance ($1/L_p$) is the sum of the resistance of a protein-lined channel, $(N/N_{max})/L_{p\ protein}$, and of a protein-depleted channel, $(1 - N/N_{max})/L_{p\ Ringer}$.

The relation between $L_p/L_{p\ Ringer}$ and C when $K = 0.1$ is shown as a solid line in Fig. 9*B*. The solid points are the data from the experiment in Fig. 4. This simple adsorption model suggests that one-half the albumin sites were filled at an albumin concentration of 0.1 g/dl. L_p is one-third the value during Ringer perfusion, a decrease somewhat larger than that actually observed. A shift in the position of the solid curve in Fig. 9*B* to the right indicates a weaker interaction between albumin and the channel, whereas a shift to the left indicates a stronger interaction.

The broken line in Fig. 9*B* is the value of $L_p/L_{p\ Ringer}$ plotted as a function of albumin concentration, using *Eq. 3,* when the affinity constant is 0.005. This curve describes our data during albumin depletion. If this model were correct, the difference in the values of K in Fig. 9*B* would be interpreted as an affinity for albumin binding sites after Ringer perfusion that is 20 times less than that after the capillary has been perfused with albumin at a concentration of 0.1 g/dl. Before investigating this idea further, we examine the assumptions implicit in the adsorption model. One assumption is that all possible binding sites for albumin are accessible to albumin concentration at the channel entrance (luminal side). A second assumption is that solute accumulation in the channel is not limited by the availability of solute near the binding site.

Limits on albumin transport to the adsorption sites. A characteristic feature of all runs in Fig. 7*B* (perfusate 0.01 g/dl after Ringer perfusion) was that the maximum rate of decrease in L_p with time occurred during the period when L_p is being measured and not immediately following the introduction of albumin into the vessel. This result is characteristic of a delay in the onset of interaction between albumin and the channel wall.

The following calculations evaluate the rate of albumin transport into the water channel after Ringer perfusion. Consider a segment of the water channel 100 nm long having the cross section shown in Fig. 8. The maximum albumin flux due to diffusion through the channel when the perfusate concentration is 0.01 g/dl is then

$$J_{s,d} = \frac{A}{\Delta x} \cdot D' \cdot \phi \cdot \Delta C \qquad (4)$$

where $A/\Delta x$ is area per unit diffusion path length, D' is the channel diffusion coefficient (free diffusion × drag factor), ϕ is the steric partition coefficient, and ΔC is the concentration difference. Substitution into *Eq. 4* yields

$$J_s = \left(\frac{140 \times 1{,}000 \times 10^{-16}}{6{,}000 \times 10^{-8}}\right) \cdot (6 \times 10^{-7} \times 0.545)\ 0.5 \left(\frac{0.01}{100}\right)$$
$$= 3.87 \times 10^{-18}\ \text{g/s}$$

The drag factor and ϕ were calculated using rectangular slit theory (19). When J_s is expressed as molecules per second, assuming a molecular weight for albumin of 68,000, the flux through the channel segment is 34 molecules/s.

The total number of albumin molecules required to cover one channel wall that is 600 nm deep and 100 nm long with a monolayer of albumin is 1,120. More than 30 s is required to accumulate all the bound molecules, assuming every molecule that diffused into the channel was taken up onto the wall. Although this calculation is based on a simplified pseudo-steady-state model to describe a time-dependent process, it makes clear that the accumulation of albumin into the cleft may be significantly limited by the rate of diffusion of albumin into the cleft.

The argument developed above neglects the accumulation of albumin in the cleft carried in by the water flow. The solvent drag flux is

$$J_{s,c} = J_v\ (1 - \sigma_f)\ C \qquad (5)$$

where J_v is the filtration flux, σ_f is the solvent drag reflection coefficient, and C is the perfusate concentration of albumin. For a pressure difference of 5–8 cmH_2O prior to occlusion, *Eq. 5* yields a flux of 6–10 albumin molecules/s when the perfusate concentration is 0.01 g/dl. On the other hand, after occlusion the pressure within the capillary is 30 cmH_2O. The convective solute flux would then be 37 molecules/s, effectively doubling the total flux into the membrane when superimposed on the diffusion flux. Further, when the perfusate concentration is 0.1 g/dl, the diffusion flux into the channel is 340 molecules/s and the convective flux at the capillary pressure of 30 cmH_2O is 370 molecules/s.

These calculations show that, during albumin replacement, transport of albumin into the cleft may limit the rate of albumin delivery to the structures in the channel. These transport limitations are a possible explanation of the slow fall in hydraulic conductivity when there is 0.01 g/dl in the perfusate. Furthermore, an ultrafiltration mechanism may account for the characteristic fall in hydraulic conductivity after beginning measurements of L_p. At a perfusate concentration of 0.01 g/dl, albumin flux into the membrane is significantly increased after the capillary is occluded. It may be important to distinguish between the period of perfusion and the period during which ultrafiltration into the membrane occurs when investigating the time course in Fig. 7*B*.

It follows from the above considerations that the affinity constant of K equal to 0.1 g/dl, which describes the ratios $L_p/L_{p\ Ringer}$ in Fig. 9*B*, is likely to be too large. A smaller value would not have been measurable during albumin replacement due to the low rate of albumin transport into the channel. For example, in six of the eight capillaries in Fig. 7*B* the mean value of $L_p/L_{p\ Ringer}$ measured with 0.01 g/dl in the perfusate after perfusion with Ringer solution, is larger than the values approached after 2 min of perfusion. Only the mean value of $L_p/L_{p\ Ringer}$ is plotted in Figs. 3–5. On the other hand, hysteresis does not simply reflect a failure to wait sufficient time for albumin to accumulate in the membrane.

In two vessels in Fig. 7*B*, in which perfusion lasts longer than 3 min after Ringer perfusion, L_p does not reach a stable level. Such instability is reminiscent of the behavior of Ringer-perfused vessels. It is also in marked contrast to the behavior of vessels perfused with 0.01 g/dl albumin prior to Ringer perfusion.

Accessibility of albumin adsorption sites. As soon as albumin begins to accumulate in the channel, the selectivity of the channel will increase. Exclusion of free albumin by adsorbed albumin near the channel entrance will reduce the albumin flux reaching the albuminal side of the cleft. As a result, to restore L_p to control values, albumin concentration in the lumen must be higher than would be the case were all adsorption sites readily available to luminal albumin. For example, if the albumin reflection coefficient in the entrance region of the channel is 0.9 as the result of albumin adsorption, the albumin concentration in the fluid reaching the albuminal portion of the channel would be 0.01 g/dl when the perfusate concentration is 0.1 g/dl. This mechanism may explain why the lumen concentration must be raised 10-fold to 1.0 g/dl to restore L_p to control levels following Ringer perfusion. This argument is supported by our recent observations that L_p can be returned to control values during perfusion with 0.1 g/dl in the perfusate if the superfusate also contains 0.1 g/dl albumin (10).

Alternate model of adsorption sites. It has been suggested that albumin may occupy sites in a network of fibrous molecules within the water pathway rather than line the walls of a rectangular slit (1, 5, 22). We may account for the increase in L_p if albumin molecules form part of the fiber matrix that has previously been described in terms of fibers (0.6 nm in radius occupying 5% of the channel volume). For example, the permeability properties of the capillary wall in the presence of albumin are described if the volume concentration of fine fibers is reduced from 5 to 3% and replaced by albumin molecules occupying 8% of the channel volume. The total surface area of the albumin-fiber matrix is the same as the surface area of the matrix containing only fine fibers. The increase in L_p when albumin is removed reflects the decrease in surface area of fibers in contact with water in the channel. Equations describing water flow through a matrix consisting of fibers and spherical protein particles are given by Turner et al. (22). If N/N_{max} in *Eq. 3* is interpreted as the fraction of the initial volume of albumin that remains in the matrix, the calculated concentration dependence of $L_p/L_{p\ Ringer}$ is similar to that in Fig. 9*B*. A network of fibers having the same volume as the cleft segment in Fig. 8 and including 8% albumin by volume will contain 1,500 albumin molecules distributed throughout the matrix. After Ringer perfusion this number of albumin molecules must be restored into the segment. Again, transport into the matrix will limit albumin availability when the perfusate concentration is 0.01 g/dl. In addition, steric exclusion near the luminal channel entrance will reduce the albumin concentration reaching the abluminal side of the channel. The conclusions drawn using the fiber matrix model are therefore similar to those reached using the rectangular slit model.

The fiber model may offer an explanation of the tendency for L_p to overshoot the Ringer value in some capillaries. A small number of albumin molecules in the channel may cause the fibers within the channel to be "clumped" together into a coarse irregular network that offers less hydraulic resistance than randomly distributed finer fibers. Hydraulic resistance will not increase until a larger amount of albumin accumulates in the channel. The possibility that adsorbed proteins may clump fibers or arrange them into rectangular arrays has been described in detail by Michel (16).

Our results indicate that albumin is a major component of the structures determining the principal resistance to water flow at the capillary wall. In both the slit and fiber matrix models the concentration of albumin molecules within the principal water pathway is more than an order of magnitude larger than that in the perfusate. Albumin is therefore preferentially accumulated in the channel. It appears likely that electrostatic forces play an important role in the accumulation of albumin at the capillary wall (18, 22). In particular, Michel and co-workers (22) have evidence that positively charged arginine groups on albumin interact with negative sites on the capillary wall to modify the hydraulic conductivity of the capillary wall. These observations may explain how albumin can modify the permeability properties of the capillary without affecting the normal ultrastructure of the capillary membrane (15).

Our most important conclusion is that the apparent threshold in albumin interaction with the capillary wall at a concentration of 0.1 g/dl after Ringer perfusion cannot be interpreted in terms of a simple adsorption model characteristic of the whole transcapillary water pathway. We began the experiments described in this paper assuming that albumin interacted with readily accessible sites on the plasma membrane of the endothelial cell. Our finding that there was a hysteresis of hydraulic conductivity on albumin perfusate concentration indicated that the affinity of albumin for its binding site before Ringer perfusion is different from that after Ringer perfusion. We have not carried out a detailed analysis of the measured affinity constants in this study because we realized that transport limitations give rise to transient phenomena that may bias the results. For example, the affinity of albumin for binding sites is probably greater than the value indicated by an affinity constant of 0.1 g/dl following Ringer perfusion in our model. On the other hand, the affinity is also probably less than that indicated by an affinity constant of 0.005 g/dl measured prior to Ringer perfusion. The reason is that transport limitations to albumin depletion will appear as decreased binding affinity constant. The question of a true difference in binding affinities requires further investigation, especially as Schneeberger et al. (21) have demonstrated a hysteresis of ferritin accumulation in vesicles and basement membranes during albumin depletion and replacement in isolated perfused rat lung. Schneeberger's demonstration of a hysteresis of ferritin filling of luminal vesicles suggests that binding and exclusion within a fiber matrix at the cell surface may be sufficient to account for hysteresis. With the information available from the present study it will be possible to

study separately the factors (such as transcapillary filtration rate, distribution of albumin) that may affect the transport processes into the membrane (transient phenomena) and the factors (such as albumin concentration, charge) that determine the steady-state interaction of albumin with binding sites within the membrane.

We thank Dr. C. C. Michel for critical reading of our manuscript. Ing-Ing Huang provided skillful technical assistance.

This work is supported by National Heart, Lung, and Blood Institute Grant HL-28607. V. H. Huxley was an American Heart Association (California Affiliate) Research Fellow, then a Giannini Foundation Fellow, and is currently the recipient of New Investigator Award HL-30827. F. E. Curry is an Established Investigator of the American Heart Association.

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Received 30 March 1984; accepted in final form 2 October 1984.

REFERENCES

1. CURRY, F. E. Is the transport of hydrophilic substances across the capillary wall determined by a network of fibrous molecules? *Physiologist* 23(4): 90–93, 1980.
2. CURRY, F. E. The effect of temperature on hydraulic conductivity of single capillaries. *Am. J. Physiol.* 240 (*Heart Circ. Physiol.* 9): H29–H32, 1981.
3. CURRY, F. E., V. H. HUXLEY, AND I. R. SARELIUS. Techniques in the microcirculation: measurement of permeability, pressure and flow. In: *Techniques in the Life Sciences. Cardiovascular Physiology,* edited by R. J. Linden. New York: Elsevier, 1983, vol. 3, part I (P309), p. 1–34.
4. CURRY, F. E., J. C. MASON, AND C. C. MICHEL. Osmotic reflexion coefficients of capillary walls to low molecular weight hydrophilic solutes measured in single capillaries of frog mesentery. *J. Physiol. London* 261: 319–336, 1976.
5. CURRY, F. E., AND C. C. MICHEL. A fiber matrix model of capillary permeability. *Microvasc. Res.* 20: 96–99, 1980.
6. DIANA, J. N., B. J. KEITH, AND B. P. FLEMING. Influence of macromolecules on capillary filtration coefficients in isolated dog hindlimbs. (Abstract). *Microvasc. Res.* 20: 106–107, 1980.
7. DRINKER, C. K. The permeability and the diameter of capillaries in the web of the brown frog (*Rana temporatia*) when perfused with solutions containing pituitary extract and horse serum. *J. Physiol. London* 63: 249–269, 1927.
8. GORE, R. W. Fluid exchange across single capillaries in rat intestinal muscle. *Am. J. Physiol.* 242 (*Heart Circ. Physiol.* 11): H337–H357, 1982.
9. HUXLEY, V. H., AND F. E. CURRY. Graded effect of albumin concentration on the hydraulic conductivity of frog capillaries (Abstract). *Microvasc. Res.* 23: 259, 1982.
10. HUXLEY, V. H., AND F. E. CURRY. The effect of superfusate albumin on the hydraulic conductivity of single capillaries (Abstract). *Microvasc. Res.* 25: 239, 1983.
11. LANDIS, E. M., AND J. R. PAPPENHEIMER. Exchange of substances through capillary walls. In: *Handbook of Physiology. Circulation.* Washington, DC: Am. Physiol. Soc., 1963, sect. 2, vol. II, chapt. 29, p. 961–1034.
12. LANGMUIR, I. Molecular layers. *Proc. R. Soc. London Ser. A* 170: 1–40, 1939.
13. MARSHALL, A. G. *Biophysical Chemistry. Principles, Techniques, and Applications.* New York: Wiley, 1978, p. 71–77.
14. MASON, J. C., F. E. CURRY, AND C. C. MICHEL. The effect of proteins on the filtration coefficient of individually perfused frog mesenteric capillaries. *Microvasc. Res.* 13: 185–204, 1977.
15. MASON, J. C., F. E. CURRY, I. F. WHITE, AND C. C. MICHEL. The ultrastructure of frog mesenteric capillaries of known filtration coefficient. *Q. J. Exp. Physiol.* 64: 217–224, 1979.
16. MICHEL, C. C. The effects of certain proteins on capillary permeability to fluid and macromolecules. In: *The Pathogenicity of Cationic Proteins,* edited by P. P. Lambert, B. Bergmann, and R. Beauwens. New York: Raven, 1983, p. 125–140.
17. MICHEL, C. C., J. C. MASON, F. E. CURRY, AND J. E. TOOKE. A development of the Landis technique for measuring the filtration coefficient of individual capillaries in the frog mesentery. *Q. J. Exp. Physiol.* 59: 283–309, 1974.
18. MICHEL, C. C., M. E. PHILLIPS, AND M. R. TURNER. The effects of chemically modified albumin on the filtration coefficient of single frog mesenteric capillaries (Abstract). *J. Physiol. London* 332: 111P, 1982.
19. RENKIN, E. M., AND F. E. CURRY. Transport of water and solutes across capillary endothelium. In: *Membrane Transport in Biology,* edited by G. Giebisch, D. C. Tosteson, and H. H. Ussing. New York: Springer-Verlag, 1978, p. 1–45.
20. SEGEL, I. H. *Enzyme Kinetics.* New York: Wiley, 1975, p. 16–89.
21. SCHNEEBERGER, E. E., M. HAMELIN, B. A. NEARY, AND J. M. MCCORMACK. Graded effect of circulating protein concentration on ferritin transport across pulmonary endothelium (Abstract). *Microvasc. Res.* 25: 255, 1983.
22. TURNER, M. R., G. CLOUGH, AND C. C. MICHEL. The effects of cationized ferritin and native ferritin upon the filtration coefficients of single frog capillaries. Evidence that proteins in the endothelial cell coat influence permeability. *Microvasc. Res.* 25: 205–222, 1983.