

INFLUENCE OF pH ON CAPILLARY FILTRATION COEFFICIENT OF RAT MESENTERIES PERFUSED WITH SOLUTIONS CONTAINING ALBUMIN

By J. GAMBLE

From the Department of Physiology, The Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ

(Received 5 July 1982)

SUMMARY

1. A preparation of rat mesentery was vascularly isolated from the intestine and perfused with a physiological salt solution containing either Ficoll 70 or bovine serum albumin, to act as colloidal agents.

2. The capillary filtration coefficient (K_f ; units, ml. min⁻¹ 100 g⁻¹ mmHg⁻¹) was measured by following the weight change after graded increases in venous pressure. At pH values greater than 7.05, K_f , during perfusion with 3 and 4% bovine serum albumin solutions, was 0.219 ± 0.023 (mean \pm s.e. of mean), ninety-eight observations in thirteen experiments, which was significantly less than the value of 0.507 ± 0.038 which was obtained during perfusion with albumin solutions at pH less than 7.05, seventy-six observations in eleven experiments, ($P < 0.05$).

3. The value of K_f obtained during perfusion with 4% Ficoll solutions was 0.267 ± 0.018 , 119 observations in sixteen experiments, and remained uninfluenced by pH over the same range that had been used with the albumin solutions; however, perfusion of the tissues with Ficoll solutions at pH > 7.05, after perfusion with albumin solution pH < 7.05, did cause the Ficoll-derived value of K_f to rise to 0.502 ± 0.055 , seventy-two observations in eleven experiments.

4. It was concluded that the changes in K_f were not due to pH alone, but were mediated by albumin at acidic pH.

INTRODUCTION

Davies & Gamble (1976) showed that when the colloid component of the solutions, used for perfusing isolated rat mesentery, was changed from 4% Ficoll solution, a non-protein co-polymer of sucrose and epichlorhydrin, to 3% bovine serum albumin (albumin), the capillary filtration coefficient (K_f) of the tissue doubled. These observations seemed to be at variance with those of other workers, that vascular exchange vessel permeability is modified by proteins, so that in their absence, non-protein colloids do not exert their full colloid osmotic pressure, e.g. Drinker (1927), Danielli (1940), W. B. Kinter & J. R. Pappenheimer, unpublished observations reported by Landis & Pappenheimer (1963).

During the early experiments on the isolated perfused mesentery, (J. Gamble,

unpublished data) inadequate gas equilibration of the albumin-containing perfusates gave rise to solutions of acidic pH; the possibility that the change in pH of the solutions might be responsible for the observed rise in K_f seemed worth investigating, especially since previous investigators of the effects of pH on the microvessels had made conflicting observations, (see Landis & Pappenheimer (1963) and Chambers & Zweifach (1940)).

The results reported in the present paper were obtained from two groups of experiments, in which the fluid flux in response to graded increases in venous pressure was determined either volumetrically, or gravimetrically in isolated perfused rat mesenteries. The tissues were perfused with physiological salt solutions containing either Ficoll 70 or bovine serum albumin as a colloid component and over the pH range 6.75–8.20. Preliminary accounts of some of these observations have already been published (Gamble, 1978*a*, *b*).

METHODS

Mesenteries were isolated from two groups of anaesthetized Wistar rats: twelve males, of mean body weight 335 g (range 230–440 g) and twenty females, of mean body weight 179 g (range 145–270 g). The mesentery weight: body weight ratios in the two groups were $1.00 \pm 0.09 \times 10^{-2}$ (s.e. of mean) and $0.80 \pm 0.04 \times 10^{-2}$ respectively.

Preparation

Following anaesthesia, induced by intraperitoneal injection of 60 mg kg^{-1} sodium pentobarbitone (May & Baker Ltd, 6% (w/v) in 0.9% NaCl solution), the trachea was cannulated and the abdomen opened with a mid-line incision. The mesoappendix, large intestine, spleen and stomach were all resected between ligatures. Branches of the abdominal aorta, between the superior mesenteric artery and the left renal artery, and branches of the hepatic portal vein, between its point of emergence from the mesentery and its entry into the liver, were divided between ligatures. The dissection procedure resulted in the mesentery and small intestine being left attached to the body by the hepatic portal vein, the superior mesenteric artery and its enveloping connective tissue bundle containing lymphatic drainage and nerve supply.

The rat was heparinized with 1000 i.u. kg^{-1} . (Weddel Pharmaceuticals Ltd) and the aorta cannulated retrogradely, below the level of the superior mesenteric artery. The vascular bundles connecting the small intestine to the mesentery were ligated close to the small intestine by passing 006 gauge ligatures around them and through the avascular sheets of mesothelium on either side. The small intestine was then cut free and the remaining mesenteric tissue studied for leaks and petechiae. The connective tissue bundle associated with the superior mesenteric artery was divided between ligatures, the hepatic portal vein cannulated retrogradely and the aorta ligated and cut free above the level of the superior mesenteric artery. The mesentery, which had been isolated without prior interruption of blood flow, was weighed and then transferred to the perfusion circuit.

Perfusates

The perfusates used in these experiments were based upon the solution of McEwen (1956) and contained (mm): NaCl, 130.0; KCl, 5.63; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2.16; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.91; NaHCO_3 , 25.0; dextrose, 11.09 and sucrose 13.15. This, when bubbled with a gas mixture of 95% oxygen and 5% CO_2 at 37 °C, had a pH of 7.3. The pH was altered by adjusting the NaHCO_3 content of the solution, the NaCl being altered by an equivalent concentration to maintain isotonicity.

pH measurement

Perfusate samples were taken into glass syringes, lubricated with silicone fluid (DC 550; Edwards Surgical Supplies Ltd) at the start and end of each perfusion session. The pH of the samples was measured anaerobically on a Radiometer M27B pH meter, using a calibrated G2973 G2 electrode, at a temperature of 37 °C.

Osmotic agents

Two substances were used as colloid osmotic agents, they were bovine serum albumin (Sigma; Cohn Fraction V, mol. wt. 69,000 Daltons) and Ficoll 70 (Pharmacia., mol. wt. 70,000 Daltons).

Colloid osmotic pressure measurement

Colloid osmotic pressures were measured using a modified Hansen osmometer coupled to a Bell & Howell physiological pressure transducer (type 4-422-0002). Amicon UM 10 unbacked membranes were used in the osmometer and had a molecular exclusion of less than 10,000 Daltons.

Ionized calcium measurement

Free (ionized) calcium was measured anaerobically at 37 °C, on an Orion Biochemical Ionized Calcium Measurer (model ss-20) at the Rayne Institute, University College Hospital. Duplicate samples were kept under identical conditions in silicone fluid-lubricated glass syringes and simultaneously measured for pH, under the same conditions.

Gas equilibration

In the early experiments, all protein-free Ficoll solutions were equilibrated with a mixture of 95 % oxygen and 5 % carbon dioxide via a sintered glass tube, whilst the solution was maintained at 37 °C. In order to avoid frothing, the protein-containing solutions were not bubbled with the gas mixture but instead the albumin was dissolved in gas-equilibrated McEwen solution and the gas mixture passed over the surface of the solution after mixing; subsequent studies showed that when adopting this procedure, the addition of 3 g albumin to 100 ml. equilibrated McEwen solution at pH 7.3 caused the pH to fall to 6.85 ± 0.03 and that during the subsequent exposure of the free surface of the solution to the gas mixture, the pH rose by 0.0026 pH units per minute.

Various types of gas equilibration procedure were tested and the method that was finally adopted is depicted in Fig. 1. Perfusate was rapidly recycled via a Starling resistor and returned to the perfusate reservoir via a vertically positioned single coiled reflux condenser maintained at 37 °C. The returning perfusate percolated down over the surface of the condenser and the gas mixture passed upwards in a counter-current manner. This equilibration procedure was rapid, did not induce frothing and avoided the use of 'anti-foam' agents.

Perfusion circuit

The perfusion circuits for both volumetric and gravimetric experiments were essentially the same and the gravimetric circuit that was used is illustrated in Fig. 1. The perfusate was drawn from a round bottomed flask, maintained at 37 °C in a water bath, and pumped, by a Watson Marlow flow inducer, via a Starling resistance, set to the required perfusion pressure, back to the perfusate reservoir. The perfusate flow in the Starling resistor circuit was greatly in excess of the tissue requirements, thus facilitating gas equilibration and temperature maintenance. The perfusate flowing to the tissue left the recycling circuit just before the Starling resistance; it passed via a silicone fluid-filled drop counter, which monitored arterial inflow and then passed via a warming coil to the mesenteric arterial cannula. The arterial pressure was measured, close to the arterial cannula, by means of a pressure transducer (Bell & Howell, type 4-222-0001).

The venous outflow pressure was measured close to the venous cannula by means of a second transducer (Bell & Howell, type 4-422-0002) and the venous outflow volume monitored by means of a transistorized 'contact' drop recorder. The height of the venous outflow could be adjusted, and it was by observing the changes in tissue weight/volume in response to imposed elevations of venous hydrostatic pressure that the relationship between venous pressure and the capillary filtration coefficient (K_f) was obtained (Mellander, 1960), see Fig. 2.

Measurement of tissue response

(a) *Volumetric.* Isolated tissues were placed in a Perspex plethysmograph, filled with McEwen solution at pH 7.3 and maintained at 37 °C. The intraplethysmographic pressure was monitored and maintained at +5 cm water. The control venous outlet pressure was maintained at +2 cm water relative to the intraplethysmographic pressure. Changes in intraplethysmographic volume were registered as changes in the force exerted on a float attached to an isometric force transducer (Devices, type 0-100 g) and immersed in a reservoir, connected to the inside of the plethysmograph. A 'bleed line' containing a Watson Marlow flow-inducer and a transistorized drop contact recorder

was connected to the reservoir to keep the control volume record constant; when in use, the rate of intraplethysmographic fluid removal was not altered, during the course of either venous pressure elevation or during the recovery period. The changes in the volume base line could be partially attributed to the imbibition of water by the tissue from the McEwen solution within the plethysmograph (see Clough & Smaje, 1978) and also to fluid formation on the serosal surface of the mesentery. It was the inability to discriminate between tissue fluid loss and tissue weight gain, which led to the development of the gravimetric system.

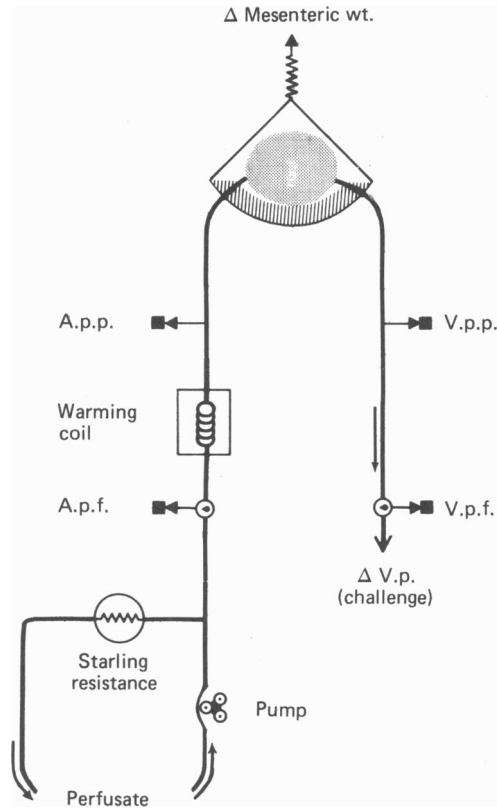


Fig. 1. Diagrammatic representation of the perfusion circuit. The perfusate was impelled by a Watson Marlow flow inducer (Pump) and the required pressure maintained by means of a Starling resistance. The perfusate was warmed and the arterial pressure (A.p.p.) and inflow (A.p.f.) monitored continuously. The venous outflow (V.p.f.) and pressure (V.p.p.) were also monitored. Venous pressure could be elevated by known increments ($\Delta V.p.$) to give the venous pressure 'challenge'. The tissue was placed on a weighing device and the changes in tissue weight (Δ mesenteric wt.) were monitored continuously.

(b) *Gravimetric.* The perfused mesentery was covered in plastic film (Snappies Cling Film Wrap) and placed on a shallow pan attached to an isometric force transducer (Devises, type 0–100 g) which enabled changes in mesenteric weight to be monitored continuously. Fluid lost from the surface of the mesenteric tissue, entered the supporting pan and was immediately removed, via tubing attached to a Watson Marlow flow inducer and voided to a tube suspended from an isometric force transducer (Devises, type 0–100 g) thus enabling fluid loss to be monitored continuously. In general, the volume/weight changes associated with imposed elevations in venous pressure were followed by 100% recovery, suggesting that neither the tissue weight gain nor the serosal fluid flux components were appreciably influenced by the venous pressure elevations during the gravimetric and therefore, presumably, during the volumetric experiments.

Viscosity. The relative viscosities of Ficoll solution and bovine serum albumin dissolved in normal saline were measured at fixed temperature and pH using an Ostwald viscometer. The pH of the solutions was varied by the addition of either 0.1 M-NaOH or HCl solution.

Determination of K_t . Alterations in tissue volume or weight following imposed elevations in venous pressure were related to the transudation of vascular fluid; measured as millilitres of fluid transuded, per minute, per 100 g tissue, per mmHg elevation of venous pressure, after the method of Mellander (1960). In each experiment, the transudation rates following elevations of both 5 and 10 cmH₂O were measured. On average, three elevations at each pressure increment were made during perfusion with each solution. The pressure elevation gave rise to a biphasic volume or weight change (see Fig. 2) the first, rapid, phase being due to the vascular capacitance response and the second slower phase being due to interstitial accumulation of transuded fluid. Where tissue weight change was measured, the volume equivalent was calculated by assuming a density of 1.0.

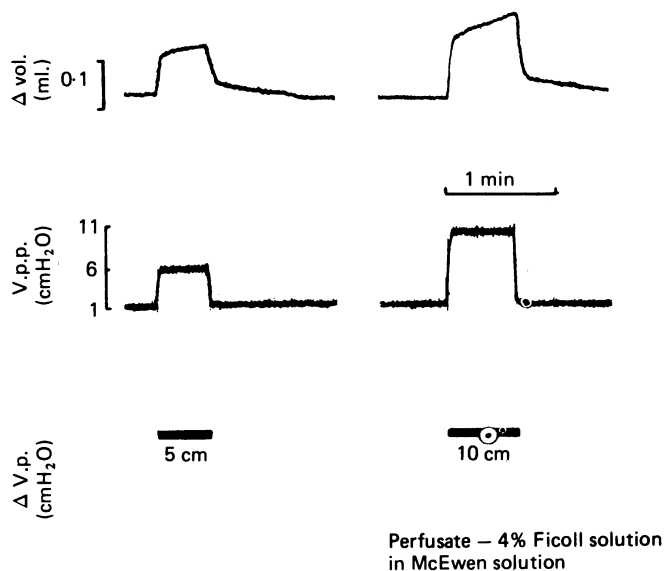


Fig. 2. Illustration depicting the change in mesenteric tissue volume (Δ vol.) with time, in response to graded elevations of venous pressure (V.p.p.). The volume response is bi-phasic, with a rapid initial capacitance component followed by a slower volume increase which is believed to represent the trans-endothelial fluid flux (J_v).

RESULTS

General observations

The mean weight of the mesenteric tissue used during the experiments was 2.13 ± 0.23 g ($n = 32$) and consisted primarily of adipose tissue, although histological examination showed that the tissue did contain lymphatic nodular masses which may have accounted for up to 15% of the total tissue mass. The lymphatic drainage had been ligated during the course of preparation so that the only routes for tissue fluid flux were across the vascular endothelium and the serosal interfaces.

Physical characteristics of the perfusates

Colloid osmotic pressure (π). The colloid osmotic pressures of the perfusates were determined in most experiments. The mean values for the albumin solutions were not

significantly different from those predicted by the formula of Landis & Pappenheimer (1963) $\pi = 2.8c + 0.18c^2 + 0.012c^3$ where c is the concentration of albumin in $\text{g } 100 \text{ ml.}^{-1}$ (Fig. 3). Equivalent concentrations of Ficoll ($\text{g } 100 \text{ ml.}^{-1}$) were observed to give higher values for colloid osmotic pressure, part of the difference being due to the presence of low molecular weight fractions in the Ficoll samples. Analysis of the mean values of colloid osmotic pressure obtained from standard concentrations

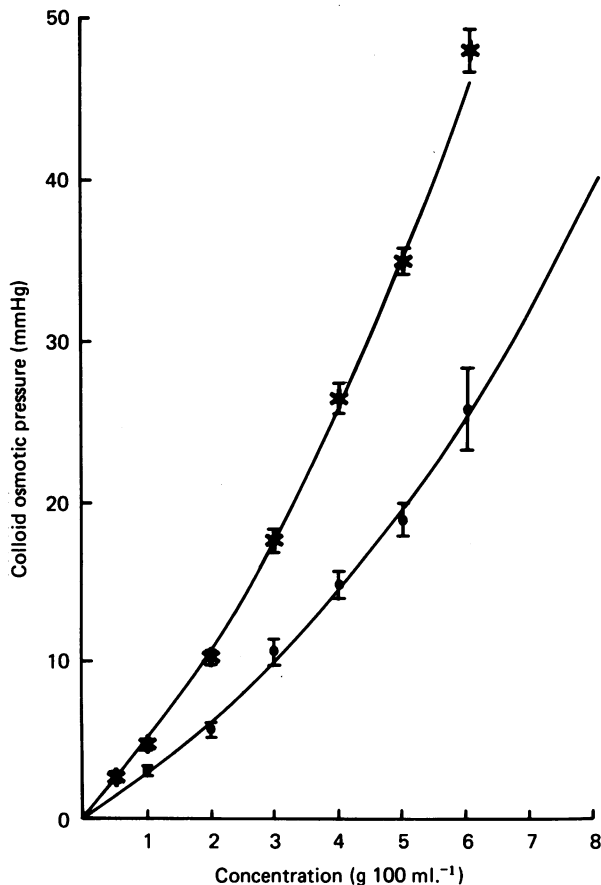


Fig. 3. The relationship between Ficoll (*) and bovine serum albumin (●) concentration and colloid osmotic pressure. The points and error bars represent the means and s.e. of means for standard solutions. The continuous line through the Ficoll points was obtained by regression analysis and that through the albumin points, is the curve derived from the formula of Landis & Pappenheimer (1963) (see text).

of Ficoll showed that a quadratic equation $\pi = 4.3c + 0.56c^2$, derived by a least squares non-linear fit, described the data obtained adequately (Fig. 3).

Viscosity. The relationship between relative viscosity and concentration for both Ficoll and albumin solutions at 37 °C is depicted in Fig. 4. It is evident that the relative viscosity of Ficoll per unit concentration is far greater than that of albumin. Variation of pH did not have any influence on the viscosity of either Ficoll or albumin solutions over the range tested in these experiments.

The effect of pH on the filtration coefficient (K_f). The mean values for K_f obtained during thirty-three perfusion sessions, using either 3 or 4% albumin, in twenty-five experiments are plotted relative to their respective pH values in Fig. 5 (standard error bars are omitted for the sake of clarity). Three main conclusions can be drawn from this graph; first that there is an inverse relationship between pH and K_f , secondly, that K_f rises markedly as pH falls below about 7.00 and thirdly, that the value of K_f is apparently uninfluenced by the different concentrations of albumin used.

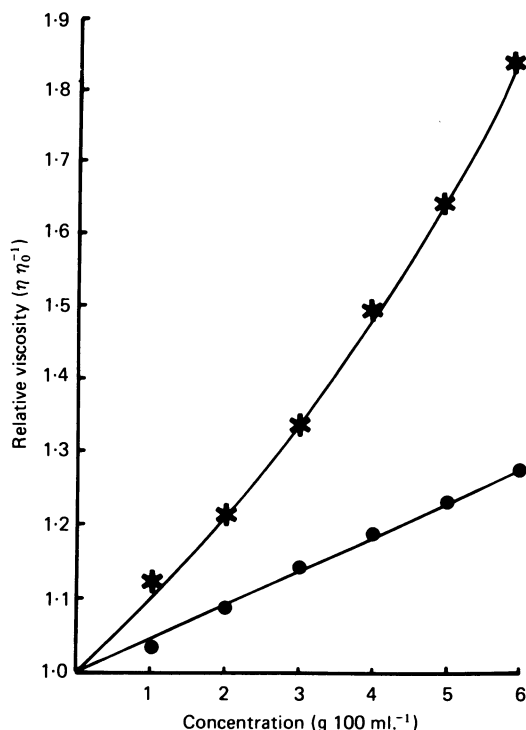


Fig. 4. The relationship between concentration (g 100 ml.⁻¹) and relative viscosity (η test solution. η_0^{-1} solvent (0.9% NaCl)), for Ficoll solution (*) and bovine serum albumin (●) solutions at 37 °C. The points represent the mean values from two studies and the lines were fitted by eye.

Statistical comparison of the K_f values obtained about an arbitrary pH of 7.05 showed that they were significantly different from one another ($K_f = 0.197 \pm 0.017$ ($n = 18$) pH > 7.05 and 0.465 ± 0.033 ($n = 15$) pH < 7.05) $P < 0.001$.

Experimental controls. Direct assessment of the effect of changing pH on the value of K_f derived during perfusion with albumin solutions was only made in one experiment and the assessment of the comparability of the preparations used in these experiments relied, in the main, on the comparability of the K_f values obtained during the preliminary perfusion with 4% Ficoll solutions at pH > 7.05.

In sixteen experiments, perfusion with albumin solutions at various pH values, was bracketed between perfusions with 4% Ficoll solution at pH > 7.05. In five of these experiments, the pH of the albumin solution was > 7.05 and the value of K_f obtained

was 0.210 ± 0.029 (thirty observations). The K_f values obtained during the initial and final 4% Ficoll perfusions at $\text{pH} > 7.05$ (0.274 ± 0.031 , $n = 34$ and 0.212 ± 0.030 , $n = 29$) were not significantly different from one another ($P > 0.1$), see Table 1.

In the remaining eleven experiments the intermediate, albumin, perfusate had a $\text{pH} < 7.05$. The value of K_f during the initial Ficoll perfusion, 0.265 ± 0.022 , $n = 85$, was not significantly different from the values obtained during Ficoll perfusion in the

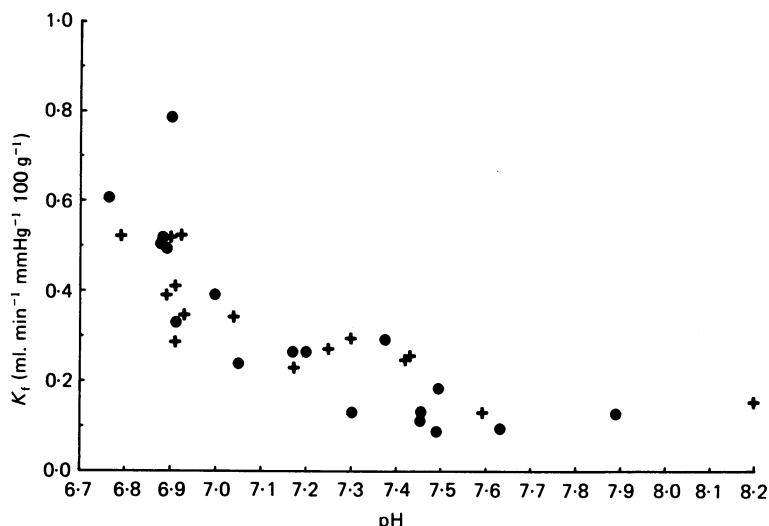


Fig. 5. The relationship between the pH of 3% (●) and 4% (+) albumin-containing perfusates and the mean values for K_f derived during the perfusion. Each point represents the mean value derived from a single experiment.

TABLE 1. Variation of K_f with pH

Experimental series 1	4% Ficoll ($\text{pH} > 7.05$) $K_f 0.274 \pm 0.031$ (34, 5)	4% albumin ($\text{pH} > 7.05$) $K_f 0.210 \pm 0.02$ (30, 5)	4% Ficoll ($\text{pH} > 7.05$) $K_f 0.212 \pm 0.030$ (29, 4)
Experimental series 2	—	3% albumin ($\text{pH} > 7.05$) $K_f 0.224 \pm 0.034$ (68, 8)	—
Grand mean	—	$K_f 0.219 \pm 0.023$ (98, 13)	$K_f 0.212 \pm 0.030$ (29, 4)
Experimental series 3	4% Ficoll ($\text{pH} > 7.05$) $K_f 0.270 \pm 0.020$ (53, 7)	3% albumin ($\text{pH} < 7.05$) $K_f 0.520 \pm 0.056$ (44, 7)	4% Ficoll ($\text{pH} > 7.05$) $K_f 0.559 \pm 0.075$ (40, 7)
Experimental series 4	4% Ficoll ($\text{pH} > 7.05$) $K_f 0.254 \pm 0.056$ (32, 4)	4% albumin ($\text{pH} < 7.05$) $K_f 0.486 \pm 0.048$ (32, 4)	4% Ficoll ($\text{pH} > 7.05$) $K_f 0.403 \pm 0.059$ (32, 4)
Grand mean	$K_f 0.267 \pm 0.018$ (119, 16)	$K_f 0.507 \pm 0.038$ (76, 11)	$K_f 0.502 \pm 0.055$ (72, 11)

Values are mean \pm s.e. of mean.

Numbers in parentheses are number of observations and number of experiments respectively.

other five experiments; however, on perfusing with albumin at $\text{pH} < 7.05$ K_f rose to 0.507 ± 0.038 and remained elevated at 0.502 ± 0.055 during subsequent perfusion with Ficoll solution at $\text{pH} > 7.05$, see Table 1.

Preliminary perfusion with Ficoll solution did not influence the value of K_f obtained during subsequent albumin perfusion. This was shown in eight experiments in which the tissues were perfused only with albumin solutions at $\text{pH} > 7.05$. The value of K_f obtained, 0.224 ± 0.034 (sixty-eight observations) was not different from the values previously found during the Ficoll solution experiments, see Table 1.

It is clear, from the results obtained in these experiments, that changing the pH of the albumin solution to a value that is less than 7.05 not only alters the hydraulic conductivity during that perfusion, but that the effect is retained during subsequent perfusion with Ficoll solution even though the pH was greater than 7.05.

Reversibility of the pH effect. The reversibility of the pH effect was only studied in one experiment. In this experiment, on changing from 4% Ficoll at pH 7.32 to 4% albumin at pH 6.79, the value of K_f rose from 0.258 ± 0.018 , $n = 8$ to 0.516 ± 0.095 , $n = 8$; then, on changing to 4% albumin at pH 7.59, K_f fell to 0.130 ± 0.038 , $n = 8$ and remained low, 0.287 ± 0.037 , $n = 8$, during a subsequent Ficoll perfusion at pH 6.79.

Effect of pH on the value K_f during Ficoll perfusion. Observations in the above experiment suggested that the pH effect was reversible; it also suggested that alterations of pH during Ficoll perfusion had no effect upon the value K_f . A direct comparison of the effects of changing pH during Ficoll perfusion was made during four experiments, the results showed that during Ficoll perfusion at pH > 7.05 , the value K_f obtained, 0.231 ± 0.030 , $n = 37$, was not significantly different from the value obtained during Ficoll perfusion at pH < 7.05 , i.e. 0.189 ± 0.021 , $n = 32$ ($P > 0.1$), see Table 1.

Vascular resistance. Vascular resistance was monitored by assessment of the perfusate pressure:flow ratio. No alteration in vascular resistance was observed when changing the pH of either Ficoll or albumin solutions.

Effect of pH and protein concentration on ionized calcium levels. Reductions in both calcium and magnesium concentration have been shown to bring about increases in vascular permeability (Clementi & Palade, 1969; Nicolaysen, 1970). Both ions compete for the same binding sites on serum albumin (Pederson, 1972) and since, in the experiments reported in this paper, magnesium was omitted from the perfusates, the ionized calcium level would be low; in consequence, small changes in ionized calcium resulting from changes in pH, might bring about significant changes in the endothelial permeability. The change in ionized calcium in response to alterations in pH of the McEwen solution, both with and without either 4% Ficoll or 4% bovine serum albumin are depicted in Fig. 6. It is apparent that Ficoll on its own has a negligible effect on the levels of ionized calcium in solution. A change from Ficoll to bovine serum albumin perfusion brings about a 50% reduction in ionized calcium. The change that occurs in the ionized calcium level of bovine serum albumin solutions when the pH is lowered from 7.1 to 6.9 is smaller than that observed in the Ficoll solution; the difference may be attributable to the calcium 'buffering' by albumin over this pH range (see Zurawski & Foster, 1974).

DISCUSSION

These experiments have shown that reduction in the pH of perfusing solutions, containing bovine serum albumin, to pH 7.05 or less causes an increase in the value of K_f (Fig. 5). This cannot be achieved by perfusing with solutions containing the non-protein colloid Ficoll, over the same pH range.

The results suggest that the changes observed in response to the alterations in pH involve the interaction of at least two components, serum albumin and the microvessel

wall; albumin, because a change of pH in the presence of the non-protein colloid Ficoll is ineffective; and the microvessel wall, because the increase in permeability is sustained, even after returning to perfusion with the non-protein colloid Ficoll solution at pHs greater than 7.05.

The importance of proteins for the maintenance of normal vascular permeability is well documented and much of the relevant literature is cited by Mason, Curry & Michel (1977). There is considerable support for the original concept of Danielli (1940) that the sites on the endothelial wall that are responsible for water movement are

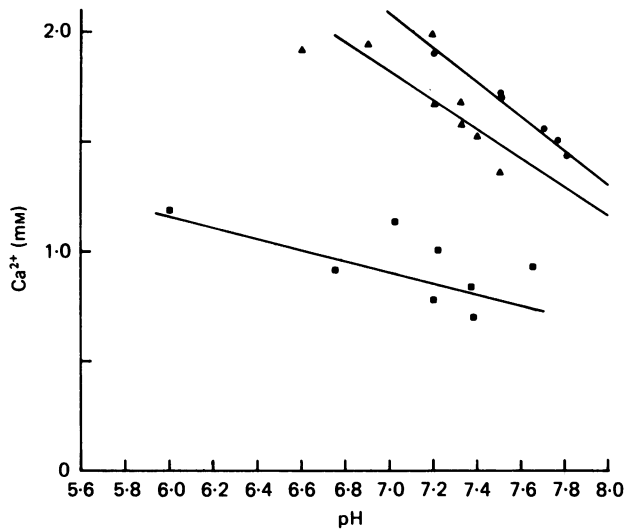


Fig. 6. Relationship between pH and ionized calcium levels in McEwen solution alone (●) and with the addition of 4% Ficoll solution (▲) or 4% bovine serum albumin (■). The continuous lines are regression lines based upon the individual values and have correlation coefficients of 0.99, 0.8 and 0.8 respectively.

lined by protein, whose presence restricts both water and further protein movement. The quantity of protein that is required for this lining effect is quite small; Mason *et al.* (1977) found that between 0.01 and 0.1 g albumin 100 ml.⁻¹ was sufficient to produce the maximal decrease in filtration coefficient in frog capillaries. In mammals, higher concentrations appear to be required, e.g. 0.2 g 100 ml.⁻¹ in the cat hind limb preparation (W. B. Kinter & J. R. Pappenheimer, unpublished observations reported by Landis & Pappenheimer, 1963) and 0.25 g 100 ml.⁻¹ in perfused rat mesentery (Gamble, 1978*b*). All of these studies showed that the protein effect was readily reversible. The evidence that the pH-protein effect is reversible is, at present, inconclusive.

The relationship between fluid movement across the microvessel walls (J_v) and the differences in hydrostatic pressure (P) and colloid osmotic pressure (π) is given by:

$$J_v = K_f(\Delta P - \sigma\pi),$$

where σ is the reflexion coefficient of the colloidal material.

The change from 4% Ficoll to 4% albumin at pH > 7.05 was not observed to give rise to an increase in tissue volume, owing to the increase in interstitial fluid resulting

from the difference in applied colloid osmotic pressure (see Fig. 3). This can probably be accounted for by the differences in the value for the reflexion coefficient (σ) and therefore the effective colloid osmotic pressure ($\sigma\pi$) for Ficoll and bovine serum albumin in this tissue system. Preliminary estimates of σ in the isolated mesentery have shown that it is in the region of 0.8 for albumin and between 0.4 and 0.6 for Ficoll (J. Gamble, unpublished data). The values for Ficoll are similar to those found by Michel & Phillips (1979) in frog mesenteric vessels but differ greatly from those of Ballard & Perl (1978) who quoted $\sigma = 0.91$ in the canine subcutaneous adipose tissue endothelium. If one applies the σ values of 0.8 for albumin and 0.5 for Ficoll, the resulting values of $\sigma\pi$ for 4% albumin and 4% Ficoll solutions would be 12 and 13 mmHg respectively.

The essential comparisons in the present paper are between albumin solutions of the same concentration, which differ only in their pH; under these circumstances, the alteration of pH should not bring about a change in either viscous resistance or capillary pressure; elevation of the venous pressure should bring about the same change in capillary pressure when either solution is used as a perfusate. Under these circumstances changes in J_v will be due either to a change in σ or to a change in K_f .

The relationship between permeability and pH depicted in Fig. 5 is reminiscent of the observations of Chambers & Zweifach (1940) who studied the onset of carbon sticking and tissue oedema in frog mesenteric capillaries that were perfused with frog Ringer solution, containing gelatin as a colloid, at a variety of pH values. They showed that as the pH was decreased, the time to the onset of carbon sticking, within the microvessels, and generalized tissue oedema was also decreased. The onset of carbon sticking always preceded the oedema formation. In Fig. 7, Chambers & Zweifach's values are compared with the values presented in this paper. (Chambers & Zweifach's values, presented as $t(\text{pH}x) : t(\text{pH } 8.0)$ where t = time to onset of either carbon sticking or oedema, are compared with the present values, represented as the ratio $K_f(\text{pH } 8.0) : K_f(\text{pH}x)$). It can be seen that these sets of data are comparable. In their paper, Chambers & Zweifach related the decrease in the time of onset of stickiness and oedema formation to the increase in ionization that occurs with more acidic pH.

There have been a number of investigations of the factors that influence the interactions between proteins and the proteoglycan material that lines the surface of both endothelial and epithelial cells. Venkatachalem & Rennke (1978), for example, demonstrated that the ability of macromolecules to penetrate the glomerular endothelial and epithelial layers depended upon the size and charge of the molecule under consideration and De Bruyn & Michelson (1979) showed that altering the pH of solutions containing cationic markers, of known isoelectric point, caused a change in the binding of these molecules to the anionic sites on the surface of the endothelium of bone marrow sinuses. More recently, several workers have suggested that albumin orders the cell coat matrix, thereby restricting the movement of other macromolecules into it (Michel & Phillips, 1981; Michel & Turner, 1981; Phillips & Turner, 1981 and Clough, Michel, Phillips & Turner, 1981). These observations show that proteins other than bovine albumin, e.g. ferritin and myoglobin could also influence the filtration coefficient and corroborated those of earlier workers by demonstrating that the efficacy of these molecules was influenced by their charge.

It is possible that the change in permeability observed when the pH of the albumin perfusate is altered is brought about by molecular transformation of the albumin molecules, see Leonard, Vijai & Foster, (1963) and Zurawski & Foster (1974). Zurawski & Foster labelled the free sulphhydryl residues of albumin to get a trifluoroacetylated albumin derivative, which was considered to behave like native albumin with respect to its neutral transition. They studied the effect of pH on the nuclear magnetic resonance spectra of the molecules and showed that a structural transformation of the albumin occurred over the range pH 7.0–9.0. The major changes

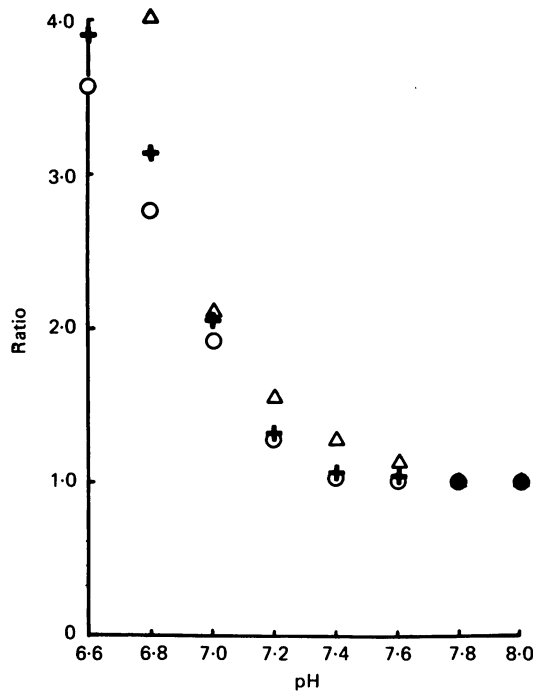


Fig. 7. Comparison of the effects of pH on the value of K_t observed in the present paper (Δ) depicted as the ratio $K_{t_{pHx}}:K_{t_{pH8.0}}$ and the observations made by Chambers & Zweifach (1940) on the time to the onset of oedema (+) and the time to the onset of carbon sticking (\circ) represented as the ratio $t_{pH8.0}:t_{pHx}$ (where t = time).

in binding capacity of the molecules were also observed over this range. These workers also found that if the observations were repeated in the presence of calcium, in concentrations approximating to those in plasma (2.0 mM), a shift in the conformational change was observed, so that a larger part of it occurred over the physiological pH range. Such changes in the molecular conformation may explain alterations in the binding capacity of albumin to free ions in the perfusate, as well as its ability to interact with sialic acid and other anionic groups in the glycocalyx.

In some experiments reported earlier (Gamble, 1978*b*) it was observed that the removal of protein from a 4% Ficoll solution at pH > 7.05, gave rise to a 70% increase in K_t in the isolated perfused rat mesentery and that these effects were reversible. Similar observations have since been made in frog mesenteric capillaries (Michel &

Phillips, 1979). In the present paper, it is shown that on returning to a Ficoll perfusion at pH > 7.05 after an intervening perfusion with albumin solution at pH < 7.05, the elevated value of K_f induced by the low pH albumin solution was sustained.

Zurawski & Foster's observations on the effects of calcium in conjunction with changes in pH may well be relevant in the interpretation of the present experimental results. They showed that the addition of 2.0 mM-calcium caused marked changes in the conformation and binding capacity of the albumin molecule over the physiological pH range. In the present experiments, 2.16 mM-calcium was added to the perfusing solutions and, during the Ficoll perfusions, about 90% of this was in the ionized form. During the albumin perfusions, the ionized calcium was only 1.0 mM (see Fig. 6) the remaining calcium being bound by the albumin itself. The reduction in the ionized calcium level, in conjunction with the low pH, may have had a significant effect upon the conformation and binding capabilities of albumin in these experiments.

The observations reported in this paper extend previous findings that the vascular exchange vessel permeability is modified by proteins by showing that the 'protein effect' is pH-dependent and it is suggested that mere perfusion of a tissue with protein free solutions does not displace all of the protein bound to the glycocalyx and therefore under these circumstances, the full potential of the protein effect may not be observed.

I would like to thank Professors M. de B. Daly and L. H. Smaje and Dr P. Knox for their helpful encouragement and criticism during the preparation of this manuscript. I would also like to thank Patricia Smart for her invaluable technical assistance, Chris Pelling for the estimations of pH, Peter Browne for his advice on statistics and for the regression analysis of the colloid osmotic pressure-concentration curves and Dr M. Jackson of the Department of Human Metabolism, University College Hospital, for the ionized calcium estimations. This project received some financial support from the Central Research Fund (University of London).

REFERENCES

- BALLARD, K. & PERL, W. (1978). Osmotic reflection coefficients of the canine subcutaneous adipose tissue endothelium. *Microvasc. Res.* **16**, 224-236.
- CHAMBERS, R. & ZWEIFACH, B. W. (1940). Capillary endothelial cement in relation to permeability. *J. cell. comp. Physiol.* **15**, 255-272.
- CLEMENTI, F. & PALADE, G. E. (1969). Intestinal capillaries. II. Structural effects of EDTA and histamine. *J. Cell Biol.* **42**, 706-714.
- CLOUGH, G. (1981). Investigation of the endothelial cell coat using cationized ferritin. *J. Physiol.* **320**, 40P.
- CLOUGH, G., MICHEL, C. C., PHILLIPS, M. E. & TURNER, M. R. (1981). Cationized ferritin reduces the permeability of frog capillaries: native ferritin does not. *J. Physiol.* **320**, 41P.
- CLOUGH, G. & SMAJE, L. H. (1978). Simultaneous measurement of pressure in the interstitium and terminal lymphatics of the cat mesentery. *J. Physiol.* **283**, 457-468.
- DANIELLI, J. F. (1940). Capillary permeability and oedema in the perfused frog. *J. Physiol.* **98**, 109-129.
- DAVIES, R. W. & GAMBLE, J. (1976). Changes in the rate of transudation of vascular fluid in the isolated rat mesentery following irradiation. *J. Physiol.* **266**, 71-72P.
- DE BRUYN, P. P. H. & MICHELSON, S. (1979). Changes in the random distribution of sialic acid at the surface of the myeloid sinusoidal endothelium resulting from the presence of diaphragmed fenestrae. *J. Cell Biol.* **82**, 708-714.
- DRINKER, Cecil K. (1927). The permeability and diameter of the capillaries in the web of the brown frog (*R. temporaria*) when perfused with solutions containing pituitary extract and horse serum. *J. Physiol.* **63**, 249-269.

- GAMBLE, J. (1978a). The effects of bovine albumin on the vascular permeability of the perfused rat mesentery. *J. Physiol.* **285**, 15–16P.
- GAMBLE, J. (1978b). The effect of low concentrations of bovine albumin on the vascular transudation coefficient of the isolated perfused rat mesentery. *J. Physiol.* **289**, 63–64P.
- LANDIS, E. M. & PAPPENHEIMER, J. R. (1963). Exchange of substances through capillary walls. In *Handbook of Physiology*, section 2, *Circulation*, vol. II, ed. HAMILTON, W. F. & Dow, P., p. 994. Washington D.C.: American Physiological Society.
- LEONARD, W. J., VIJAI, K. K. & FOSTER, J. F. (1963). A structural transformation in bovine and human plasma albumins in alkaline solution as revealed by rotary dispersion studies. *J. biol. Chem.* **238**, 1984–1988.
- LUNDVALL, J. & HOLMBERG, J. (1974). Role of tissue hyperosmolarity in functional vasodilatation in the submandibular gland. *Acta. physiol. scand.* **92**, 165–174.
- MASON, J. C., CURRY, F. E. & MICHEL, C. C. (1977). The effects of proteins on the filtration coefficient of individually perfused frog mesenteric capillaries. *Microvasc. Res.* **13**, 185–202.
- MC EWEN, L. M. (1960). The effect on the isolated heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol.* **131**, 678–689.
- MELLANDER, S. (1960). Comparative studies on the adrenergic neurohumoral control of resistance and capacitance blood vessels in the cat. *Acta. physiol. scand.* **50**, (suppl. 176) 1–86.
- MICHEL, C. C. & PHILLIPS, M. E. (1979). The effects of Ficoll 70 and bovine albumin on the permeability properties of individually perfused frog mesenteric capillaries. *J. Physiol.* **291**, 39P.
- MICHEL, C. C. & PHILLIPS, M. E. (1981). The effects of Ficoll 70 on the filtration coefficient of single frog mesenteric capillaries. *J. Physiol.* **315**, 12P.
- MICHEL, C. C. & TURNER, M. R. (1981). The effect of molecular charge on the permeability of frog mesenteric capillaries to myoglobin. *J. Physiol.* **316**, 51P.
- NICOLAYSEN, G. (1970). Permeability of lung capillaries: Role of Ca^{++} and Mg^{++} . In *Capillary Permeability*, ed. CRONE, C. & LASSEN, N. A., pp. 400–412. Copenhagen: Munksgaard.
- PEDERSEN, K. O. (1972). Binding of calcium to serum albumin. III. Influence of ionic strength and ionic medium. *Scand. J. clin. Lab. Invest.* **29**, 427–432.
- PHILLIPS, M. E. & TURNER, M. R. (1981). The effects of haemoglobin and myoglobin on the filtration coefficient of single frog capillaries. *J. Physiol.* **320**, 39P.
- VENKATACHALAM, M. A. & RENNKE, H. G. (1978). The structural and molecular basis of glomerular filtration. *Circulation Res.* **43**, 337–347.
- ZURAWSKI, V. R. & FOSTER, J. F. (1974). The neutral transition and the environment of the sulphhydryl side chain of bovine plasma albumin. *Biochemistry, N. Y.* **13**, 3465–3471.