Biochimica et Biophysica Acta, 551 (1979) 85 —94

BBA 78278

STUDIES ON THE FILTRATION PROPERTIES OF ISOLATED RENAL BASEMENT MEMBRANES

GARTH B. ROBINSON and THOMAS G. COTTER

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU (U.K.)

(Received July 18th, 1978)

Key words: Filtration properties; Proteinurea; (Basement membrane)

Summary

1. The filtration properties of films of renal basement membrane were studied in vitro using pressure filtration chambers.

2. Retention of cytochrome *c* by the films was found to be dependent upon the filtration pressure indicating that it was transferred across the films by convective as well as diffusive flow. In contrast, serum albumin was transferred by diffusive movement only.

3. When solutions containing both cytochrome *c* and IgG were filtered it was found that increasing the filtration pressure reduced the flux of cytochrome *c* across the films. A similar phenomenon occurred when serum was filtered, less protein passed through the films at high filtration pressures. These phenomena are explained by concentration-polarisation effects.

4. The flux of cytochrome *c* through the films was found to decrease in a non-linear manner as the film thickness was increased. With thin films, the flux of cytochrome *c* increased in a non-linear manner as the concentration of the protein in the overstanding solution was increased. With thicker films the flux was linearly dependent on concentration. These findings are interpreted as supporting the view that movement of cytochrome *c* occurs, at least in part, by convective flow.

Introduction

The initial step in the formation of urine, the ultrafiltration of blood in the glomerulus, has been studied widely in vivo [1,2]. Filtration is a complex process [3] since movement of solute through a filter barrier may result from diffusion, or from convective flow, and both processes may occur currently

depending upon the natures of the filter and of the solutes. In general, diffusion occurs through channels similar in size to the mean free path of the solute molecules while convective flow occurs through larger channels.

Flux of solute through filters may be formalised as

$$J_s = \frac{A}{t} \cdot \overline{D} \cdot (C_b - C_f) + \alpha \cdot C_b \cdot J_w \tag{1}$$

where J_s is the flux of solute; A, area of the filter; $\overline{D}$, the mean diffusion coefficient of solute in the membrane; C_b, solute concentration in the overstanding solution; C_f, solute concentration in the filtrate; t, membrane thickness; α, the fraction of the total solvent flux carried by convective channels; J_w, water flux.

Flux of solvent (water) is given by:

$$J_w = \frac{A}{t} \cdot \overline{P} \cdot (\Delta P - \Delta\pi) \tag{2}$$

where $\overline{P}$ is the mean permeability coefficient for water; ΔP, hydrostatic pressure difference; $\Delta\pi$, osmotic pressure difference. It should be noted that J_s is, in part, independent of J_w so that solute rejection ($\sigma = (C_b - C_t)/(C_b)$, where $C_f = (J_s)/(J_w)$) is not an absolute value but varies with changes in filtration conditions.

When solutions of macromolecules are filtered across microporous membranes filtration does not proceed in accord with theory, concentration-polarisation effects modify the behaviour of the filters [4]. Concentration-polarisation results from the build-up of a boundary layer of rejected macromolecules at the filter surface and this layer serves as a secondary filtration barrier. The formation of the polarisation layer depends upon the rate at which solute is carried to the filter face by solvent, the rate at which solute passes through the filter, and upon the rate at which solute diffuses back into bulk solution assisted by stirring. These relationships have been formally expressed [4] as

$$\frac{C_w}{C_b} = \exp \frac{J_w}{k_s^0} \tag{3}$$

where C_w is concentration of solute in the boundary layer and k_s^0 is a mass transfer coefficient defined by the stirring conditions and the diffusion coefficient of the solute. This polarisation phenomenon has been examined in some detail [4] and it has been established that both solvent flux and solute flux are affected by the formation of a polarisation barrier.

Returning to glomerular filtration, it is difficult to assess how the filtration behaviour of the glomerular capillary wall might change as filtration conditions are altered. Experiments are normally carried out in vivo where it is impossible to alter the filter conditions markedly without consequential changes in other parameters that might affect the preparation. Equally it is impossible to predict how polarisation might affect filtration across the barrier since there is no theory which describes polarisation effects when complex solutions such as plasma are filtered. Recently we developed a procedure for studying filtration in vitro across films composed of fragments of isolated renal basement mem-

branes [5] and this method was used to demonstrate that concentration-polarisation markedly influenced the rejection of serum proteins by these films [6]. Here we extend these observations by examining the effects of changing both filtration pressure and the thickness of the films on their filtration properties.

Materials and Methods

Basement membranes were isolated from rabbit renal cortex as described previously [7]. The preparations consisted mainly of tubular basement membrane rather than glomerular membrane, and were contaminated to a minor extent with interstitial collagen fibres. Filter films were constructed from the membrane fragments [6] by packing known amounts of basement membrane onto Millipore membranes (0.45 μm exclusion, Millipore, Ltd. London, U.K.) in pressure filtration chambers of 65 ml capacity with a filtration area of 13.8 cm^2 (Amicon Ltd., High Wycombe, Bucks., U.K.). All experiments were conducted at 20°C, the cells were pressurised with nitrogen gas and the contents were stirred magnetically at 1200 rev./min; solutions used for filtration studies were first filtered through Millipore membranes (described above). During filtration the filtrates were collected for timed intervals and experiments were terminated when no more than 20% of the volume of the overstanding solution had passed through the filter bed.

For filtration experiments, pure proteins (Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K.) were dissolved in 0.15 M NaCl containing 0.01 M Tris-HCl buffer, pH 7.4. Whole serum was obtained from rabbit blood taken by heart puncture immediately after death. Concentrations of pure proteins were measured by absorbance (A_{280nm}) of the solutions, when cytochrome *c* was mixed with horse immunoglobulin G (IgG), cytochrome *c* concentrations were measured as A_{540nm} values. When protein concentration was low, and in the case of serum proteins, the folin procedure was used [8]; with serum, proteins were precipitated with 5% (w/v) trichloroacetic acid and the precipitates were dissolved in reagent C [8] prior to colour development.

Gel exclusion chromatography was effected using 1 X 100 cm columns of Sephadex G-150 developed with buffered NaCl solution.

During filtration experiments and chromatography, NaN_3 (0.01%, w/v) was used as a bacteriostat.

Samples were prepared for electron microscopy using conventional fixation and staining procedures [7].

Results

The diffusive flow of solute through the filter films can be distinguished from convective flow since the latter is pressure dependent while the former is pressure independent (Eqn. 1). Accordingly the effects of increasing filtration pressure on the flux of proteins through membrane films was measured and the results are shown in Fig. 1; for these experiments each film was constructed using 1.5 mg of basement membrane protein. The flux of cytochrome *c* increased in a non-linear fashion with pressure, whereas the flux of bovine serum albumin was unaffected. In contrast, the flux of protein from whole

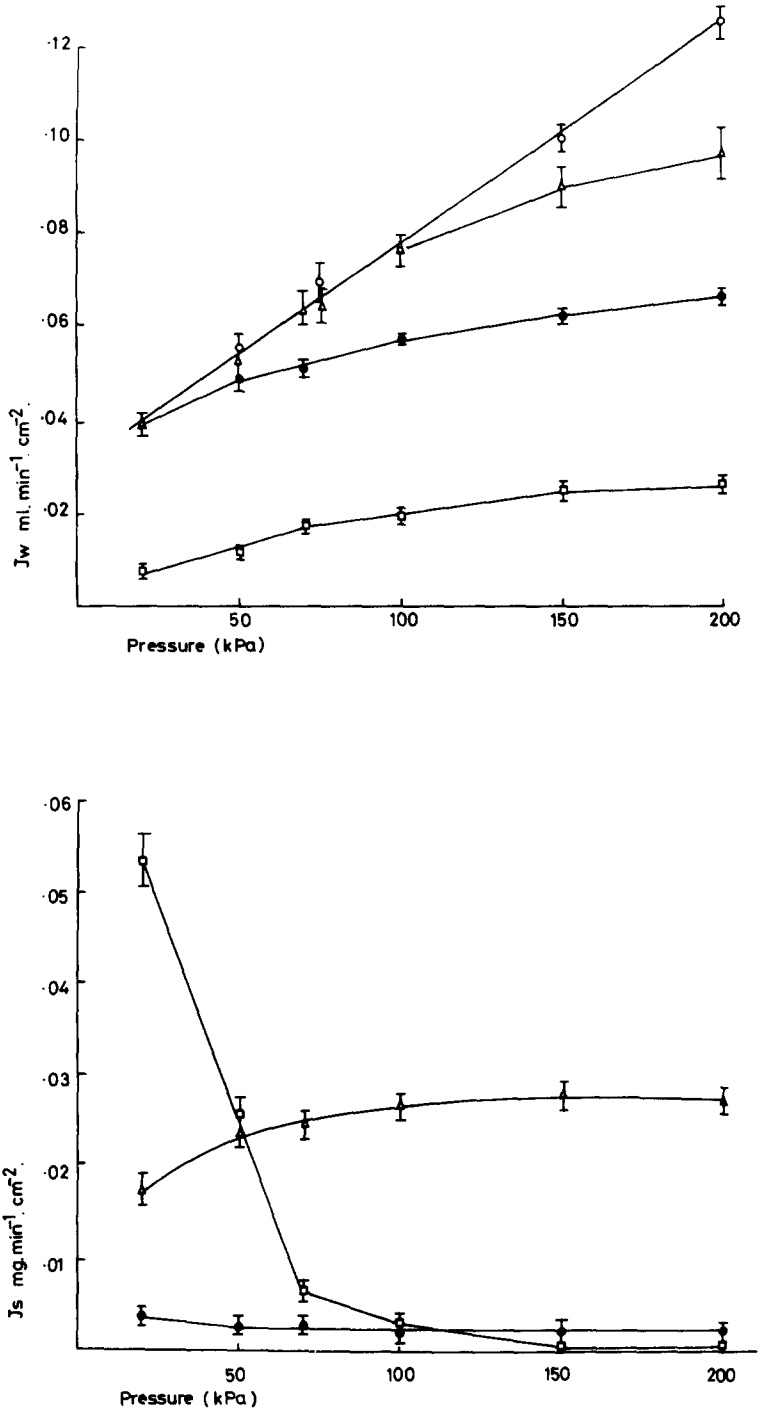

Fig. 1. Filtration of solutions of different proteins through films of renal basement membranes (1.5 mg of basement membrane protein) at different applied pressures, in stirred filtration chambers. Each result is the mean of three observations on each of three films. The bars represent ±1 S.D. Upper figure, water flux (J_W) ○, buffered saline; △, 0.5 mg cytochrome *c*/ml; ●, 0.5 mg bovine serum albumin/ml; □, whole rabbit serum. Lower figure, protein flux (J_S). Symbols as for the upper figure.

serum declined as the pressure was increased to reach very low values at pressure >50 kPa. When saline was filtered alone the flux of water increased linearly with pressure but the water flux rates increased more slowly with pressure when protein solutions were filtered; the lowest water flux rates were observed with serum.

The very low water fluxes obtained with serum indicated that a polarisation layer was being formed and was serving to reduce the permeability of the filter to water; it seemed that such a barrier might also obstruct the passage of protein. This notion was tested by examining the filtration of cytochrome *c* in the presence of a much larger protein, IgG. When a mixture of the two proteins was filtered it was found that the flux of cytochrome *c* diminished with increasing pressure (Fig. 2), in contrast to the results obtained when cytochrome *c* was filtered alone; the flux of water was also much reduced when the mixture was filtered. Thus filter behaviour was appreciably modified when mixtures of proteins were filtered as compared with solutions containing only one species of macromolecule.

When thicker membrane films were used (5.5 mg of basement membrane protein) essentially similar results were obtained with serum although the fluxes were reduced. However when cytochrome *c* was filtered alone the flux of protein was found to be unaffected by pressure (Fig. 3) in contrast to the results obtained with the thinner films.

A systematic investigation of the effects of increasing film thickness on the filtration of cytochrome *c* revealed that protein flux diminished in a non-linear manner as the thickness was increased, although the flux of water showed a linear regression; for convenience the thickness of the films was expressed in

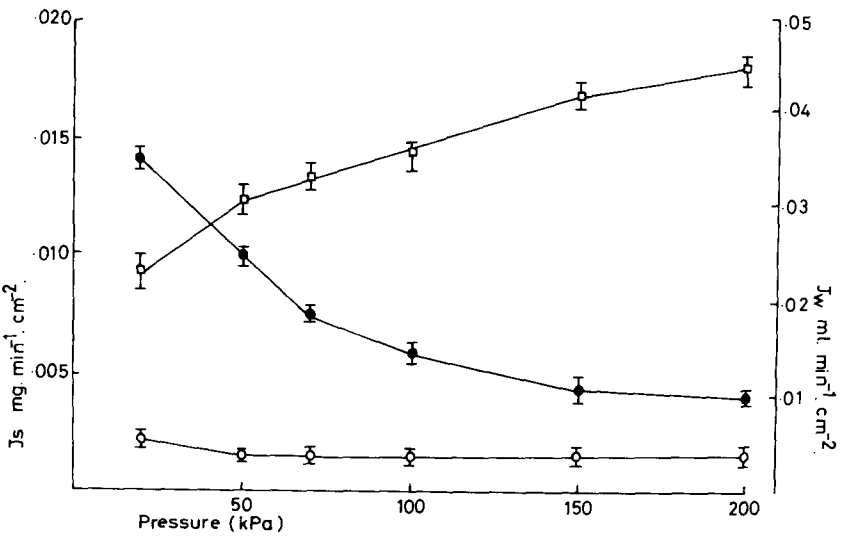

Fig. 2. Filtration of a solution containing 0.5 mg cytochrome *c*/ml and 2.0 mg IgG/ml at different pressures through basement membrane films in stirred filtration chambers. Each result is the mean of three observations on each of three films prepared from 1.5 mg of basement membrane protein; the bars represent ±S.D. Left ordinate, protein flux (J_S); right ordinate, water flux (J_W). □, water flux; ●, cytochrome *c* flux; ○, IgG flux.

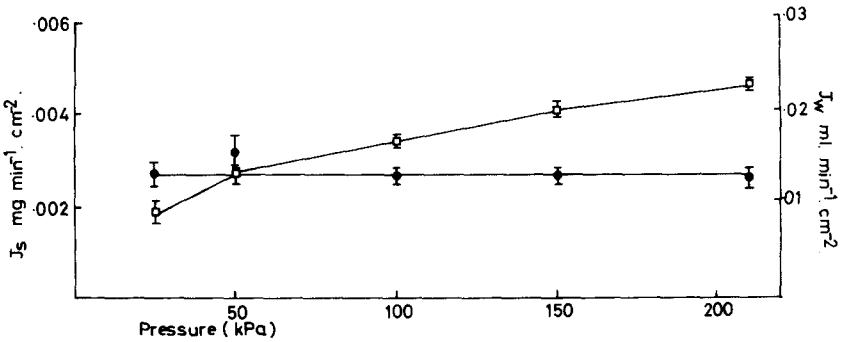

Fig. 3. Filtration of 0.5 mg cytochrome *c*/ml through films of basement membrane (prepared using 5.5 mg of basement membrane protein) at different pressures in stirred filtration chambers. Each result is the mean of three observations on three separate films ±1 S.D. Left ordinate, protein flux; right ordinate, water flux. •, protein flux; □, water flux.

terms of the amount of protein used to form the films (Fig. 4). Electron micrographs of the films showed them to be reasonably even in thickness (Fig. 5) but it proved impossible to measure the thickness in terms of the number of individual membrane layers since the close packing at the surface of the Millipore membrane rendered individual membranes indistinguishable. The average thickness could be estimated by measuring the thickness of the compacted layer and dividing this value by the mean thickness for an individual membrane. Films formed from 1.5 mg of protein were estimated to be

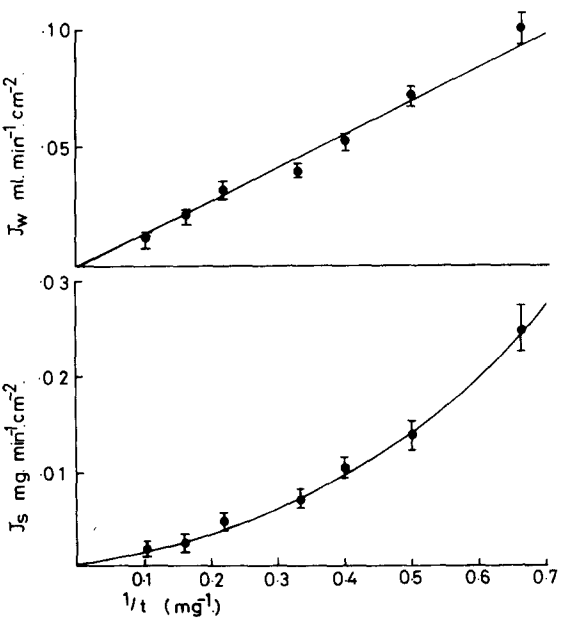

Fig. 4. Filtration of 0.5 mg cytochrome *c*/ml through films of basement membrane of different thickness in stirred filtration chambers at a pressure of 150 kPa. Each result is the mean of three observations on three films ±1 S.D. Lower figure, protein flux; upper figure, water flux.

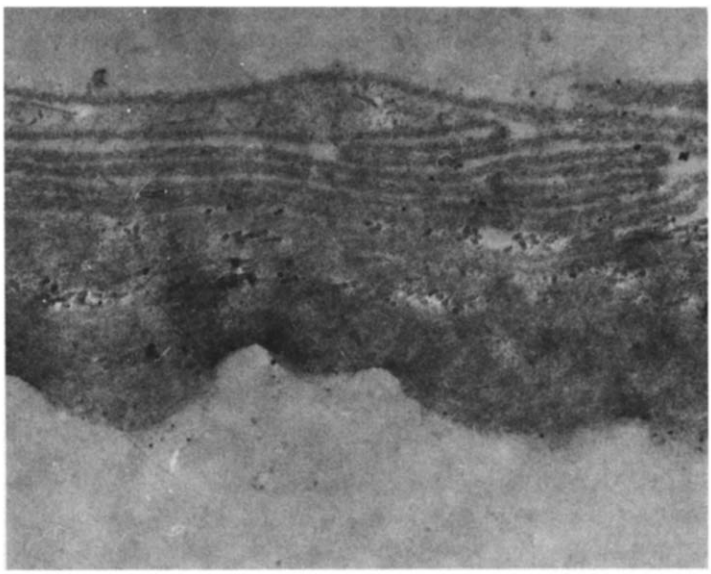

Fig. 5. Electron micrograph of a transverse section of a basement membrane film lying on a Millipore membrane. Some collagen can be seen lying between the layers of basement membrane. Magnification ×18 000.

comprised of thirty layers of individual membranes on average.

The effect of increasing the concentration of cytochrome *c* on flux through the films was studied. With thin films (1.5 mg of protein) the flux declined relative to the increasing concentration of cytochrome *c* in the overstanding solution becoming a linear function of concentration when the concentration

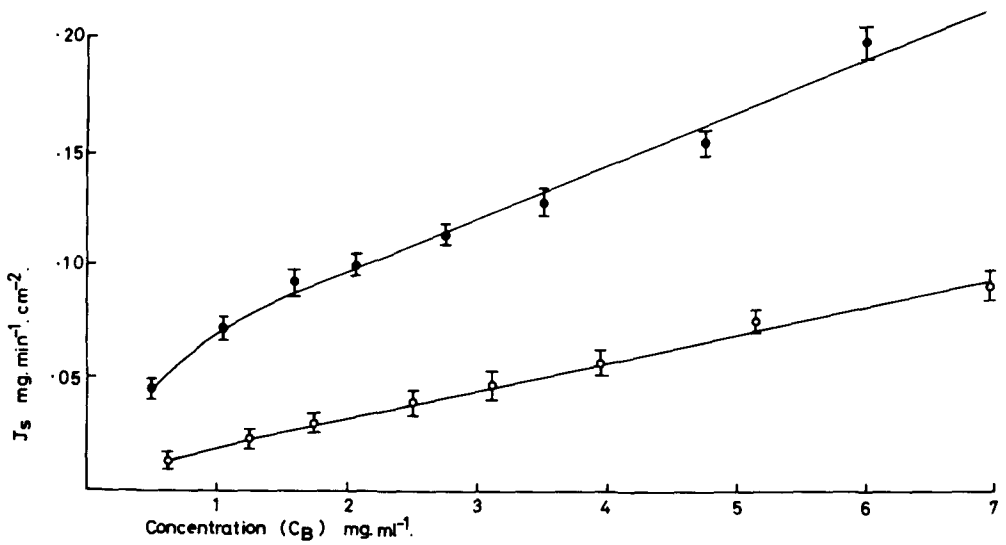

Fig. 6. The effect of increasing the concentration of cytochrome *c* on the flux of the protein through basement membrane films in stirred filtration chambers operated at 150 kPa pressure. •, film prepared using 1.5 mg of basement membrane protein; ○, film prepared using 2.5 mg of basement protein.

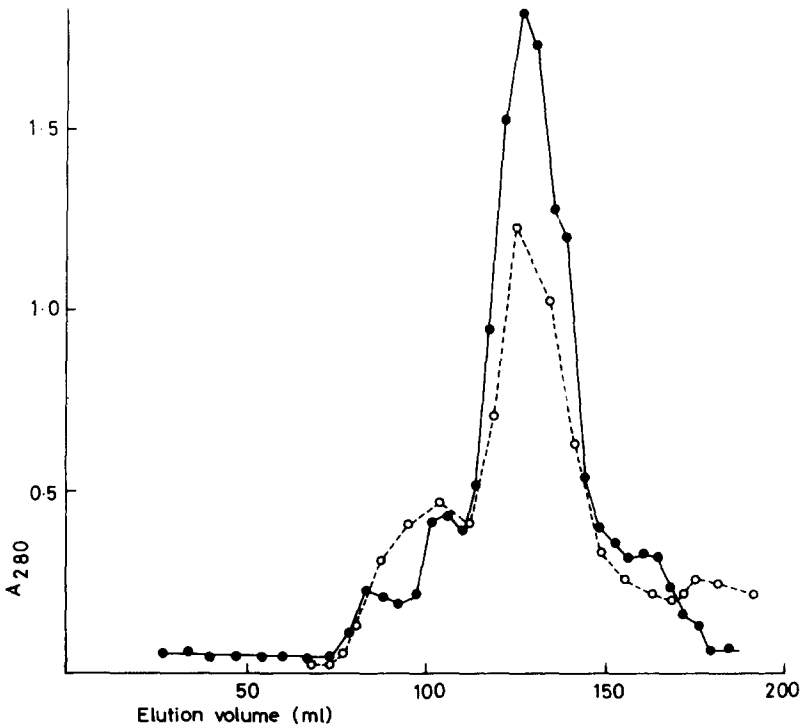

Fig. 7. Comparison of the molecular weight range of proteins in whole serum and in filtrates obtained from whole serum at a filtration pressure of 50 kPa, with filtration films prepared from 1.5 mg of basement membrane protein in stirred filtration chambers. Samples were separated on columns of Sephadex G-150, 1 X 100 cm, eluted with buffered saline. •, filtrate; ○, whole serum.

was >1.0 mg/ml (Fig. 6). With thicker membranes (2.5 mg of basement membrane protein) the flux showed a linear dependence on concentration over almost the whole of the concentration range (Fig. 6).

It was of interest to examine the size of the proteins which passed through the films when serum was filtered at low pressures. This was done by gel chromatography. The elution patterns (Fig. 7), when compared with those obtained using whole serum, revealed that large molecular weight material had passed through the films although the concentration of this material was less than that in whole serum. Thus at low filtration pressures the films were restricting but not preventing the passage of high molecular weight proteins.

Discussion

Perhaps the most striking result to emerge from this study was the observation that the flux of serum proteins through the basement membrane films decreased markedly as the filtration pressure was increased (Fig. 1). There could be several reasons for this. The membrane films act as molecular sieves because the membrane fragments overlap to form a coherent sealed matrix; at lower pressures the sealing may be inadequate so permitting protein to seep through. This seems unlikely as both albumin (Fig. 1) and IgG (Fig. 2) showed consistently low fluxes at all but the lowest filtration pressure. Alternatively

the membrane films might become compressed as the pressure increases so narrowing channels; if this had occurred changes should have been observed in the fluxes of albumin and IgG. The strikingly similar results obtained when cytochrome *c* was filtered in the presence of IgG suggest a further explanation. From a comparison of the results obtained when cytochrome *c* was filtered alone (Fig. 1) and when it was filtered with IgG (Fig. 2), it seems that the passage of cytochrome *c* through the filters was impeded by IgG, impedance increasing with increasing filtration pressure; such behaviour could result from concentration-polarisation. As the pressure is increased, more solute is carried to the filter face by the increased solvent flux (Eqn. 3). Consequently the rejected protein, primarily IgG, tends to become more concentrated there so occluding channels which admit cytochrome *c*. The notion that channels do become occluded is supported by the finding that the flux of water was reduced when IgG was present (Fig. 2). Such an effect could explain the results obtained when serum was filtered.

A curious result which emerged from these pressure studies was the finding that while the flux of saline through the films increased linearly with pressure (Fi.g 1) as predicted (Eqn. 2), the line did not extrapolate to zero flux at zero pressure. A similar phenomenon, termed surface-flow, has been observed in gas permeation [9].

In experiments where the effects of film thickness on filtration were studied, cytochrome *c* was used since it passed through the films more readily than larger proteins. As membrane thickness increased, the flux of cytochrome *c* diminished in a non-linear fashion (Fig. 4) although the flux of water diminsihed in a linear manner as expected (Eqn. 2). Flux of cytochrome *c* through thin films (Fig. 1) is pressure dependent indicating a convective flow component while flux through thick films (Fig. 3) is pressure independent indicating that it is diffusive. Thus it appears that the convective channels become obstructed in the thicker films; presumably either the continuity of the convective channels is lost as a result of the overlapping of increased numbers of membranes or increasing channel resistance counters the driving force.

When the flux of cytochrome *c* was measured with increasing concentrations of the protein in the overstanding solutions, it was found that the flux was linear with concentration for thicker films but not for thinner films. Flux through the latter films appeared to exhibit a saturable component which saturated at concentrations >1.0 mg/ml. This behaviour, seen only with thin films, might reflect some property of the convective channels. An increased concentration of solute in these channels would result in an increased viscous drag so limiting flux through the channels.

It is difficult to judge to what extent, if any, studies with this artificial system relate to glomerular filtration. Ryan and Karnovsky [10] have shown that the permeability of the glomerular filtration barrier alters when blood flow is stopped, as would be expected if concentration-polarisation influences filtration in vivo. Thus the phenomenon may well be important in vivo as well as in vitro. Clearly more studies are needed to confirm the suggestion that concentration-polarisation does modify filtration in vivo; however the phenomenon is an inevitable consequence of ultrafiltration and it seems reasonable to propose that it must influence glomerular filtration.

Acknowledgements

T.G.C. is grateful to the Pirie-Reid Trustees, University of Oxford, for financial support. We are indebted to Jennifer Byrne for technical assistance.

References

1 Renkin, E.M. and Gilmore, J.P. (1973) Handbook Physiol. 8, 185—248
2 Brenner, B.M., Bohrer, P., Baylis, C. and Deen, W.M. (1977) Kidney Int. 12, 229—237
3 Hwang, S.T. and Kammermeyer, K. (1975) Membranes in Separations, John Wiley and Sons, New York
4 Blatt, W.F., Dravid, A., Michaels, A.S. and Nelson, L. (1970) in Membrane Science and Technology (Flynn, J.E., ed.), pp. 47—97, Plenum Press, New York
5 Robinson, G.B. and Brown, R.J. (1977) FEBS Lett. 78, 189—193
6 Cotter, T.G. and Robinson, G.B. (1978) Clin. Sci. Mol. Med. 55, 113—119
7 Ligler, F.S. and Robinson, G.B. (1977) Biochim. Biophys. Acta 468, 327—340
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
9 Bartholemew, R.F. and Flood, E.A. (1965) Can. J. Chem. 43, 1968—1972
10 Ryan, G.B. and Karnovsky, M.J. (1976) Kidney Int. 9, 36—45