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EFFECT OF EXTRAVASCULAR PLASMA PROTEIN ON PRESSURE–FLOW RELATIONS ACROSS SYNOVIUM IN ANAESTHETIZED RABBITS

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SUMMARY

1. The effect of extravascular plasma protein on fluid flux through interstitial matrix was investigated *in vivo* by studying the pressure–flow relation across synovium during intra-articular infusions of protein solutions (usually bovine serum albumin). Synovium is a sheet of non-epithelial cells separated by interstitium-filled gaps, beneath which are fenestrated capillaries: synovium regulates synovial fluid volume and composition.

2. Albumin solutions (10–150 g l^{-1}) of measured oncotic pressure and viscosity were infused at known pressure into the synovial cavity of knees of anaesthetized rabbits. Flow across the synovial lining in the steady state (absorption rate $\dot{Q}_s$) was recorded at a series of joint pressures (P_j) to define the pressure–flow relation. Krebs solution was infused into the opposite knee as a control (26 animals).

3. Infusion of a low albumin concentration (10 g l^{-1}, bovine or rabbit) or diluted rabbit serum revealed no specific effect of plasma protein on interstitial matrix permeability (cf. specific protein effect on capillary glycocalyx permeability). Physiological (22·5 g l^{-1}) and higher concentrations reduced trans-synovial absorption rate. The slope of the pressure–flow relation was reduced and the pressure intercept displaced to the right (i.e. P_j at zero flow was raised).

4. Slope $d\dot{Q}_s/dP_j$ correlated negatively with intra-articular viscosity (P= 0·001–0·04), in keeping with viscous interstitial flow. The reduction in normalized slope, however, did not equal the reduction in fluidity (1/viscosity) quantitatively. It is proposed that apparent fluidity within the interstitial matrix is higher than in the bulk phase due to steric exclusion of albumin (radius 3·55 nm) by the interstitial glycosaminoglycans. The latter form spaces of estimated mean hydraulic radius 14–18 nm in synovium.

5. The joint–pressure intercept at zero net trans-synovial flow was displaced 0·015 cmH_2O per cmH_2O intra-articular oncotic pressure (π_j; S.E.M. ± 0·006). Thus large trans-synovial osmotic gradients were not maintained at physiological flow velocities. The 1·5% displacement of the P_j intercept by π_j was attributed principally to interstitial albumin exerting pericapillary oncotic pressure and enhancing net Starling filtration pressure. Indeed, net trans-synovial flow at low

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joint pressure sometimes reversed from absorption to filtration into the joint cavity at high intra-articular oncotic pressures.

6. The displacement of the trans-synovial flow intercept per unit change in intra-articular oncotic pressure, $(d\dot{Q}_s/d\pi_j)_{P_j=0}$, was 18 ± 3 nl min^{-1} cmH_2O^{-1}. This is significantly less than the change in trans-synovial flow per unit change in intra*vascular* oncotic pressure (51 ± 7 nl min^{-1} cmH_2O^{-1}; $P < 0{\cdot}001$). The asymmetry may result in part from interstitial albumin gradients around filtering capillary fenestrations.

7. Extravascular albumin thus influenced interstitial flow by both viscous and pericapillary oncotic mechanisms. The oncotic effect is the more important of the two under physiological conditions, where pressures and flows are small; but because the absolute magnitude of a viscosity-induced flow change is proportional to the size of the flow, viscosity becomes relatively more important at high flows (pathological conditions such as arthritis).

INTRODUCTION

Fluid and macromolecular transport through the extracellular matrix is a process of fundamental physiological and pathological importance, but the biophysics of interstitial fluid and macromolecule interactions is not well understood, partly because there have been relatively few experimental investigations (Bert & Pearce, 1984). Theoretically, extravascular plasma proteins may influence flow by modifying both the interstitial fluid viscosity and the water's chemical potential gradient (Taylor, Bert & Bowen, 1990), as well as by promoting microvascular filtration. A good opportunity to study such interactions, and to do so in a physiologically important context, is provided by the synovial lining (synovium) of a diarthrodial joint. Synovium generates and regulates the joint's synovial fluid, which is vital to lubrication and cartilage nutrition. Synovium is a thin, well vascularized sheet of mesenchymal tissue whose lining cells are separated by gaps 1–2 μm wide. The gaps contain a complex interstitial matrix comprising several types of collagen fibrils and glycosaminoglycans, and these create hydraulic resistance. Embedded within the matrix about 4–11 μm from the surface there is a rich network of fenestrated capillaries.

Fluid movement into or out of the joint cavity is a passive process dependent on local intra- and extravascular pressures. Trans-synovial flow was first studied systematically by Edlund (1949), who recorded the rate of absorption of saline from the cavity of the rabbit knee. At supra-atmospheric pressures some saline is absorbed by the synovial capillaries, but mostly the flow passes through the synovial intercellular spaces into the subsynovium, a zone of areolar connective tissue with lymph vessels. Edlund described an unusual pressure–flow relation in which the slope (i.e. synovial hydraulic conductance) increased steeply above ~ 9 cmH_2O intra-articular pressure (yield pressure), and this has been confirmed (Levick, 1979). Trans-synovial absorption rate is also proportional to plasma colloid osmotic pressure; and the hydraulic conductance of the plasma-to-cavity pathway increases at intra-articular pressures above yield pressure (Levick & Knight, 1988). The cause of the conductance increase is partly deformation of the intercellular pathway and perhaps also displacement of matrix components (Levick, 1991).

In the above studies, endogenous synovial fluid was replaced by a physiological saline solution. Normal synovial fluid, however, contains plasma protein (23 g l^{-1} in rabbit knee) and hyaluronan (5·5 g l^{-1}). Albumin is present at 54 % of the plasma concentration and accounts for most of the oncotic pressure, which is 13 cmH_2O (Knox, Levick & McDonald, 1988). The synovial lining is permeable to albumin (Simkin & Benedict, 1990). Hyaluronan contributes more to the fluid's viscosity than to its oncotic pressure. The question thus arises whether the endogenous macromolecules influence trans-synovial flow. This study attempts to answer that question for the protein component, and in doing so to gain some insights into a broader issue, namely how interstitial plasma proteins influence the universal process of tissue fluid percolation through the extracellular matrix.

METHODS

Methods in vitro

Measurement of colloid osmotic (oncotic) pressure. A lagged Hansen electronic membrane osmometer with a 5 mm diameter sample well was fitted with a PM10 membrane (exclusion > 10000 Da; Amicon, Lexington, USA) and calibrated with a water column; for construction details see Knox *et al.* (1988). Readings at room temperature (20–23 °C) and osmotic equilibrium (2–3 min) were adjusted to intra-articular temperature (35 °C) by the ratio of absolute temperatures (van't Hoff's law). Repeated measurements on the same sample gave a relative standard deviation of 2·9 % ($n = 9$) at 10·8 cmH_2O and 2·0 % at 24·5 cmH_2O ($n = 6$).

Capillary viscometry. Albumin increases a solution's viscosity as well as its oncotic pressure. The capillary viscometer comprised a horizontal glass capillary tube with horizontal graduated entrance and exit reservoirs designed to minimize errors from kinetic energy changes and entrance effects (Van Wazer, Lyons, Kim & Colwell, 1963). The capillary, cut from an Ostwald 2S90 kinematic viscometer, was 118 mm long and had a lumen radius of 0·174 mm (calculated from Poiseuille's law and mercury weighings). The large length/radius ratio rendered entrance effects negligible and ensured a fully developed parabolic velocity profile over > 99 % of the capillary length (Barr, 1931). The entry and exit reservoirs comprised 10 cm lengths of graduated volumetric tubing (0·5 ml E-mil gold-line glass pipette, Gallenkamp, UK) with 0·005 ml graduations and a lumen radius of 0·95 mm. Reservoir resistance was < 0·25 % of total viscometer resistance. The entry reservoir was connected by air-filled tubing to a 1 l air tank at a set pressure, measured to ± 0·1 cmH_2O by a water-filled U-tube manometer; the pressure-head generated Reynolds numbers of < 10, to ensure laminar flow. To minimize counterpressure from differences in meniscal curvature between retreating and advancing air–liquid interphases, sample volume was adjusted until the menisci formed in wide-bore plastic tubing (radius 2·5 mm) at the end of each reservoir.

Measurements were made in a thermostated water bath at 35 °C (mean intra-articular temperature). The capillary, entry and exit reservoirs were filled with test liquid and a small air bubble introduced into the entry reservoir as a flow marker. After connection to the pressure head an acceleration distance of 1 cm was allowed, then the time for the bubble to move a distance equivalent to 0·2 ml was recorded with a stop watch (> 20 s). The volume ΔV driven through a capillary tube of radius r in time t under a pressure gradient $\Delta P/\Delta x$ is related to viscosity η by Poiseuille's law (Pappenheimer, 1984):

$$\Delta V/t = \pi r^4(\Delta P/\Delta x)/8\eta. \quad (1)$$

For a constant ΔV, r and $\Delta P/\Delta x$, the viscosity of solution 1 relative to solution 2 (water or Krebs solution) is thus t_1/t_2. Accuracy was checked with sucrose solutions of documented viscosity (200–600 g l^{-1}; Barr, 1931). Pressure–flow plots were linear and the measured relative viscosities (1·9–37·0) were within ± 1·5 % of tabled values. Repeated determinations of flow time on the same albumin solution gave a standard deviation of ± 1·4 s around a mean of 85·2 s ($n = 6$, relative standard error ± 1·6 %).

In vivo

Animal preparation. This has been detailed previously (Levick 1979). New Zealand white rabbits (2·0–3·2 kg) of either sex were anaesthetized (I.V. sodium pentobarbitone, 30 mg kg^{-1}, plus urethane, 500 mg kg^{-1}, maintained by smaller half-hourly doses) and tracheostomized. Core temperature was controlled by a Havard animal blanket and rectal thermistor. With the animal supine, the hindlimbs were secured with the knees at 100–130 deg extension, an angle naturally adopted by the relaxed limb. Two cannulae (21-gauge hypodermic needles with terminal lateral perforations) were advanced into the suprapatellar joint space. One was connected to a water-calibrated Gould–Statham P23 pressure transducer at joint level to measure intra-articular fluid pressure (P_j, $\pm$ 0·2 cmH_2O). The other was connected to an infusion reservoir, the vertical height of which controlled intra-articular pressure. Flow of test solution from the reservoir into the cavity was recorded by an Elcomatic photoelectric drop counter (drop size 10·2 μl at flows < 300 $\mu l\ min^{-1}$). Total hydraulic resistance of the infusion system, 0·0178 $cmH_2O\ min\ \mu l^{-1}$, was ~ 2 % of the resistance of the cavity lining at low intra-articular pressure and ~ 12 % at the highest intra-articular pressure. Outputs were recorded on a SE6008 ultraviolet oscillograph (SE Laboratories, Feltham, UK). Correct insertion of cannulae was confirmed by dissection postmortem.

At the end of one experiment the radial pressure gradient in the tissue surrounding the joint was explored using a 23-gauge hypodermic needle with a lateral terminal perforation. The saline-filled cannula was preconnected to a second Gould–Statham pressure transducer and gradually advanced from the limb surface towards the joint cavity while maintaining intra-articular pressure at 21 cmH_2O. Tissue pressure was recorded continuously until finally the cannula broke through into the joint cavity.

Calculation of trans-synovial flow, $\dot{Q}_s$. Flow into the joint cavity after a step rise in infusion pressure consists of two phases: a rapid, declining inflow over 1–3 min due to elastic expansion of the cavity, followed by a slower sustained inflow that is due chiefly to trans-synovial absorption of fluid. A small part of the second phase, however, is caused by viscous creep of the cavity walls. The volumetric creep rate after 15–20 min ($\dot{Q}_{creep}$, $\mu l\ min^{-1}$),measured by experiments with non-absorbed oils, was:

$$\dot{Q}_{creep} = 0{\cdot}23\ P_j + 0{\cdot}4, \tag{2}$$

where P_j is joint fluid pressure in cmH_2O (Levick, 1979). Inflows 15–20 min after a pressure step were corrected by subtraction of $\dot{Q}_{creep}$ to give the steady-state trans-synovial flow ($\dot{Q}_s$). The relative magnitude of this correction depended on the absolute flow; at 60 $\mu l\ min^{-1}$ ($P_j = 20\ cmH_2O$) it represented an 8 % correction, whereas at 3·5 $\mu l\ min^{-1}$ ($P_j = 2\ cmH_2O$) the creep rate of 0·86 $\mu l\ min^{-1}$ constituted a 25 % correction.

In a few cases where intra-articular oncotic pressure was high and P_j was low, trans-synovial flow reversed direction and became a net filtration into the joint cavity. To measure this, a tap on the infusion cannula was closed to convert the joint cavity into a closed chamber, and the initial rate of rise of intra-articular pressure (dP_j/dt) was recorded. The reversed trans-synovial flow, $-\dot{Q}_s$, was then calculated as:

$$-\dot{Q}_s = (dP_j/dt)\,(dV/dP_j), \tag{3}$$

where dV/dP_j is the mean compliance of the rabbit synovial cavity at that pressure (Knight & Levick, 1982).

Determination of pressure–flow relation. In each animal one knee was infused with Krebs solution (control) and the other with test solution: the order was random. An initial infusion of 250–500 μl raised P_j from the endogenous subatmospheric pressure to between 0 (atmosphere) and 2·5 cmH_2O, the lowest pressure that generated a recordable absorption rate. Since endogenous fluid volume is ~ 24 μl (Knox *et al.* 1988), endogenous fluid was thereby diluted 10- to 20-fold and did not influence the experiment. This was confirmed by subsidiary experiments in which synovial fluid was flushed out by repeatedly filling and draining the cavity, a process that did not alter the results.

Intra-articular pressure was then increased in steps, usually 1·5–2·0 cmH_2O, by raising the infusion reservoir every 15–20 min. Trans-synovial flow was calculated from the inflow at the end of each period. The experiment continued until P_j reached ~ 24 cmH_2O. After the last flow measurement (3–4 h) a sample of intra-articular fluid was aspirated for analysis and comparison with infusate.

Protein solutions. Most test solutions consisted of bovine serum albumin (Cohn fraction V, Sigma Chemical Co., UK) dissolved in Krebs solution adjusted to pH 7·4 (for composition see Knight, Levick & McDonald, 1988). Five concentrations were investigated (10, 22·5, 55, 110, 150 g l^{-1}) and five to six animals were studied at each concentration. The oncotic pressure and viscosity of both the infusate and end-experiment aspirate were measured.

A 1:5 dilution of rabbit serum in Krebs solution was used as test solution in one animal (total protein ~ 10 g l^{-1}); and in another animal 10 g l^{-1} rabbit serum albumin (Sigma Chemical Co.) in Krebs solution was infused.

Subsequent to this study it was reported that infusion of commercial radiolabelled goat albumin caused inflammation in goat joints i.e. redness, warmth, swelling and leucocytosis, owing to endotoxin contamination (Bassett, Simkin, Jacobs & Roux, 1992). Commercial human albumin, however, was not contaminated. Several observations argue against an analogous problem with the bovine albumin used here. (i) The above signs of inflammation were not seen in the rabbit knees. (ii) Fresh diluted rabbit serum gave a result indistinguishable from that with commercial rabbit or bovine albumin. (iii) Results with 10 g l^{-1} commercial rabbit or bovine albumin were indistiguishable from results with Krebs solution alone. (iv) The rabbit synovial microvascular reflection coefficient (0·8) determined with Sigma Cohn fraction V bovine albumin was in the normal range for non-inflamed capillary beds (Knight *et al.* 1988).

Intra-articular temperature and heat transfer. Since intra-articular temperature affects osmotic pressure and viscosity, it was monitored in four experiments by a needle thermistor ($\pm$ 0·1 °C, Light Laboratories, Brighton). Normal rabbit knee temperature is 35·1 °C (Knox *et al.* 1988) and core temperature (rectal) was 37·5–40·0 °C. The infusate was not pre-heated above room temperature (21–24 °C), but intra-articular temperature nevertheless averaged 34·6 $\pm$ 0·4 °C ($n = 47$) at infusion rates of 2–100 μl min^{-1} in the steady state, and did not correlate with infusion rate (correlation coefficient, −0·072). The initial rapid filling with cool liquid after a step rise in infusion pressure (1–3 min) caused a transient fall in temperature by at most 1 °C at high inflows (> 300 μl min^{-1}), and temperature then quickly rose to normal. These observations indicated an efficient heat transfer from the adjacent vascularized tissues.

Analysis of pressure–flow relation and other statistical methods

The issue of how best to characterize trans-synovial pressure–flow relations mathematically (to allow statistical comparisons) was considered by Edlund (1949) and Levick (1979). Since the plots commonly showed a sharp bend at a certain pressure (yield pressure) with little or inconsistent curvature above and below this pressure, the empirical expedient was adopted of representing the relation by two straight lines, one above and the other below the yield point. In conformity with this practice, the relations above and below yield pressure here were fitted by linear regression analysis. Yield pressure was determined by eye, from inspection of the plotted relation. If the slope change developed over a small range of pressures rather than at a single point, the mid-range was adopted as yield point. Regression slopes were compared by Student's paired t test with $P < 0{\cdot}05$ accepted as a significant difference.

To describe the curvilinear viscosity–concentration relation, non-linear regression analysis by the simplex optimization method was used, as implemented in Fully Interactive Regression Statistics (Serious Statistical Software, Lynwood, South Wirral). Means are followed by standard errors throughout, and R denotes the correlation coefficient.

RESULTS

Oncotic pressure and viscosity curves in vitro

The polynomial relating concentration C (g l^{-1}, corrected for specified water content) to colloid osmotic pressure π (cmH_2O, 35 °C) for Cohn fraction V albumin was:

$$\pi = 0{\cdot}345C + 2{\cdot}66 \times 10^{-3}C^2 + 2{\cdot}26 \times 10^{-5}C^3. \quad (4)$$

The modest deviation from the Landis & Pappenheimer (1963) polynomial was discussed by Knight *et al.* (1988).

Albumin solution behaved *in vitro* as a Newtonian fluid (i.e. the pressure–flow relations were linear), but the relative viscosity η_{rel} increased as a non-linear function of concentration (Fig. 1*A*). Einstein's expression for the viscosity of a solution containing a low volume fraction of spherical solute is $\eta_{rel} = 1 + 2{\cdot}5(\theta)$,

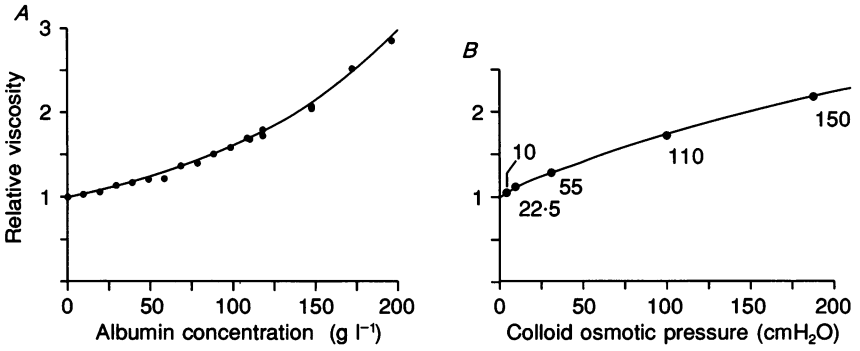

Fig. 1. *A*, viscosity of bovine serum albumin dissolved in Krebs solution as fraction of viscosity of Krebs solution alone. Continuous line is the Vand expression (see eqn (5)) fitted to the results by non-linear regression analysis. *B*, relation between viscosity and colloid osmotic pressure (oncotic pressure, 35 °C) of bovine serum albumin; concentrations infused *in vivo* are marked in grams per litre.

where θ is solute volume fraction; θ equals $C \times V'$, V' being the effective rigid specific volume of the hydrated solute. This linear relation did not describe the concentrations of interest here, however, and albumin viscosity relative to Krebs solution (35 °C) was better described by the Vand expression:

$$\eta_{rel} = \exp(K_{ES}\theta/(1-K_2\theta)), \tag{5}$$

where K_{ES} is the Einstein–Simha shape factor and K_2 is an arbitrary solute interaction parameter (Robinson & Stokes, 1959; Tanford 1961). Shape factor K_{ES} is 2·5 for a sphere but 4·17 for a prolate ellipsoid of axial ratio 3·5 (Mehl, Oncley & Simha, 1940), which is the axial ratio for serum albumin measured by small-angle X-ray scattering (Anderegg, Beeman, Shulman & Kaesberg, 1955). Non-linear regression analysis to find the values of V' and K_2 that best described the results gave 1·00 ml g^{-1} and 1·241 respectively (continuous line, Fig. 1*A*) i.e.:

$$\eta_{rel} = \exp(4{\cdot}17 \times 10^{-3}C/(1-1{\cdot}24 \times 10^{-3}C)), \tag{6}$$

where C is concentration in grams per litre. The value of V' (1·00 ml g^{-1}) is larger than the *partial* specific volume of albumin (0·733 ml g^{-1}; Putnam, 1977) indicating that albumin's solvation or 'fixed' water component is 0·267 millilitres per gram albumin in solution (~ 990 water molecules per albumin molecule). These represent water molecules within and close to the albumin molecule that are held sufficiently firmly to resist shearing stress. Oncley, Scatchard & Brown (1947) estimated the fixed water to be 0·264 ml (g albumin)$^{-1}$.

The relation between oncotic pressure and viscosity (Fig. 1*B*) deviated only modestly from a straight line. The tight coupling between the two factors was important in interpreting observations *in vivo*.

Reduced oncotic pressure and viscosity of solutions aspirated from joint cavity

For infused protein concentrations up to 22·5 g l^{-1}, the oncotic pressure of fluid aspirated from the cavity at the end of the experiment was not significantly different from that infused. With higher concentrations, the intra-articular fluid underwent a small but significant dilution in the steady state; the oncotic pressure of the 110 g l^{-1} infusate for example was reduced from 92·7 ± 3·2 cmH_2O (input) to 80·3 ± 4·0 cmH_2O (aspirate; $n = 6$, $P < 0{\cdot}01$, paired t test), corresponding to an intra-articular dilution of 7·9 % (eqn (4)). This tallied with a larger study of dilution *in vivo* by McDonald & Levick (1992), who attributed it primarily to a local influx of plasma ultrafiltrate via synovial intercellular spaces overlying capillaries. Results presented below are related to the final intra-articular values rather than infused values.

Aspirate viscosity was analysed to test whether viscosity had been raised by hyaluronan secretion during the experiment. The aspirate viscosities were found to lie around the curve in Fig. 1*B*, and a plot of measured aspirate viscosity against theoretical viscosity at the same oncotic pressure had a regression slope of 1·07 ± 0·05 ($R = 0{\cdot}986$), which was not significantly different from equality. This indicated that neither residual endogenous hyaluronan nor hyaluronan secretion by the synovial lining during the experiment had significantly altered the viscosity.

The term relative viscosity below refers to the aspirate viscosity relative to Krebs solution.

Pressure–flow relations for Krebs and low protein solutions

Results with Krebs solution (Fig. 2) were broadly similar to those with mammalian Ringer solution (Edlund, 1949) i.e. the relations were characterized by low slopes at low pressure and steep slopes at high pressure, and the change from one to the other was often abrupt, appearing as a kink in the pressure–flow plot. As noted previously (Levick, 1979) the plots displayed some variation in shape and in abruptness of slope change, raising the question of how best to describe them mathematically. Although it might be possible to fit a single continuous curve in certain cases, this would be difficult in cases with abrupt slope changes; the type of curve chosen would be empirical, and curve fitting would conflict with separate evidence that synovial hydraulic conductance alters little if at all below 8 cmH_2O (Knight & Levick, 1985). The latter observation supported the use of linear regression analysis at low pressures. Above yield pressure linear regression offered a standardized, statistically good description of relations that on inspection were sometimes linear (e.g. Fig. 3*A*, open circles above 8 cmH_2O; Fig. 3*B*, filled circles above 8 cmH_2O) and sometimes slightly curved, either towards the flow axis (e.g. Fig. 2, filled circles above 10 cmH_2O) or towards the pressure axis (e.g. Fig. 3*A*, filled circles above 12 cmH_2O). Description of the relation by two regression lines is illustrated in Fig. 3*A* (open circles).

At low pressures, the slope $d\dot{Q}_s/dP_j$, determined by linear regression analysis averaged $1{\cdot}84 \pm 0{\cdot}17$ μl min^{-1} cmH_2O^{-1} (intercept $-0{\cdot}28 \pm 0{\cdot}43$ μl min^{-1}, 43 joints). Above the mean yield pressure of $7{\cdot}4 \pm 0{\cdot}4$ cmH_2O, the mean slope was more than twice as steep, namely $3{\cdot}82 \pm 0{\cdot}35$ μl min^{-1} cmH_2O^{-1} ($P < 0{\cdot}001$). The mean slope below yield point was higher than in an earlier series (Levick, 1979), with substantial variation between animals.

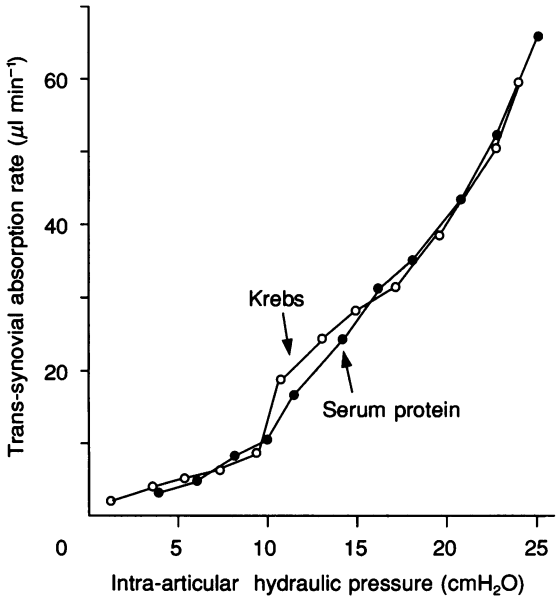

Fig. 2. Rate of absorption of liquid from the joint cavity as a function of joint fluid pressure in a pair of knees from the same animal. One joint was infused with Krebs solution (○) and the other with diluted rabbit serum (10 g l^{-1}, ●)

The pressure–flow relation for Krebs solution was first compared with that of a dilute albumin solution (10 g l^{-1}) to test for any specific effect of protein on the shape of the relation, or on synovial interstitial conductivity analogous to the major reduction in capillary permeability by albumin (the 'protein effect' e.g. Landis & Pappenheimer, 1963; Levick & Michel, 1973; Michel, Phillips & Turner, 1985). The concentration of 10 g l^{-1} was above that which elicits a 4- to 5-fold reduction in endothelial permeability (0·1–1 g l^{-1}; Huxley & Curry, 1991) but low enough to affect the solution's physical properties only slightly ($\eta_{rel} = 1{\cdot}02 \pm 0{\cdot}001$, $\pi = 3{\cdot}6 \pm 0{\cdot}10$ cmH_2O). In paired comparisons the yield pressure and pressure–flow relation were not significantly altered by bovine albumin (10 g l^{-1}), rabbit serum albumin (10 g l^{-1}) or diluted rabbit serum (Fig. 2). In six pairs of joints with a mean yield pressure of $8{\cdot}6 \pm 1{\cdot}0$ cmH_2O (albumin $9{\cdot}8 \pm 2{\cdot}0$ cmH_2O; Krebs $7{\cdot}8 \pm 1{\cdot}4$ cmH_2O, $P = 0{\cdot}26$) the pressure–flow relations were as follows ($P > 0{\cdot}05$).

Below yield pressure:

Albumin (10 g l^{-1}) $\dot{Q}_s = (1{\cdot}16 \pm 0{\cdot}47)P_j - (0{\cdot}79 \pm 0{\cdot}96)$.

Krebs solution $\dot{Q}_s = (0{\cdot}82 \pm 0{\cdot}28)P_j - (1{\cdot}18 \pm 1{\cdot}01)$.

Above yield pressure:

Albumin (10 g l^{-1}) $\dot{Q}_s = (4{\cdot}05 \pm 0{\cdot}58)P_j - (26{\cdot}78 \pm 7{\cdot}10)$.

Krebs solution $\dot{Q}_s = (3{\cdot}52 \pm 0{\cdot}53)P_j - (15{\cdot}88 \pm 6{\cdot}63)$.

Thus absence of endogenous plasma proteins did not cause the yield phenomenon. The experiments here did not test the protein effect at the capillary wall (cf. synovial lining) because endothelium was in contact with plasma throughout.

TABLE 1. Relation between trans-synovial absorption rate ($\dot{Q}_s$) and intra-articular pressure (P_j) in pairs of rabbit knees containing albumin solution or Krebs solution

Infusate	No. of animals (pairs of joints)	Slope $d\dot{Q}_s/dP_j$ (μl min^{-1} cmH_2O^{-1}) Below yield	Slope $d\dot{Q}_s/dP_j$ (μl min^{-1} cmH_2O^{-1}) Above yield	Intercept at zero pressure (μl min^{-1}) Below yield	Intercept at zero pressure (μl min^{-1}) Above yield
Albumin, 10 g l^{-1}	6	1·16 ± 0·47	4·05 ± 0·58	−0·79 ± 0·96	−26·8 ± 7·1
Krebs solution		0·82 ± 0·28	3·52 ± 0·53	−1·18 ± 1·01	−15·9 ± 6·6
Albumin, 22·5 g l^{-1}	5	2·41 ± 0·66	5·18 ± 1·02	−1·13 ± 1·02	−19·9 ± 8·7
Krebs solution		2·88 ± 0·59	6·41 ± 1·62	−1·18 ± 0·65	−29·4 ± 9·6
Albumin, 50 g l^{-1}	5	1·66 ± 0·16	3·41 ± 0·91	−1·59 ± 1·38	−11·3 ± 4·3
Krebs solution		3·21 ± 0·55	5·29 ± 0·96	−2·92 ± 1·80	−13·8 ± 7·8
Albumin, 110 g l^{-1}	5	1·02 ± 0·28	2·53 ± 0·78	−1·71 ± 1·40	−11·4 ± 5·1
Krebs solution		2·21 ± 0·54	4·24 ± 0·96	−0·93 ± 1·09	−16·1 ± 8·0
Albumin, 150 g l^{-1}	5	1·17 ± 0·39	1·96 ± 0·37	−3·96 ± 2·08	−8·4 ± 3·1
Krebs solution		2·01 ± 0·58	4·97 ± 0·78	−1·65 ± 0·52	−25·5 ± 6·6

All values are means ± S.E.M.

To assess extra-articular pressure at the end of an experiment (see Methods), Krebs solution was infused to a final intra-articular pressure of 21 cmH_2O ($\dot{Q}_s = 54$ μl min^{-1}), then the 23-gauge cannula was advanced manually in small steps from the limb surface through the medial quadriceps muscle towards the suprapatellar pouch. Some distance from the joint cavity, recorded pressure was atmospheric. As the probe approached the cavity, extra-articular pressure rose gradually to 1 cmH_2O. A further small advance caused pressure to jump suddenly to intra-articular pressure and dissection postmortem confirmed that the tip had just penetrated the synovial lining into the joint cavity. There was thus no evidence of a major build up of counter-pressure in the peri-articular tissues during the experiment.

Pressure–flow relations at physiological and high albumin concentrations

Protein concentrations of 22·5 g l^{-1} and above raised intra-articular oncotic pressure (π_j) and relative viscosity sufficiently to alter trans-synovial flow by detectable amounts. Concentrations of 22·5 g l^{-1} (aspirate mean $\eta_{rel} = 1{\cdot}06$,

$\pi_j = 8{\cdot}1$ cmH$_2$O) and 55 g l^{-1} (aspirate $\eta_{rel} = 1{\cdot}22$, $\pi_j = 24{\cdot}9$ cmH$_2$O) covered the physiological and pathological ranges. Because these concentrations produced smaller changes than expected, the concentration range was extended to 110 g l^{-1} (aspirate $\eta_{rel} = 1{\cdot}69$, $\pi_j = 80{\cdot}3$ cmH$_2$O) and 150 g l^{-1} (aspirate $\eta_{rel} = 2{\cdot}04$, $\pi_j =$ 137 cmH$_2$O). Five paired comparisons with Krebs solution (opposite knee) were

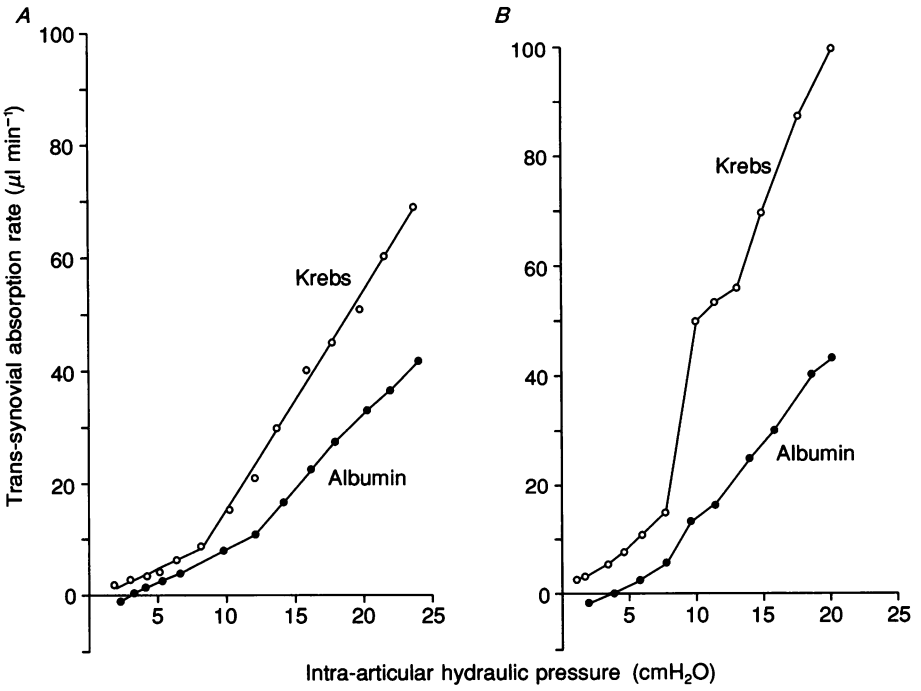

Fig. 3. *A*, rate of absorption of liquid across synovial lining in a pair of knees from the same animal. When a high concentration of intra-articular albumin was infused (110 g l^{-1}, ●), absorption rate was depressed, and at 2 cmH$_2$O joint pressure the direction of trans-synovial flow was reversed. Method of analysis by two regression lines is illustrated for the upper plot; the fitted lines were $\dot{Q}_s = 1{\cdot}10\ (\pm 0{\cdot}17)\ P_j - 0{\cdot}56\ (\pm 0{\cdot}89)$ and $\dot{Q}_s = 3{\cdot}75\ (\pm 0{\cdot}10)\ P_j - 22{\cdot}5\ (\pm 1{\cdot}7)$, with correlation coefficients of 0·95 ($P = 0{\cdot}004$) and 0·99 ($P < 0{\cdot}001$) respectively. *B*, similar experiment at a higher albumin concentration (150 g l^{-1}); note reduction in slope and shift in intercept.

carried out at each concentration, plus sixteen unpaired experiments. Figure 3 illustrates the changes in the Edlund curve caused by albumin and Table 1 summarizes the results of paired experiments. Raised intra-articular protein concentrations reduced the rate of fluid absorption from the joint cavity, and both the intercept and the slope of the pressure–flow relation were affected. Yield pressure was not altered significantly (mean $7{\cdot}7 \pm 0{\cdot}7$ cmH$_2$O for all albumin experiments) and did not correlate significantly with concentration ($R = 0{\cdot}24$, $P = 0{\cdot}2$). The effect on slope is analysed next.

Reduction in slope $d\dot{Q}_s/dP_j$ and relation to viscosity

Slope reduction was obvious above yield pressure (Fig. 3; $P < 0{\cdot}005$ at 150 g l^{-1}, $n = 5$) and bore a graded relation to albumin concentration. The slope of the

pressure–flow relation across a macroscopic porous medium depends *inter alia* on fluid viscosity η according to Darcy's law:

$$\dot{Q} = \kappa\,(\mathrm{d}P/\mathrm{d}x)(A/\eta), \tag{7}$$

where κ is specific hydraulic conductivity, $\mathrm{d}P/\mathrm{d}x$ is pressure gradient and A is area. In keeping with this, the experimental slopes correlated negatively with intra-articular viscosity over the range 1·02–2·04, both above yield pressure ($R = -0{\cdot}51$, $P < 0{\cdot}001$, $n = 41$) and below yield pressure ($R = -0{\cdot}32$, $P = 0{\cdot}04$, $n = 41$). The low but significant correlation coefficients reflected the large inter-animal variation.

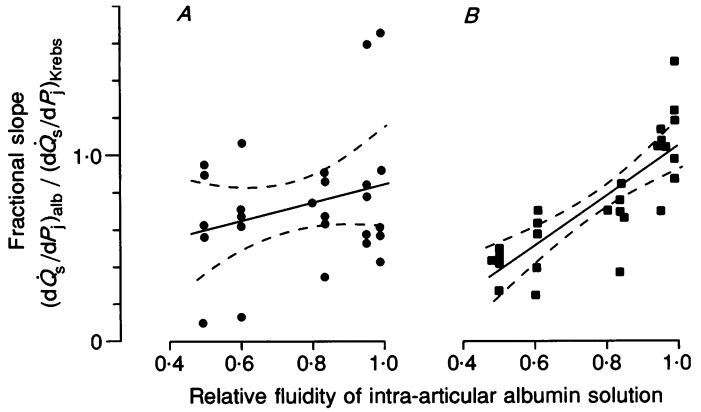

Fig. 4. Ratio of slope $\mathrm{d}\dot{Q}_s/\mathrm{d}P_j$ in presence of albumin to that in its absence (opposite knee) as a function of relative fluidity (fluidity of aspirated albumin solution relative to Krebs solution). Fitted lines are regression line and 95 % confidence intervals for mean (see text). Circles (*A*) and squares (*B*) denote slopes below yield pressure or above it respectively.

To test the agreement with Darcy's law quantitatively, the fractional reduction in slope (i.e. test slope/control slope) was compared with the fractional reduction in fluidity ψ (reciprocal of viscosity). Manipulation of eqn (7) shows that for a simple porous bed these two terms should be equal if other terms are constant:

$$\frac{(\mathrm{d}\dot{Q}/\mathrm{d}P)_{test}}{(\mathrm{d}\dot{Q}/\mathrm{d}P)_{control}} = \frac{\psi_{test}}{\psi_{control}} = \psi_{rel} = 1/\eta_{rel}. \tag{8}$$

Figure 4 shows the fractional slope, $(\mathrm{d}\dot{Q}_s/\mathrm{d}P_j)_{albumin}/(\mathrm{d}\dot{Q}_s/\mathrm{d}P_j)_{Krebs}$, plotted against the intra-articular relative fluidity ψ_{rel} at the end of the experiment. A slope of one (equality) would represent the Darcy relation (eqn (8)), but the results differed substantially from this. The regression line through the results above yield pressure (slope $1{\cdot}42 \pm 0{\cdot}20$, $R = 0{\cdot}82$, $P = 0{\cdot}001$) was significantly steeper that the Darcy slope of 1 ($P = 0{\cdot}05$). The regression line through the results below yield pressure had a smaller slope, only $0{\cdot}47 \pm 0{\cdot}37$ ($R = 0{\cdot}26$), and although not statistically different from 1 ($P = 0{\cdot}15$) it was significantly less steep than the line above yield pressure ($P = 0{\cdot}025$). The lack of agreement with Darcy's law is considered further in the Discussion.

Displacement of the pressure intercept; unequal effects of intra-articular oncotic and hydraulic pressures

The second effect of albumin was to shift the entire pressure–flow relation down and to the right by displacing the intercept. Figure 3 shows that to maintain zero net trans-synovial flow in the presence of raised intra-articular albumin, a raised

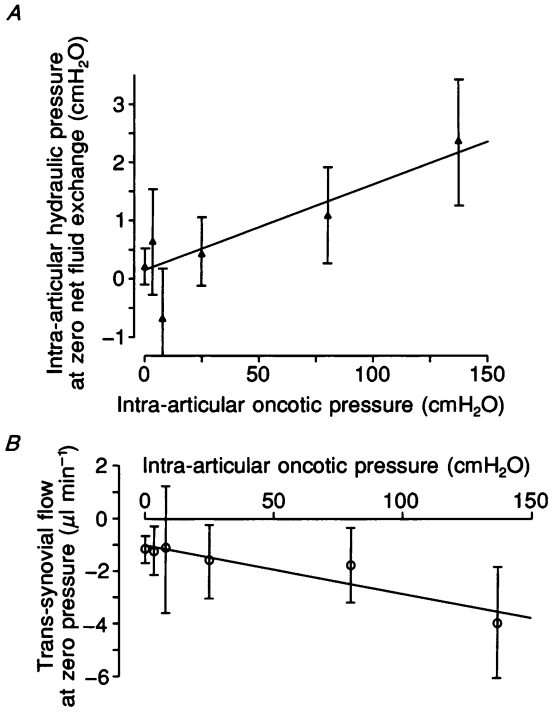

Fig. 5. Displacement of pressure–flow relation as function of intra-articular oncotic pressure. Symbols are means with standard error bars for infusions of 0, 10, 22·5, 55, 110 and 150 g l^{-1} albumin. *A*, intercept with the joint pressure axis (pressure at zero flow) as function of colloid osmotic pressure in the joint cavity at the end of the experiment. Slope of regression line is $0{\cdot}015 \pm 0{\cdot}006$ ($P = 0{\cdot}02$). *B*, plot to determine change in flow at zero pressure (intercept on flow axis) per unit change in colloid osmotic pressure in the joint cavity. Slope $(d\dot{Q}_s/d\pi_j)_{P_j=0}$ was -18 ± 3 nl min^{-1} cmH_2O^{-1} ($P < 0{\cdot}05$).

joint pressure was necessary. To analyse this effect further, the pressure intercept at zero flow was calculated from each of the regression lines fitted to sixty-six sets of pressure–flow data below yield pressure. The shift in intercept proved to be a graded effect, but as Fig. 5*A* shows the extra joint pressure needed to offset the rise in intra-articular oncotic pressure was very small and averaged only 1·5 % of the oncotic pressure i.e. $(dP_j/d\pi_j)_{\dot{Q}_s=0}$ was $0{\cdot}015 \pm 0{\cdot}006$ cmH_2O per cmH_2O oncotic pressure ($R = 0{\cdot}28$, $n = 66$, $P = 0{\cdot}02$). There was thus little, if any, effective osmotic pressure exerted by albumin across the synovial lining itself. The reflection coefficient of the synovial capillary wall, however, is ~ 0·8 (Knight *et al.* 1988) and the intercept increase was compatible with increased net capillary filtration pressure due to a rise in synovial interstitial oncotic pressure (see Discussion).

Flow intercept; asymmetry of flow response to intra-articular and intravascular oncotic pressure

Flow intercepts at zero pressure were calculated from the linear regression equations fitted to the results below yield pressure. The flow intercept became increasingly negative as intra-articular oncotic pressure was raised, i.e. there was

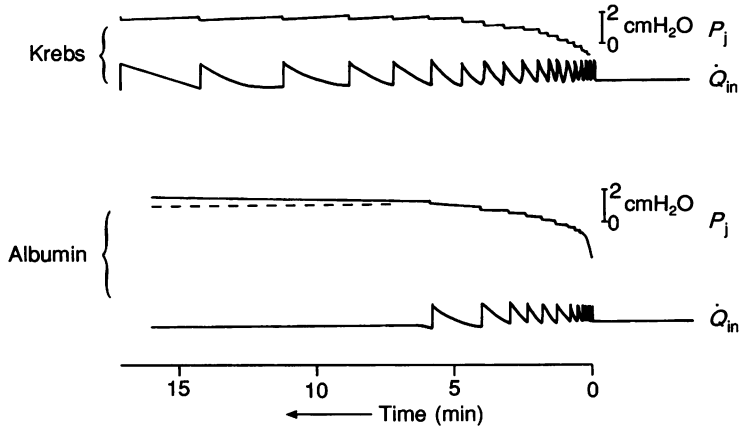

Fig. 6. Oscillograph records showing reversed trans-synovial flow at high intra-articular oncotic pressure (infused albumin 150 g l^{-1}); control infusion shown in upper pair of traces, same animal. Time axis runs from right to left. Upper trace in each pair shows joint fluid pressure; minor oscillations correspond with drop fall, and initial rise (right) is effect of raising infusion pressure. Lower trace in each pair is drop counter signal, each vertical step marking fall of a 10 μl drop. For the albumin solution, inflow ceases after inflation of the cavity, then pressure begins to rise, indicating net filtration into the joint cavity from the synovial lining (rate 3 μl min^{-1} here, from $dP/dt \times$ compliance).

increasing filtration into the joint cavity at zero pressure as oncotic pressure was increased (Fig. 5*B*). The change in flow per unit change in intra-articular oncotic pressure, $(d\dot{Q}_s/d\pi_j)_{P_j=0}$, was $-18{\cdot}2 \times 10^{-3} \pm 3 \times 10^{-3}$ μl min^{-1} cmH_2O^{-1} ($R = -0{\cdot}92$, $P < 0{\cdot}05$). By contrast, in a study of perfused synovial microcirculation the change in trans-synovial fluid absorption induced by intra*vascular* albumin was nearly three times larger, $d\dot{Q}_s/d\pi_p$ being $51 \times 10^{-3} \pm 7 \times 10^{-3}$ μl min^{-1} cmH_2O^{-1} below yield pressure (Levick & Knight, 1988). Comparison of the two regression coefficients showed that the asymmetry of the intra-articular and intravascular responses to albumin was highly significant ($P < 0{\cdot}001$, *t* test); see Discussion.

The response of trans-synovial flow to intra-articular oncotic pressure, $(d\dot{Q}_s/d\pi_j)_{P_j=0}$, was only 1–2% of its response to intra-articular hydraulic pressure below yield point $(d\dot{Q}_s/dP_j)_{\pi_j=0}$, as expected from the low value of $(dP_j/d\pi_j)_{\dot{Q}_s=0}$, namely 0·015.

Reversal of flow by high intra-articular oncotic pressure at low P_j

The most direct evidence that intra-articular albumin could influence trans-synovial flow via oncotic pressure was obtained by infusing high concentrations of albumin at low pressures. This often resulted in reversal of the measured net trans-synovial flow. Net filtration into the cavity against opposing intra-articular

pressures of 0·8–2·5 cmH_2O were observed in two of five experiments with 110 g l^{-1} albumin and three of five experiments with 150 g l^{-1} albumin. Net filtration was recognized by the cessation of inflow through the drop counter followed by a rising intra-articular pressure (Fig. 6*B*) – an event also reported in implanted Guyton capsules when protein concentration is raised (Stromberg & Wiederhielm, 1970). Filtration rates calculated from dP_j/dt (see Methods) ranged up to 8·5 $\mu l\ min^{-1}$. When Krebs solution alone was infused, net filtration into the cavity was not seen at supra-atmospheric joint pressures (Fig. 6*A*). The filtration induced by intra-articular albumin was attributed to increased Starling imbalance across the synovial capillary wall; see Discussion.

DISCUSSION

Intra-articular protein reduced the rate of trans-synovial fluid absorption by viscous and oncotic mechanisms, manifested as slope and intercept changes; but it did not alter the fundamental shape of the pressure–flow relation (yield phenomenon). The failure to detect any specific effect of protein on intrinsic hydraulic permeability, akin to that in capillaries, may be because the interstitial glycoproteins and glycosaminoglycans have different binding properties to those forming the endothelial glycocalyx, or are less free to alter their conformation upon protein binding. A third possibility is that endogenous plasma proteins were not adequately displaced from the Krebs-perfused matrix despite it being flushed with Krebs solution continuously for several hours.

Relation to previous studies

There is little previous work on the physiological effect of intra-articular protein, although viscosity-related reductions in interstitial flow by albumin have been described in artery walls (Tarbell, Lever & Caro, 1987). Edlund (1949) injected haemoglobin into rabbit knees (55 g l^{-1}, $\pi_j = 25$ cmH_2O) and found no change in the characteristic shape of the trans-synovial pressure–flow relation: but quantitative comparisons between the haemoglobin and control curves were not made. Palmer & Myers (1968) observed that replacement of a protein-rich effusion in human rheumatoid knees by an equal volume of normal saline was followed by partial fluid absorption, which is compatible with the effects observed here; however, the simultaneous removal of endogenous hyaluronan may have contributed to their result. Newbould (1983) found that injection of hyperosmotic dextran solution into dog knees caused a small but progressive increase in pressure, indicating filtration into the joint cavity (as in Fig. 6*B*); and the rate of increase depended on the concentration and molecular weight of the dextran. The present findings are thus compatible with the small existing literature.

Causes of the slope and intercept changes

The synovial lining itself is evidently not a semi-permeable membrane since $d\dot{Q}_s/d\pi_j$ (flow response to intra-articular oncotic pressure) was only 1–2 % of $d\dot{Q}_s/dP_j$ (response to hydraulic pressure). This tallies with the lining's permeability to albumin (Simkin & Benedict, 1990). The synovial capillary on the other hand is a semi-permeable membrane with an albumin reflection coefficient of 0·8 (Knight *et*

al. 1988). The effect of intra-articular albumin on slope was therefore attributed primarily to albumin permeating the interstitial matrix and increasing interstitial fluid viscosity; and the effect on intercept was attributed primarily to interstitial albumin exerting osmotic pressure at the synovial capillary wall, thereby

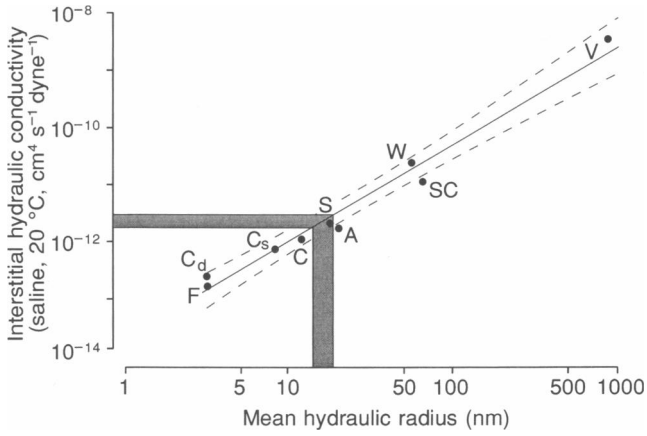

Fig. 7. Logarithmic relation between measured hydraulic conductivity of interfibrillar interstitial matrix to saline at 20 °C (k) and mean hydraulic radius (r_H) calculated from biochemical density as void volume/fibre surface area, for a range of tissues; data from Levick (1987 *b*). Regression line, with 95% confidence intervals for mean, was log $(k) = 1{\cdot}74\ (\pm 0{\cdot}11) \log r_H - 13{\cdot}85\ (\pm 0{\cdot}172)$; $R = 0{\cdot}98$, $P < 0{\cdot}001$. Shaded zone is synovial interstitial conductivity below yield pressure (Levick 1991) and the interpolated hydraulic radius is 14·1–18·0 nm. A, aorta; C, corneal stroma; C_s and C_d, condylar cartilage (femoral), superficial and deep respectively; F, femoral head cartilage; S, scleral stroma; SC, subcutis; V, vitreous body; W, Wharton's umbilical jelly

increasing net capillary filtration pressure. Evidence for enhanced capillary filtration in the presence of intra-articular albumin was presented by McDonald & Levick (1992) and supported here by flow reversal (Fig. 6).

There are indirect reasons for believing that the interstitial reflection coefficient to albumin may be > 0·12 (McDonald, 1988), which at first sight seems at variance with the finding that the shift in pressure intercept, $(dP_j/d\pi_j)_{\dot{Q}_s = 0}$, was only 0·015. The two values are reconciled, however, by the low velocity of the interstitial fluid ($\sim 8 \times 10^{-5}$ cm s^{-1} at 50 μl min^{-1}), which allows diffusional relaxation of interstitial concentration gradients and therefore osmotic gradients. The ratio of solute velocity caused by solvent drag to restricted diffusional velocity, the Peclet number, was estimated to be $\ll 1$ in these experiments (McDonald, 1988).

Relation of slope $d\dot{Q}_s/dP_j$ to bulk-phase fluidity

If the above view is correct (i.e. slope reduction is due primarily to albumin-induced increases in interstitial viscosity), the lack of agreement with Darcy's law in Fig. 4 requires explanation. Since the deviation above yield pressure was in the opposite direction to that below yield pressure and the two were statistically significantly different, they will be considered separately.

Below yield pressure. When intra-articular albumin concentration was raised at low pressures, fractional slope fell approximately only half as much as intra-articular fluidity, raising the possibility that fluidity within the synovial interstitium might be greater than in the joint cavity. Although the deviation from Darcy's law did not achieve statistical significance ($P = 0{\cdot}15$), deviation in the observed direction can be predicted on the basis of known interactions at the molecular level. Pappenheimer (1953) pointed out that in order for flow to obey a simple viscous flow law, solute radius must be less than 1/10th channel radius. For larger solute:channel ratios, Renkin (1954) suggested that intrapore viscosity is related to solute concentration within the pore and consequently is lowered by steric exclusion. The ratio of solute to channel size can be estimated roughly from albumin's hydrodynamic radius (3·55 nm) and the mean hydraulic radius of the intramatrix spaces (r_H), which is defined as the ratio of void volume to fibre surface area. Interpolation from a plot of interstitial hydraulic permeability against r_H for a range of tissues (Fig. 7) gives a synovial r_H of 14–18 nm below yield pressure. This is equivalent to a cylindrical channel radius of 28–36 nm. The estimated solute:channel ratio is thus $> 0{\cdot}1$ and falls in the range where steric exclusion and deviation from Darcy's law may be expected.

Steric exclusion of albumin from substantial fractions of the interstitial water space is well documented in other tissues (e.g. Bert & Pearce, 1984). For a synovial interstitial glycosaminoglycan concentration of ~ 16 mg ml^{-1} the excluded volume fraction for albumin would be 0·35, calculated by the random fibre theory of Ogsten (1958). If part of the water flux passes through albumin-excluded space (fluidity = 1) then the effective interstitial fluidity will be higher than bulk-phase fluidity. (An analogy may be drawn here with the anomalous fluidity of particulate suspensions such as blood flowing along a narrow tube, fluidity being raised by the flow of solvent along a marginal particle-excluded annulus (the Fahreus-Lindquist effect).) There are thus biophysical grounds for proposing that fractional hydraulic conductance should decline less than bulk-phase fluidity in a dense random fibre matrix.

Other factors that might contribute to deviation from Darcy's law are as follows. (i) Interstitial albumin concentration (and hence fluidity) may be reduced locally near capillary fenestrations due to capillary filtration. (ii) Transcapillary flow, which affects the net observed trans-synovial flow, has an effective fluidity of nearly 1 because albumin is largely excluded from the capillary small pore system. Calculations indicate, however, that effects (i) and (ii) had rather small effects here. (iii) Intra-articular fluidity below yield pressure may have been greater than that measured at the end of the experiment when joint pressure was high. This emerged later, in studies stimulated by the discovery here of end-experiment dilution. The later work showed that infusate dilution is more pronounced at low pressure (McDonald & Levick, 1992). Dilution is thought to be caused by localized capillary filtration into the cavity even when net flow is out of the cavity (simultaneous bidirectional flow; Levick, 1991). Nevertheless, dilution is unlikely to explain fully the raised apparent fluidity, because subsequent experiments with concurrent fluid sampling, conducted postmortem (to eliminate capillary filtration), confirmed the

existence of a raised fluidity anomaly unrelated to intra-articular dilution (McDonald, 1988). (iv) The possibility of osmotic shrinkage of the interstitial pathway by albumin is noted, altering pathlength and fibre concentration; but the compliance of synovial interstitium, $\Delta V/\Delta P$, is very small, at least over the range 5–25 cmH_2O (Levick & McDonald, 1989).

Above yield pressure. Here the fractional slope declined more than the relative fluidity (ratio 1·4, Fig. 4) in contrast to Darcy's law and in contrast to the biophysical prediction in the preceding section. The reason relates to the fact that slope $d\dot{Q}_s/dP_j$ above yield pressure is a compound parameter; it does not represent a simple hydraulic conductance, because conductance increases as a continuous function of joint pressure above yield point (Knight & Levick, 1985). Even with a minimalistic one-dimensional model (a variable intimal interstitial resistance in series with two parallel resistances, one across the capillary wall and one through deeper interstitium into subsynovium), the expression for $d\dot{Q}_s/dP_j$ above yield pressure is a composite term involving oncotic pressure gradients as well as conductances, as expressed by eqn (4A) of Knight & Levick (1985). Re-expressing interstitial conductance as conductivity × fluidity, the one-dimensional model predicts that the relation between fractional slope and fluidity is steeper above yield pressure than below it, in keeping with the experimental observations in Fig. 4. The reason is that the interstitial resistance located between capillary and cavity declines as P_j is raised above yield pressure. This progressively increases the relative importance of capillary filtration induced by pericapillary albumin. As a result the net trans-synovial flow increases with pressure less steeply than it would in a simple porous medium, i.e. the slope of the pressure–flow relation is depressed in the presence of albumin more than would be predicted from simple fluidity considerations.

Asymmetry between effect of intra-articular albumin ($d\dot{Q}_s/d\pi_j$) and intravascular albumin ($d\dot{Q}_s/\pi_p$)

The reversal of trans-synovial flow by high intra-articular oncotic pressure (Fig. 6) supported the view that intra-articular albumin enhances microvascular filtration into the cavity by raising pericapillary oncotic pressure (Starling's principle of fluid exchange). Rabbit synovium is one of the few tissues where responses to all four Starling pressures have been systematically investigated in the same tissue, and an interesting difference emerged between the magnitude of $(d\dot{Q}_s/d\pi_j)_{P_j=0}$ and $d\dot{Q}_s/d\pi_p$. The former (response to intra-articular albumin) was only 35 % of the latter (response to intravascular albumin), even though the nearest point on the capillary wall was only a few micrometres from the joint cavity (Levick & McDonald, 1989) and the intervening tissue is permeable to albumin.

There have been few analogous studies with which to compare this finding. No asymmetry was reported between the filtration responses to intravascular oncotic pressure and extravascular albumin up to 50 $g\,l^{-1}$ in rat omentum continuous capillaries (Smaje, Zweifach & Intaglietta, 1970). A comparable phenomenon, however, has been described *in vitro* (Comper & Williams, 1987) and predicted from

theory (Pedley & Fischbarg, 1978). Comper & Williams found that when dextran solutions acted across a semi-permeable membrane, the osmotically induced flow increased by less than the expected amount in the steady state, when osmotic pressure was raised. This was because dextran solution immediately adjacent to the pore mouth was continuously diluted by the emerging solvent stream, dextran diffusion velocity being too slow to prevent dilution. Similarly, the interstitial albumin concentration around the capillary fenestrae may be less than in the joint cavity due to local dilution by the stream of ultrafiltrate emerging from the small-pore system. High current velocities near the fenestrae (Levick, 1991) raise the local Peclet number and create a race between albumin diffusion towards the fenestrae and convective transport away from them. A broadly analogous situation was described by Tedgui & Lever (1985): protein-free fluid emerging from aortic endothelium into the tunica media met albumin diffusing in from the adventitial side, and this resulted in a steady-state gradient of albumin concentration across the wall. Preliminary modelling studies in synovium indicate that this could reduce the pericapillary oncotic pressure in extreme cases to less than half that in the joint cavity, explaining much of the asymmetry between intravascular and intra-articular effects of albumin.

Additional factors that may have buffered the response to extra-vascular albumin are as follows. (i) Extravascular albumin reduces interstitial fluidity, causing viscous damping of fluid exchange. Substitution of conductivity × fluidity for interstitial conductance in the heuristic model of Knight & Levick (1985; eqn (1A)) confirms that $(d\dot{Q}_s/d\pi_j)_{P_j=0}$ contains an interstitial fluidity term and decreases as fluidity decreases: but numerical substitution indicates that fluidities of 0·8–0·5 reduce $(d\dot{Q}_s/d\pi_j)_{P_j=0}$ by at most 5–18 % relative to $d\dot{Q}_s/d\pi_p$, when pericapillary albumin dilution is not considered. (ii) A rise in mean capillary π_p as plasma filtration fraction rises (haemoconcentration) may also buffer $(d\dot{Q}_s/d\pi_j)_{P_j=0}$. The synovial filtration fraction is normally only ~ 2–4 % (Levick, 1987*a*; Simkin & Benedict, 1990), but could rise substantially under the influence of high pericapillary albumin concentrations. Estimated synovial plasma flow in the knee is 40 μl min^{-1}, capillary reflection coefficient is 0·8 and capillary filtration capacity is 0·23 μl min^{-1} cmH_2O^{-1} (Levick, 1991), so a 50 cmH_2O rise in pericapillary oncotic pressure would raise filtration rate by 9·2 μl min^{-1} if unbuffered, producing a filtration fraction of 0·23. Buffering by haemoconcentration may thus be important at high albumin concentrations. (iii) Intra-articular albumin concentration at low pressures may have been overestimated by the end-experiment aspirate as discussed earlier. In this event the effect of π_j on $\dot{Q}_s$ will have been underestimated here. This is unlikely to explain the asymmetry fully, however, because experiments using a different protocol in which joint fluid was sampled immediately after each flow measurement gave a value for $d\dot{Q}_s/d\pi_j$ of $29{\cdot}7 \times 10^{-3}$ μl min^{-1} cmH_2O^{-1} which was still significantly lower than $d\dot{Q}_s/d\pi_c$ (authors' unpublished results). (iv) $d\dot{Q}_s/d\pi_p$ was determined in a maximally vasodilated, artificially perfused hindquarter preparation (cf. intact animal here) so it is possible that the perfused surface area of synovial exchange vessels was not identical in the two situations.

Physiological and pathophysiological significance of intra-articular plasma protein

Under *physiological* conditions, intra-articular protein concentration is 23 g l^{-1} in rabbit knees, oncotic pressure is 12·8 cmH_2O, hydraulic pressure is just below or above atmospheric pressure (depending on joint angle) and the average trans-synovial flow is estimated to be of the order 0·3–0·7 μl min^{-1} (Levick, 1987 *a*). The displacement of the flow intercept by 23 g l^{-1} protein at atmospheric pressure is 0·23–0·38 μl min^{-1}. Since this is of comparable magnitude to the normal flow, the oncotic effect (intercept shift) is physiologically significant and likely to be an important factor in synovial fluid turnover.

The relative importance of slope change and intercept change depends on flow magnitude, because the effect of a slope change on flow is multiplicative in nature whereas that of an intercept shift is additive. Under *pathological* conditions the protein concentration in joint fluid *in vivo* can approach plasma concentration (55 g l^{-1} in the rabbit), and the associated effusion has a high pressure. The present results show that when pressure is 20 cmH_2O, a protein concentration of 55 g l^{-1} reduces trans-synovial absorption rate from $\sim$60 μl min^{-1} (Krebs solution) to 48 μl min^{-1}. This 20% reduction is chiefly due to the reduced slope of the pressure–flow relation; the intercept shift being relatively insignificant at these high flows (0·5–0·75 μl min^{-1} for 55 g l^{-1}). Fluidity exerts the dominant influence under these conditions because its effect is proportional to the size of the flow (eqns (7) and (8)). This raises the question of the role of hyaluronan, an important but distinct issue.

To summarize, extravascular albumin modified synovial interstitial flow by raising interstitial fluid viscosity, though the rise in interstitial viscosity was probably less than in the bulk phase. Albumin also modified interstitial flow by raising the net Starling filtration force across the synovial capillaries. The osmotic effects of intravascular protein and intra-articular protein were unequal, however, and this is attributed in part to local protein gradients around filtering capillary fenestrations.

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