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SYNOVIAL CAPILLARY DISTRIBUTION IN RELATION TO ALTERED PRESSURE AND PERMEABILITY IN KNEES OF ANAESTHETIZED RABBITS

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SUMMARY

1. The hydraulic conductance of the synovial lining of the rabbit knee increases greatly at intra-articular pressures (IAP) above 9 cmH_2O. A structural cause was sought by fixing synovium *in situ* at $\leqslant 5$ cmH_2O IAP (ten animals) or 25 cmH_2O IAP (five animals) and examining histological sections morphometrically.

2. The synovial lining was found to be a highly deformable sheet of very vascular connective tissue, with 47×10^3–73×10^3 capillaries per cm^2 section, 150–260 cm^2 endothelial surface per cm^3 tissue and a vascular volume of 2·4–5·7 %.

3. The thickness of the lining averaged 14–19 μm at low IAP and was reduced at high IAP; in suprapatellar synovium, where changes were most marked, thickness fell by 24–47 %. The loose subsynovial space expanded.

4. The average distance separating capillary near-edges from the joint cavity approximately halved from 3·75 and 7·47 μm at low IAP (harmonic and arithmetical means respectively) to 1·82 and 3·35 μm at high IAP. Capillaries remained patent and their number density did not change significantly at high IAP.

5. It is concluded that a reduction in the extravascular path length for fluid exchange contributes to the increase synovial conductance at high IAP, but the path length changes were not sufficient to account fully for the conductance changes.

INTRODUCTION

The cavity of a diarthrodial joint is lined by a thin sheet of modified connective tissue, the synovial lining or synovium, which comprises a loose array of cells in a dense interstitium with a rich microvascular network (Hasselbacher, 1981; Jilani & Ghadially, 1986; Henderson & Edwards, 1987). The synovial lining backs onto the subsynovium, a broader layer of loose areolar connective tissue or, depending on site, adipose/fibrous tissue (Key, 1932). The space enclosed by the lining contains synovial fluid, which is important for intra-articular nutrition and lubrication. The volume of this fluid is regulated by exchange between synovial capillaries and the cavity, and by drainage from the cavity into the lymphatic vessels in the subsynovium (Levick, 1987*a*). Two pathways are thus involved in fluid regulation

– a superficial plasma-to-cavity route comprising capillary wall and overlying synovial interstitium, and a cavity-to-subsynovium path traversing the full thickness of the synovial lining. Measurements of trans-synovial flow reveal that the hydraulic conductance of each route increases at intra-articular fluid pressures above a critical value of ~ 9·5 cmH_2O, called the yield pressure. The change in trans-synovial flow per unit change in intra-articular pressure (IAP) is 4–6 times greater above yield

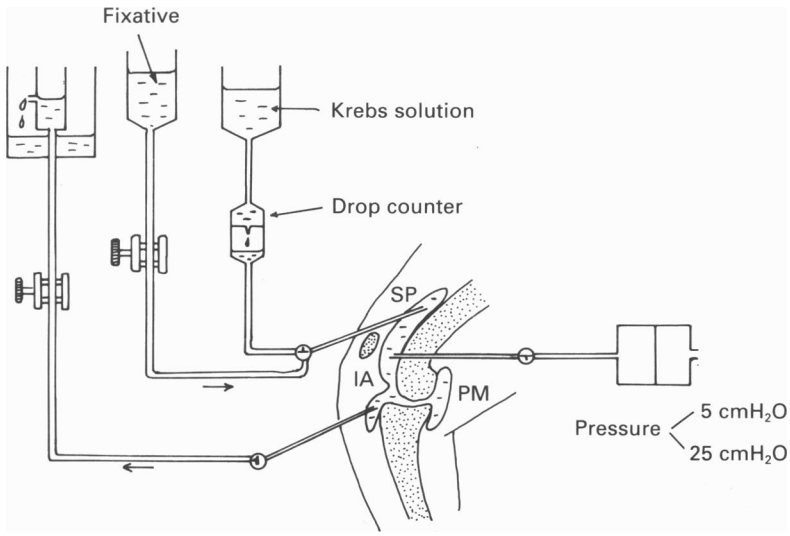

Fig. 1. Apparatus used to determine the effect of intra-articular pressure (IAP) on rate of absorption of Krebs solution into joint cavity, and to fix the synovial lining *in situ* at a controlled IAP. Tap settings are shown for the determination of the pressure–flow relation. Screw clamps on the fixative inflow and outflow lines allowed fine adjustment of IAP after switching to fixative. Samples were taken of suprapatellar areolar–muscular synovium (SP), infrapatellar adipose synovium (IA) and posteromedial synovium (PM).

pressure than below it (Edlund, 1949; Levick, 1979); and similarly the response of trans-synovial flow to changes in capillary hydraulic or oncotic pressure is about 5 times greater at 25 cmH_2O IAP than at 6 cmH_2O IAP (Knight & Levick, 1985; Levick & Knight, 1988).

Physiological evidence indicates that the increased hydraulic conductance arises extravascularly, i.e. in the interstitium, not at the capillary wall (Levick, 1980). In support of this a scanning electron microscope study showed marked deformation of the synovial surface at high IAP (McDonald & Levick, 1988). For any porous matrix the hydraulic conductance depends on the ratio of area to thickness as well as on the specific hydraulic conductivity of the material (Darcy's law; review Levick, 1987*b*). The present histological study was therefore undertaken to quantify the change in thickness of the interstitial pathway at raised IAP, and a parallel ultrastructural study (Levick & McDonald, 1989) was undertaken to assess changes in the area term. In brief we found that elevation of IAP reduces synovial lining thickness, reduces the thickness of the plasma-to-joint pathway and increases the interstitial area available for exchange.

METHODS

Outline of protocol

New Zealand rabbits (2–3 kg) were anaesthetized by intravenous pentobarbitone (30 mg kg^{-1}) and urethane (500 mg kg^{-1}), tracheostomized and the right knee prepared for cannulation (Levick, 1979). The joint was then treated in one of three ways. (a) Samples were excised immediately post-mortem from non-cannulated joints and fixed by immersion *in vitro* (five rabbits). (b) Synovium was fixed *in situ* at 5 cmH_2O IAP in five animals. (c) Synovium was fixed *in situ* at 25 cmH_2O in five animals. Conditions (a) and (b) involved fixation below yield pressure, while condition (c) was above yield pressure.

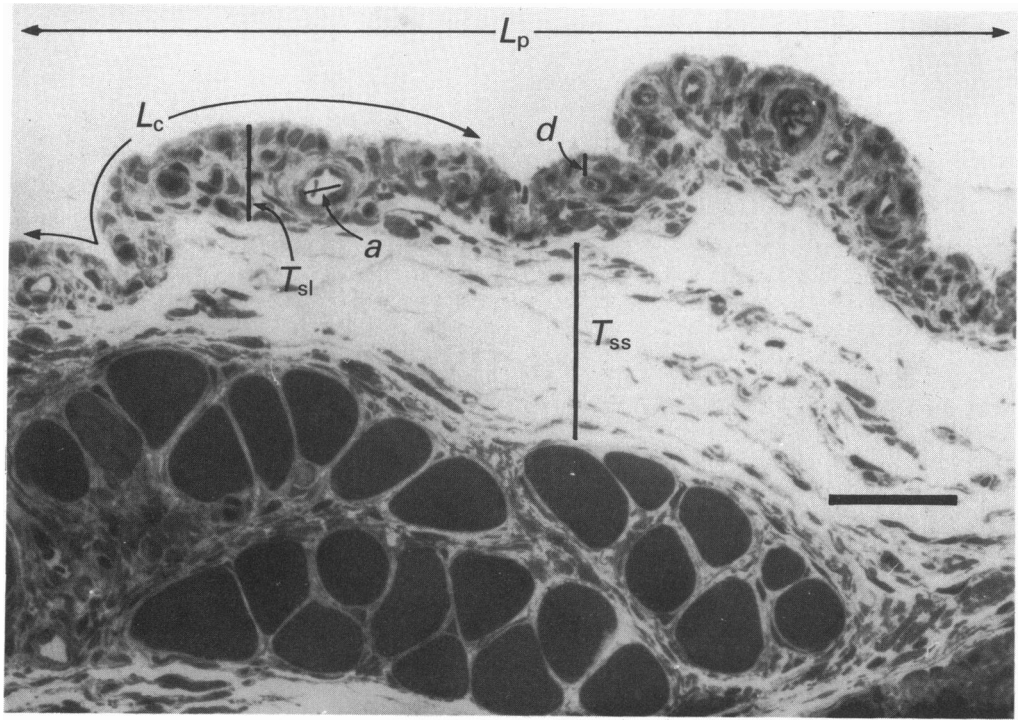

Fig. 2. Photomicrograph of synovial lining in suprapatellar region of rabbit knee, where synovium overlies areolar subsynovium and quadriceps muscle; immersion-fixed sample. Measurements included straight section length (L_p), contouring length (L_c), thickness of synovial lining (T_{sl}) and of subsynovium (T_{ss}), depth of capillary's nearest edge beneath the synovial surface (d) and lumen major axis (a). Scale bar = 50 μm.

Pressure and flow measurements prior to fixation

To ascertain whether synovial conductance had, as expected, increased at 25 cmH_2O IAP and not at 5 cmH_2O IAP, the pressure–flow relation was determined before fixation. Three perforated 21-gauge steel cannulae were inserted into the anterior aspect of the knee cavity with the joint at 100–120 deg extension (Fig. 1). A pressure transducer connected to one cannula recorded IAP. A raised reservoir of Krebs solution (pH 7·4, 283 mosM) was connected to the second cannula to control IAP, and absorption from this reservoir into the joint cavity was recorded by an interposed drop counter. Control experiments have shown that ~ 90% of the steady-state inflow is due to trans-synovial absorption provided 15 min is allowed for volume expansion (Levick, 1979). Flow was measured at 2·0, 3·5 and 5·0 cmH_2O IAP before fixation at 5 cmH_2O, and at 2·5, 5·0, 16·0 and 25·0 cmH_2O IAP before fixation at 25 cmH_2O.

Fixation techniques

The primary fixative was half-strength Karnovsky solution (2% paraformaldehyde and 2·5% glutaraldehyde in 0·05 mM-sodium cacodylate buffer, pH 7·4) with 5% sucrose as osmotic buffer (Bone & Denton, 1971), the osmotic pressure of the impermeant solutes (sucrose and cacodylate) being 231 mosM (Advanced Instruments osmometer 3W). Fixation at 5 or 25 cmH_2O IAP was accomplished by switching from the Krebs infusion reservoir to a reservoir of fixative at a slightly higher level (Fig. 1). At the same time a tap on the third cannula was opened, allowing the fixative to flow through the joint cavity and out to a collection reservoir with an overspill. By adjusting the overspill height and the ratio of inflow to outflow resistance by screw clamps, IAP was held constant while the cavity was perfused at 1 ml min^{-1} for 1 h. The rate constant (flow/volume) for the exponential rise in intra-articular fixative concentration was 1·1 min^{-1} at 5 cmH_2O, and 0·5 min^{-1} at 25 cmH_2O, based on intra-articular volumes of 0·9 ml and 2·0 ml respectively (Knight & Levick, 1982*a*). After an hour the joint was sufficiently rigid to allow incision without collapse.

Unfixed synovium from the non-cannulated left knee (control) was excised for immersion fixation *in vitro*.

Sampling and processing

Three samples of area ~ 15 mm^2 and thickness 2 mm were excised from each joint post-mortem. Areolar synovium was taken from the lateral suprapatellar pouch over the quadriceps; adipose synovium from the infrapatellar fat pad; and synovium of intermediate type from the posteromedial pouch (Knight & Levick, 1983). The suprapatellar and posteromedial samples were from concave locations, while infrapatellar adipose synovium was convex (and presumably experienced different hoop stresses). Samples were fixed for a total of 2 h in half-strength Karnovsky solution, washed, finely diced and post-fixed in 1% osmium tetroxide for 3 h. After dehydration through an ascending series of ethanol solutions tissue was embedded in low-viscosity resin (Spurr, Agar Aids, Cambridge) at 60 °C for 18 h. Tissue shrinkage, which occurs mainly at the stage of dehydration (Glauert, 1975) was assessed in three samples by linear measurement with a Vernier travelling microscope, immediately after fixation *in situ* and excision, and again after completion of ethanol dehydration. Linear shrinkage was 4% (areolar synovium) to 8·5% (adipose and posteromedial samples) as in many other tissues (e.g. Weibel & Knight, 1964).

The same joints were investigated by electron microscopy too (Levick & McDonald, 1989) and for this reason certain ultrastructural markers (Ruthenium Red/ferritin/glycogen/ferrocyanide) were used in some experiments. These tracers had no detectable effect on the morphometric results and are not pursued further here.

Embedded material was oriented under a binocular microscope for sectioning normal to the synovial surface (±10 deg). Semi-thin 1·5 μm sections were cut with a Sorvall JB-4A microtome, stained with 1% Toluidine Blue in borax buffer and mounted in Dammar xylene.

Morphometric measurements (see Fig. 2)

At least 2000 μm of sectioned surface was quantified per sample. Measurements were made with a calibrated eyepiece graticule in a Nikon Optiphot microscope, the smallest graticule division representing 1·0 μm at the highest magnification (× 1000).

Surface length was measured both in a straight line parallel to the surface (L_p) and in a line contouring around surface bumps and folds (L_c) in order to estimate surface irregularity (L_c/L_p).

The thickness of the synovial lining (T_{sl}) was measured at 100 μm intervals. The lower boundary, which is not defined by any limiting membrane (Ghadially, 1978), was taken to be the point where cellularity and staining intensity declined sharply.

The thickness of subsynovium (T_{ss}) was measured at 100 μm intervals and was defined as the distance between the synovial lining undersurface and non-synovial tissue (skeletal muscle, fibrous retinaculum or fat).

Capillaries, defined as blood vessels of minor axis $\leqslant 10$ μm, were counted both within the synovial lining (N_{sl}; 'synovial capillaries') and beneath it (N_e; 'extrasynovial capillaries'), down to an arbitrary contour line 100 μm below the surface. The capillary profiles stood out very distinctly against the Toluidine Blue background in semi-thin sections (Figs 2 and 4) and special staining methods were considered both unnecessary and undesirable.

To assess capillary compression and endothelial surface area, the major (a) and minor axes (b) of each capillary were measured. The presence of cells in the capillary lumen was also recorded.

To assess the thickness of tissue interposed between the joint cavity and the microvasculature, the depth of each capillary beneath the synovial surface was measured as the shortest straight line between the surface and the nearest point on the outer surface of the capillary perimeter (the capillary 'near-edge').

Calculations and statistical procedures

The number density of synovial capillaries (N_A) is:

$$N_A = N_{sl}/A_{sl},$$

where A_{sl} is cross-sectional area of synovial lining, i.e. length L_c multiplied by mean thickness T_{sl}. The density of extrasynovial capillaries within 100 μm of the surface was N_l/L_c $(100 - T_{sl})$. The circumference of a capillary profile (C), the mean diameter normal to the vessel axis (D') and the endothelial surface area per unit volume of synovium (A_V) were calculated using morphometric formulae from Underwood (1970) and Weibel (1969) as described by Knight & Levick (1983):

$$C = 4{\cdot}44\sqrt{[(a/2)+(b/2)^2]},$$

$$D' = 2\Sigma C/\pi^2 N_{sl},$$

$$A_V = 2N_A \pi D'.$$

Capillary volume fraction was obtained from Delesse's principle (equivalence of area fraction and volume fraction) as the net area of capillary profiles ($\pi ab/4$ for ellipses) divided by the section area A_{sl}.

Arithmetic means are followed by standard error throughout. Differences between tissue types and between fixation conditions were assessed by one-way analysis of variance (Snedecor & Cochran, 1967) and Scheffé's conservative test for multiple comparisons (Colquhoun, 1971). The distribution of capillary depths, being skewed, was normalized by a reciprocal (harmonic) transformation prior to analysis of variance. A harmonic analysis is also of greater physiological relevance to membrane transport (see Results). The standard error of the harmonic mean (S.E.H.M.) was evaluated from a general expression for the variance of any function of x (Kendall & Stuart, 1969) whose solution for $z = 1/x$ is:

$$\text{S.E.H.M.}(x) = \text{S.E.M.}(z)/(\bar{z})^2$$

where S.E.M.(z) is the conventional standard error of the mean of z ($\bar{z}$). Where only two populations were involved, comparisons were evaluated by the distribution-insensitive Mann–Whitney U test or χ^2 test as appropriate, and correlations by Spearman's ranking test (Siegel, 1956). Significance was accepted at $P < 0{\cdot}05$.

RESULTS

General observations

The pressure of endogenous synovial fluid averaged $-5{\cdot}2 \pm 0{\cdot}7$ cmH_2O relative to atmospheric pressure ($n = 8$) as in a previous study (Knox, Levick & McDonald, 1988). The rate of absorption of Krebs solution increased markedly above yield pressure (Fig. 3), the slope of the pressure–inflow relation increasing on average 3·4-fold between 5 and 25 cmH_2O IAP. These results were as expected and showed that the yield phenomenon had indeed occurred in the joints fixed at 25 cmH_2O.

When unfixed areolar synovium was excised it folded and collapsed, whereas unfixed adipose synovium and areolar synovium fixed *in situ* were stiffer. Posteromedial synovium did not seem well fixed at 5 cmH_2O *in situ*, as might be anticipated from the hydraulic isolation of the posteromedial compartment at low IAP (Knight & Levick, 1982*b*). At 25 cmH_2O IAP all three sites were well fixed and the suprapatellar and posteromedial samples remained concave after excision. On

inspection of the sections it was clear at once that synovium fixed at the higher IAP was thinner, contained stretched cells and had more superficial microvessels than at lower pressure (Figs 2 and 4). Subsynovial tissue often appeared broader and oedematous at 25 cmH_2O IAP but the synovial lining did not. None of the nineteen sections at high IAP (length 51 100 μm) showed any rupture of the lining layer, in agreement with radiographic and other evidence (Edlund, 1949; Levick 1979, 1980).

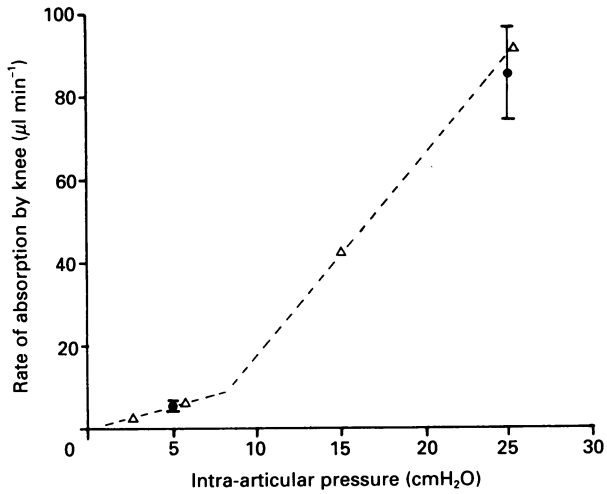

Fig. 3. Rate of absorption of Krebs solution into the rabbit knee joint as a function of intra-articular pressure prior to fixation at 5 or 25 cmH_2O. △, results for a single joint. ●, mean flows immediately prior to fixation (±S.E.M.). Mean slope increased from $1{\cdot}1 \pm 0{\cdot}4$ to $3{\cdot}7 \pm 0{\cdot}6$ $\mu l\ min^{-1}\ cmH_2O^{-1}$ above yield point. The flows are plotted as observed, without the small correction for viscous creep of the cavity walls.

Changes in surface convolution (Table 1)

Seventy sections containing 170 mm of surface were analysed. In areolar synovium the contoured surface length (L_c) exceeded the straightline measurement (L_p) by only 8% at 25 cmH_2O in contrast to a 16% convolution at 5 cmH_2O and a 20% convolution after fixation *in vitro* (Fig. 2; cf. Fig. 4). The negative correlation between the convolution index L_c/L_p and pressure (assigning atmospheric pressure to immersion-fixed tissue) was significant for areolar synovium ($P < 0{\cdot}01$; $r = -0{\cdot}50$) and posteromedial synovium ($P < 0{\cdot}05$; $r = 0{\cdot}42$). Scanning electron microscope confirmed that areolar synovium wrinkles up after immersion fixation (McDonald & Levick, 1988).

Reduced thickness of the synovial lining (T_{sl}) at high IAP (Table 2)

Areolar synovium was thicker than other synovia ($P < 0{\cdot}05$, Scheffé's test) except at high IAP. The lining at all three sites was thinner at 25 cmH_2O IAP than at 5 cmH_2O ($P < 0{\cdot}05$), and the thickness of areolar synovium fell by 47% ($P < 0{\cdot}01$). Immersion-fixed areolar synovium was 17% thicker than tissue fixed *in situ* at 5 cmH_2O ($P = 0{\cdot}05$) but there were no significant differences for the other tissues.

Synovial thickness was very uneven (Fig. 5); Weibel & Knight (1964) have pointed

out that the resistance to transport across an uneven membrane is more closely related to the harmonic mean thickness (the reciprocal of the mean of reciprocals) than to the arithmetic mean, because an uneven membrane is in effect a set of unequal resistances in parallel array. This is why the harmonic means are presented here. The synovial harmonic mean thickness was heavily weighted by the thinner regions of membrane and was therefore always smaller than the arithmetic mean (Table 2) but it again revealed a fall in thickness upon raising IAP from 5 to 25 cmH_2O.

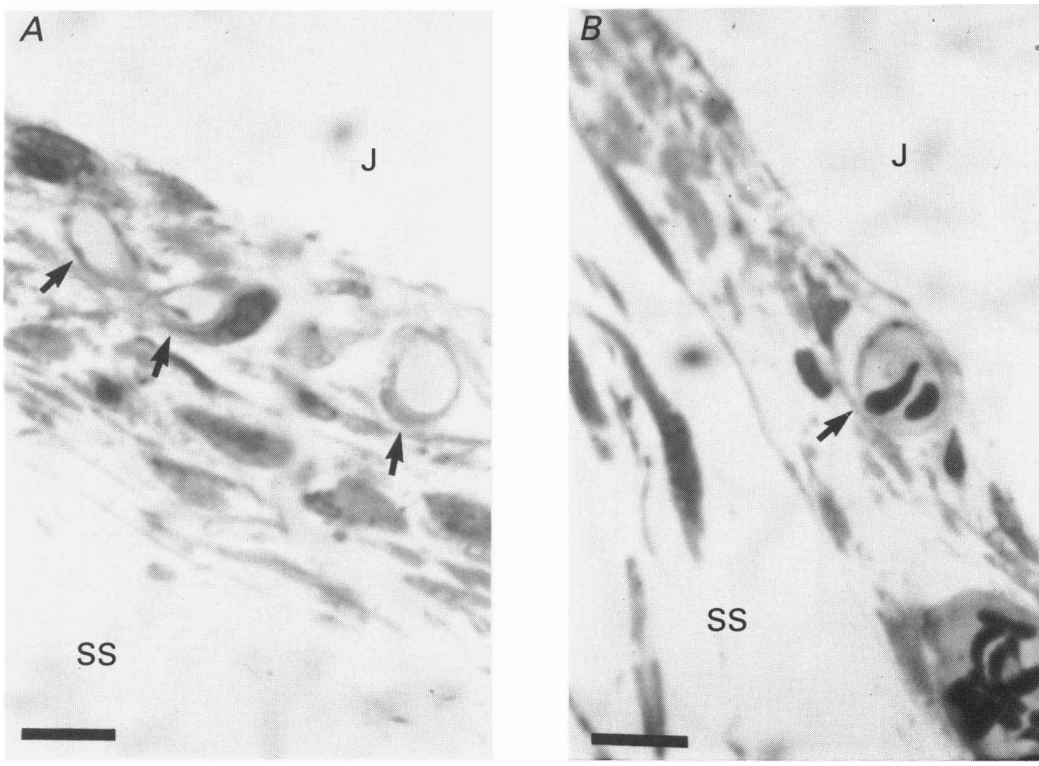

Fig. 4. Photomicrographs of areolar synovium from suprapatellar region of rabbit knee fixed *in situ* by intra-articular perfusion at a pressure of 5 cmH_2O (*A*) and 25 cmH_2O (*B*). J marks joint cavity and SS the subsynovium. Arrows point to synovial capillaries. Both prints are the same magnification; scale bar = 10 μm.

Increased thickness of the subsynovium (T_{ss}) after fluid infusion

When fluid is driven across the synovial lining by raising IAP, the portion of the fluid not absorbed by the synovial microcirculation passes into the subsynovial tissue, so it was of interest to measure subsynovial thickness. The areolar subsynovium separating suprapatellar synovium from quadriceps muscle was significantly thicker than subsynovium at the other sites, being ~ 3 times thicker below yield pressure ($P < 0{\cdot}05$, Scheffé's test). After absorption of fluid from the joint cavity at 25 cmH_2O the areolar subsynovial space expanded by $\sim 70\%$ relative to 5 cmH_2O ($P < 0{\cdot}01$, Scheffé's test) owing to a patchy subsynovial oedema, i.e. increased separation of formed elements. The expansion was very uneven, T_{ss} being

TABLE 1. Synovial surface: lengths analysed and convolution index (mean ± S.E.M., n)

		Fixation condition*		
			IA perfusion	
Tissue	Parameter†	Immersion	5 cmH_2O	25 cmH_2O
Areolar	L_c (μm)	18325	15832	14442
	L_c/L_p	1·20 ± 0·06 (10)	1·16 ± 0·08 (10)	1·08 ± 0·03 (8)
Adipose	L_c (μm)	16010	15991	18769
	L_c/L_p	1·17 ± 0·06 (6)	1·11 ± 0·05 (9)	1·46‡ ± 0·16 (5)
Posterior	L_c (μm)	18531	19905	17898
	L_c/L_p	1·45 ± 0·21 (6)	1·32 ± 0·14 (6)	1·11 ± 0·03 (6)

* Five joints under each condition

† L_c is aggregate contoured length of surface. L_p is a straight-line measurement. L_c/L_p is an index of the degree of convolution (see Methods), the ratio being calculated for each of n sections. The mean ratio was very similar if calculated from log ratios.

‡ This high value is attributable to two sections containing major acute-angle folds in the surface.

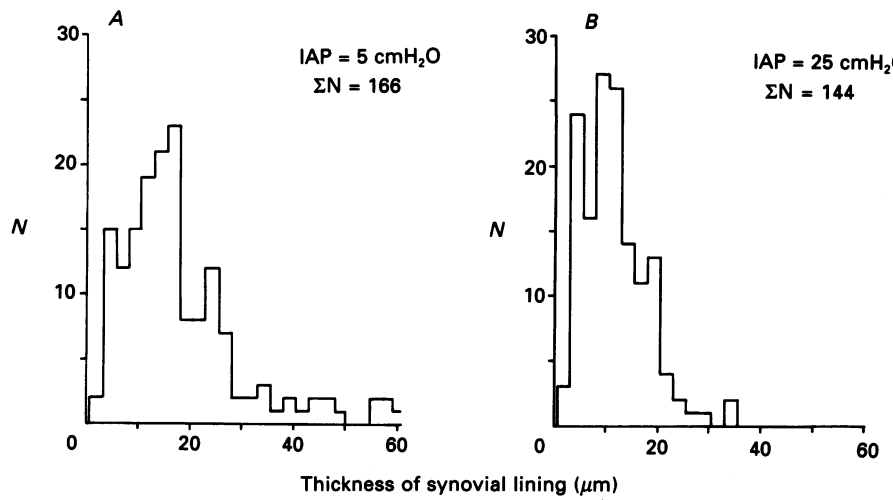

Fig. 5. Histograms of thickness of areolar synovium from suprapatellar region of rabbit knee fixed *in situ* at 5 cmH_2O (*A*) and 25 cmH_2O intra-articular pressure (*B*). Histogram resolution is 2·5 μm. The synovial lining is significantly thinner at the higher pressure ($P < 0·01$, U test).

> 400 μm in some places but close to control mean in others. By contrast T_{ss} at the other sites did not change significantly at 25 cmH_2O (analysis of variance).

Capillary density (N_A, Table 3)

A value was calculated for capillary number density in each joint and the mean of these is given in Table 3. Capillary density within the lining was 2–3 times greater

TABLE 2. Thickness (μm) of the synovial lining and subsynovium (mean ± S.E.M., n)

		Fixation condition		
			IA perfusion	
Tissue	Parameter*	Immersion	5 cmH_2O	25 cmH_2O
Areolar	T_{sl} AM	23·2 ± 0·7 (171)	19·3 ± 1·2 (166)	12·2 ± 0·5 (144)
	HM	20·0 ± 0·7 (171)	12·0 ± 0·7 (166)	9·1 ± 0·5 (144)
	T_{ss}	82·8 ± 6·4 (145)	99·6 ± 6·7 (147)	168·5 ± 13·0 (131)
Adipose	T_{sl} AM	13·8 ± 0·7 (157)	13·9 ± 0·6 (164)	11·4 ± 0·5 (184)
	HM	9·3 ± 0·6 (157)	9·5 ± 0·6 (164)	7·3 ± 0·5 (184)
	T_{ss}	28·7 ± 4·7 (157)	28·9 ± 3·9 (161)	20·4 ± 2·2 (188)
Posterior	T_{sl} AM	16·1 ± 0·8 (172)	18·8 ± 1·0 (195)	14·8 ± 0·8 (179)
	HM	11·0 ± 0·7 (172)	11·5 ± 0·8 (195)	9·5 ± 0·6 (179)
	T_{ss}	27·4 ± 3·7 (126)	39·0 ± 4·8 (150)	30·0 ± 3·6 (146)

* T_{sl} is synovial lining thickness and T_{ss} is thickness of subsynovium (see Methods). AM and HM are arithmetic mean and harmonic mean respectively; n denotes number of measurements. T_{ss} is an arithmetic mean.

TABLE 3. Periarticular capillary densities* (cm^{-2}, mean ± S.E.M., n = 5 joints)

	Fixation condition		
		IAP	
Tissue	Immersion	5 cmH_2O	25 cmH_2O
Areolar			
Synovium	53 530 ± 5280	53 660 ± 11 630	51 900 ± 7860
Deeper tissue	7330 ± 770	6290 ± 2400	3750 ± 1300
Adipose			
Synovium	73 060 ± 7430	69 520 ± 14 640	69 670 ± 6520
Deeper tissue	5220 ± 2410	6260 ± 1230	5290 ± 430
Posteromedial			
Synovium	59 680 ± 10 830	46 570 ± 15 920	57 680 ± 17 420
Deeper tissue	3940 ± 1150	3700 ± 1110	4350 ± 800

* Synovium capillary density = $N_{sl}/L_c \bar{T}_{sl}$. Deeper tissue capillary density = N_e/L_c $(100 - \bar{T}_{sl})$. Deeper tissue is non-synovial tissue within 100 μm of joint cavity – mainly subsynovium plus some skeletal muscle, fat or fibrous tissue.

than in rabbit hamstring muscle (21 900–24 700 cm^{-2}, Perry, 1980). Differences between synovial sites were not statistically significant but subsynovial capillary density was roughly 1/10th of synovial capillary density ($P < 0{\cdot}005$; Mann–Whitney U test). Synovial capillary density did not change significantly at 25 cmH_2O IAP (analysis of variance), an important negative finding which is pursued in the Discussion. Subsynovial capillary density declined at 25 cmH_2O in the suprapatellar site as expected from the subsynovial expansion, but the difference was not significant statistically.

Increased proximity of capillaries to synovial surface at high IAP (Table 4)

The most striking change of all was an increased proximity of the areolar synovial capillaries to the joint cavity at high IAP. Even under immersion fixation the distribution of capillary near-edges was remarkable with a sharp modal depth of only

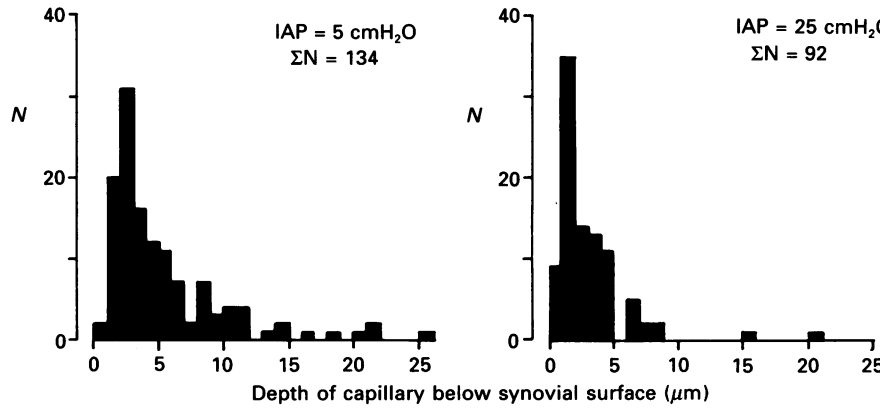

Fig. 6. Histograms of depths of capillaries beneath the surface of the areolar synovial lining at low and high intra-articular pressures. 'Depth' represents the shortest straight line from the synovial cavity to the nearest point on the capillary perimeter. Histogram resolution is 1 μm. The distribution is significantly more superficial at 25 than at 5 cmH_2O ($P < 0{\cdot}01$, U test).

TABLE 4. The mean depths (μm) of 2280 synovial capillaries beneath the synovial surface† (mean ± S.E.M., n)

		Fixation condition		
			IAP	
Tissue	Measure of central tendency‡	Immersion	5 cmH_2O	25 cmH_2O
Areolar	AM	9·60 ± 0·63 (224)	7·47 ± 0·81 (134)	3·35 ± 0·32 (92)**
	HM	5·57 ± 0·31 (224)	3·75 ± 0·22 (134)	1·82 ± 0·21 (92)**
Adipose	AM	10·57 ± 0·52 (161)	9·91 ± 0·49 (170)	8·78 ± 0·43 (151)
	HM	6·66 ± 0·47 (161)	6·44 ± 0·37 (170)	6·04 ± 0·34 (151)
Posteromedial	AM	12·65 ± 0·83 (167)	10·94 ± 0·76 (133)	8·59 ± 0·52 (150)*
	HM	7·52 ± 0·40 (167)	6·78 ± 0·46 (133)	4·33 ± 0·46 (150)**

*, ** denotes significant difference from values at or below 5 cmH_2O IAP at 5 or 1% level respectively.

† Shortest straight line from synovial surface to nearest point on capillary perimeter ('near-edge').

‡ AM = arithmetic mean, HM = harmonic mean.

6·3 μm (areolar synovium) to 11·3 μm (adipose synovium). At 25 cmH_2O IAP the capillary distribution became even more superficial, with a modal depth of 1·5 μm and a harmonic mean depth of 1·8 μm in areolar synovium, the most altered tissue (Fig. 6). As explained earlier the harmonic mean is the appropriate measure for

assessing the resistance of an uneven membrane, and the harmonic mean capillary depth at 25 cmH_2O IAP was significantly less than at 5 cmH_2O ($P < 0.01$, Scheffé's test), both in areolar synovium (2·1-fold change) and in posteromedial synovium (1·6-fold change) though not in adipose synovium. The depth distribution at 5 cmH_2O IAP was not significantly different from the control distribution.

Other capillary parameters (Table 5)

Shape and 'collapse'

The ratio of the major to minor axis served as an index of circularity. When cylinders of circular normal section are cut randomly only 25% of the profiles have an axial ratio greater than 2 (Elias, Henning & Schwartz, 1971), whereas the percentage of capillary profiles with an axial ratio greater than 2 was significantly greater than this, namely 40% at 5 cmH_2O IAP and 39% at 25 cmH_2O IAP, implying a slightly elliptical profile ($P < 0.05$, χ^2 test). Statistical analysis of the ratios and log ratios revealed no significant change at 25 cmH_2O IAP, providing no support for the view that capillaries might collapse under an IAP of 25 cmH_2O. On the contrary Fig. 4 shows clearly that an extremely superficial capillary actually bulges out into the joint cavity at 25 cmH_2 IAP, indicating that capillary pressure was greater than 25 cmH_2O.

Capillary diameter and surface area

The only statistically significant alteration in capillary diameter was in immersion-fixed tissue, which consistently had smaller capillaries (diameter 4·1–4·9 μm) than tissue fixed *in situ* (diameter 5·3–7·8 μm), presumably because capillary pressure falls prior to immersion in fixative. Capillary surface area was large, 150–350 cm^2 $(cm^3$ synovium$)^{-1}$, providing the large exchange area needed for the nutrition of the avascular articular cartilage and the cruciate ligaments as well as synovium (Renzoni, Amiel, Harwood & Akeson 1984; Levick, 1984).

Capillary volume fraction

Capillaries formed between 2·4 and 5·7% of the synovial lining by volume (Table 5), though this was reduced to 1·9% in immersion-fixed tissue owing to the decrease in vessel diameter. Vascular volume fraction was not significantly changed between 5 and 25 cmH_2O IAP, supporting the testimony of the axial ratios that capillaries do not collapse at the higher pressure.

Occupancy of capillary profiles by blood cells

Only 1·4% profiles contained a leucocyte, supporting the physiological and ultrastructural evidence that intra-articular infusion of Krebs solution over an hour or more does not provoke an acute inflammatory response.

Other vessels

A striking feature in some sections was the existence of broad venules (diameter 20–30 μm) at the border between the synovial lining and subsynovium. Their distribution was not quantified but was obviously deeper than that of the capillary

TABLE 5. Synovial lining capillaries – size, shape and areas (mean ± S.E.M., n)

		Fixation condition		
			IAP	
Tissue	Measurement	Immersion	5 cmH_2O	25 cmH_2O
Areolar	Shape a/b*	1·98 ± 0·09 (224)	2·02 ± 0·14 (134)	2·29 ± 0·17 (92)
	Diameter D' (μm)†	4·50 ± 0·23 (5)	6·17 ± 0·45 (5)	7·08 ± 0·27 (5)
	Area A_V (cm^{-1})‡	149 ± 10 (5)	219 ± 41 (5)	234 ± 40 (5)
	Volume V_V (%)§	1·83 ± 0·14 (5)	3·35 ± 0·58 (5)	4·05 ± 0·76 (5)
	Cells (%)‖	54 ± 7 (5)	65 ± 4 (5)	64 ± 11 (5)
Adipose	Shape a/b	2·34 ± 0·15 (161)	3·08 ± 0·18 (170)	2·40 ± 0·20 (151)
	Diameter D' (μm)	4·09 ± 0·43 (5)	5·27 ± 0·66 (5)	5·79 ± 0·32 (5)
	Area A_V (cm^{-1})	194 ± 34 (5)	225 ± 41 (5)	256 ± 32 (5)
	Volume V_V (%)	1·94 ± 0·52 (5)	2·60 ± 0·55 (5)	3·65 ± 0·45 (5)
	Cells (%)	40 ± 5 (5)	38 ± 7 (5)	43 ± 9 (5)
Posteromedial	Shape a/b	2·81 ± 0·18 (167)	3·13 ± 0·26 (133)	2·72 ± 0·15 (150)
	Diameter D' (μm)	4·91 ± 0·84	7·41 ± 0·92	5·71 ± 0·53
	Area A_V (cm^{-1})	170 ± 26 (5)	213 ± 55 (5)	196 ± 43 (5)
	Volume V_V (%)	1·83 ± 0·34 (5)	3·37 ± 0·89 (5)	2·38 ± 0·46 (5)
	Cells (%)	39 ± 7 (5)	49 ± 13 (5)	39 ± 5 (5)

* a/b is the ratio of the lumen major axis (a) to minor axis (b). The tabled values are arithmetic means. The geometric means were a little smaller, e.g. 1·75, 1·71, 1·96 for areolar synovium fixed by immersion perfusion at 5 and 25 cmH_2O IAP respectively.

† D' is the capillary diameter normal to the long axis (see Methods).

‡ A_V is the capillary endothelial area (cm^2) per cm^3 synovial lining.

§ V_V is volume percentage of synovial lining comprising capillaries.

‖ 'Cells' refers to the percentage of microvessel profiles containing blood cells, almost exclusively red cells.

network. Occasional lymphatic capillaries of similar diameter were also seen at the synovium–subsynovium interface.

DISCUSSION

Fixation in situ versus fixation in vitro

Fixation *in situ* proved to be superior to fixation after excision. Areolar–muscular synovium in particular tended to crumple if not fixed prior to excision (Fig. 2; also McDonald & Levick, 1988) and, much more seriously, capillary diameter and therefore apparent exchange area were reduced in tissue fixed *in vitro*. This is attributed to the finite compliance of the capillary wall (Levick & Michel, 1977; Smaje, Fraser & Clough, 1980) coupled with the abolition of the transmural pressure gradient upon excision. Nevertheless the capillaries remained patent in such samples, perhaps due to the support offered by the surrounding interstitium and/or basement membrane (Carlson, 1988). Despite its disadvantages immersion-fixation did not alter the thickness of the blood–joint barrier significantly and did not alter lining thickness except in the case of areolar synovium (relative to 5 cmH_2O IAP).

Our finding that suprapatellar areolar synovium is significantly thicker than other adipose synovium matches that of Shaw & Martin (1962) but conflicts with our earlier series (Knight & Levick, 1983).

Relation between structural changes and increased hydraulic conductance

The hydraulic conductance of the combined pathways from joint cavity to capillary plasma and subsynovium was calculated as the slope of the pressure–flow relation (Fig. 3) after applying a correction for the small inflow produced by viscous creep of the cavity walls. This conductance increased 4·0-fold on average between 5 and 25 cmH_2O. In the same joints the areolar synovium showed a 47% reduction in the thickness of the cavity-to-subsynovium pathway and a 55% reduction in the thickness of the cavity-to-plasma pathway. Since the hydraulic conductance of a material is inversely related to its thickness (Darcy's law) it seems that the geometrical changes must have contributed to the increased conductance above yield pressure. The thinning of the lining (cavity-to-subsynovium pathway) was only sufficient, however, to increase the conductance of this pathway 1·32 times, based on harmonic means. Moreover, the halving of the harmonic mean thickness of the cavity-to-plasma pathway will not halve the resistance of that pathway, as might at first be imagined, because the resistance of the interstitial component accounts for only 53% of the pathway's resistance below yield pressure, the remainder being the fixed resistance of the capillary wall (Knight & Levick, 1985). Halving the interstitial resistance will only lower the cavity-to-plasma resistance by 26%, or increase its conductance (1/resistance) 1·36 times. While this is a substantial effect it is insufficient in itself to explain fully the large conductance changes in the cavity-to-plasma pathway described by Knight & Levick (1985). Since, however, a widening of the synovial intercellular spaces occurs simultaneously and amplifies the geometric alteration (Levick & McDonald, 1989), further consideration of the structure–conductance relationship is deferred to the following paper.

Mechanical considerations

Thinning and increased area are mechanical consequences of changes in wall stress when a deformable concave structure is pressurized, as for example in the aorta (Tedgui & Lever, 1984). Here the changes were least pronounced in adipose synovium, where the convex geometry does not give rise to tensile hoop stresses as in the concave suprapatellar and posterior regions. Additional studies at intermediate IAPs would be needed to check whether the geometrical deformation increases progressively above yield pressure, as the hydraulic conductance does (Levick, 1980; Knight & Levick, 1985).

The question must also be asked: 'Why does a yield point arise at a specific IAP or, as is often the case, a narrow range of IAPs? If conductance depends on synovial geometry and stress, why is it relatively insensitive to IAP below $\sim 9{\cdot}5$ cmH_2O and very sensitive at higher IAPs?' The convolution of the normal synovial lining *in situ* (Table 1) offers one possible explanation. The lining must normally clothe both large and small arcs during a cycle of joint flexion and extension, and if the synovial area is sufficient to cover the largest arc without undue stress and strain there may be a redundancy of surface at smaller arcs (Simkin, 1985) creating the surface convolution detected at low IAP (Table 1; also McDonald & Levick, 1988). The skin over the back of the elbow provides a familiar analogy. There may thus be little increase in synovial lining stress when intra-articular volume is increased only modestly in joints at a neutral angle. Once the expanding joint has fully absorbed the 'slack' (excess surface), synovial stress will increase steeply with further volume expansion, because the lining must now stretch. As the lining area increases there will be a proportional thinning of the lining and a rise in conductance.

It is implicit in the above discussion that the synovial lining itself carries most of the stress generated by raising IAP to 25 cmH_2O. This is supported by the observation that areolar subsynovium was expanded at 25 cmH_2O IAP, not compressed. It must be appreciated that, contrary to the general view that synovial joints are enclosed by a fibrous 'capsule', there is no anatomical confining collagenous sheath around areolar–muscular synovium other than the collagen within the synovial lining itself – see Fig. 2.

Patency of microvessels at 25 cmH_2O IAP

It has been suggested to us, in relation to earlier studies of transynovial flow at IAPs around 25 cmH_2O, that synovial capillaries may collapse under the raised extravascular pressure. Physiological evidence did not support that view and here direct evidence of capillary patency was obtained. At 25 cmH_2O IAP there was no change in the number of patent capillaries per unit area synovium (Table 3) or in the capillary volume fraction (Table 5), and only a marginal change in the capillary axial ratio (Table 5). There was also a reassuring lack of evidence of any inflammatory response.

Observations bearing on synovial lining hydration

The absence of any significant fall in capillary number density or volume fraction at 25 cmH_2O IAP is an important negative finding for another reason too; it indicates that there was no substantial increase in synovial lining volume despite

the increased water flux through it. This is important because a powerful potential mechanism for raising the conductance of a fibrous matrix is an increase in hydration (Granger, 1981; Levick, 1987*b*; Urban, 1987). The absence of any significant change in the number of anatomical bodies (capillaries) per unit cross-sectional area of synovium argues against a substantial increase in synovial interstitial volume. The possibility remains, however, that small but functionally important changes in hydration might be concealed by the variance of the data.

If synovial lining volume did not change yet lining thickness decreased, the lining area must increase proportionately. Suprapatellar area, for example, would have to increase 1·57 times between 5 and 25 cmH_2O to maintain a constant volume of synovial lining and constant capillary density. Measurement of intra-articular area is difficult but an increase of roughly this magnitude has been noted in casts of the knee cavity (Knight & Levick, 1983).

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