Osmotic flows across the blood-joint barrier

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SUMMARY The effective osmotic pressure across the blood-joint barrier is a key factor controlling synovial fluid volume and joint effusions. The effect of plasma colloid osmotic pressure (COP) on trans-synovial flow was studied directly in rabbit knees expanded by intra-articular fluid infusion. The synovial microcirculation was perfused with blood of varying COP. Absorption of fluid from the joint cavity was a linear function of COP; but albumin COP was only 78% effective across the blood-joint interface. Hyperosmolar solutions of small solutes (e.g., glucose) generated transient osmotic flows across the blood-joint barrier, but far less effectively than albumin. The hydraulic permeability of synovium increased at pathological intra-articular pressures—a phenomenon of potential importance to effusion kinetics.

Key words: osmotic pressure, synovial absorption, fluid exchange.

Synovial fluid is derived primarily from plasma, and disturbances in the balance of forces between plasma and the joint cavity can create large clinical effusions. Fluid movement between blood and the joint cavity is considered to obey Starling's hypothesis,¹ which treats the intervening tissues ('blood-joint barrier') as a semipermeable membrane. The quintessential feature of the hypothesis is that flow should be governed by the osmotic pressure of plasma proteins (colloid osmotic pressure, COP). Certain clinical observations provide indirect support for osmosis across the blood-joint barrier. For example, replacement of a protein-rich knee effusion by an equal volume of saline causes a net absorption of fluid from the joint cavity.² Trans-synovial osmotic effects, however, have never been measured. Here the effect of plasma osmotic pressure, both colloid and 'crystalloid', (i.e., small solutes) upon trans-synovial fluid exchange was investigated directly in rabbit knees. The study also assessed the osmotic reflection coefficient of the blood-joint barrier to protein—a coefficient which characterises the molecular selectivity of the barrier.

Materials and methods

To obtain full control over hydraulic and osmotic pressures on each side of the blood-joint barrier the cavity of the rabbit knee was cannulated and infused

with Krebs' solution at a known hydrostatic pressure; and the synovial microcirculation was perfused with blood of known COP at controlled pressures from one of two extracorporeal pump-oxygenator circuits, via the cannulated abdominal aorta. Perfusion of the knee in isolated rabbit hindquarters has been fully described previously.³ The essential features (Fig. 1) were as follows.

ARTERIAL PRESSURE (P_A)

Pressure was generated by a Harvard peristaltic pump; regulated by a Starling resistor; and measured by a Gould-Statham pressure transducer. The blood was equilibrated with 95% O_2 –5% CO_2 in a foam exchanger and maintained at 36–39°C by a heat exchange coil. Control pressures were 80–100 mmHg.

VENOUS PRESSURE (P_V)

A cannula in the inferior vena cava drained into an open drop chamber whose height governed venous pressure (P_V) (control level 2 cmH₂O). This was recorded by a Gould-Statham transducer.

SYNOVIAL CAPILLARY PRESSURE (P_C)

Synovial capillary pressure was calculated from P_A , P_V , and the precapillary to postcapillary resistance ratio.³ P_C was 23–48 cmH₂O at control P_A , P_V .

PRESSURE IN THE JOINT CAVITY (P_J)

Pressure in the joint cavity was recorded by an intra-articular (IA) 21G cannula in the suprapatellar

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Fig. 1 Schematic representation of artificially perfused knee of isolated rabbit hindquarters. The joint was perfused via tap A connected to a cannula in the abdominal aorta; and drained via tap V connected to the abdominal vena cava. The two extracorporeal circuits contained bloods of different colloid osmotic pressure and consisted of peristaltic pump, foam oxygenator, heat exchanger, bubble trap, Starling resistor, and venous drop chamber. Arterial pressure (P_A) and venous pressure (P_V) were measured by transducers. Intra-articular fluid pressure (P_J) was measured via an intra-articular cannula. An infusion reservoir (IR) of Krebs' solution controlled P_J . Absorption rate was recorded in the steady state ($\dot{Q}_S$) by a drop counter (DC).

region, connected to a SE 100/WG pressure transducer. P_J was set by the height of an infusion reservoir of Krebs' solution connected to the joint cavity by a second IA cannula (Fig. 1). Most experiments were at P_J 18 cmH₂O, a pressure typical of a moderate effusion. Dilution of the endogenous synovial fluid ($\sim 24 \mu\text{l}$)⁴ by Krebs' solution reduced the IA COP to less than 2 cmH₂O (osmometry of aspirates).

TRANS-SYNOVIAL FLOW ($\dot{Q}_S$)

A drop counter between the infusion reservoir and IA cannula recorded the flow of Krebs' solution into the joint cavity. Published studies with non-absorbed oils at 18 cmH₂O showed that joint expansion (creep) contributed only 10% of the inflow at 15–20 min and declined slowly (e.g., 4% at 80 min).³ The creep component was subtracted by analysing differences between inflow within two to three minutes of a step in COP. Observations began at 20 min and continued for 120–180 min.

THE VASCULAR PERFUSATE

The perfusate comprised red cells (human group O, Rh negative) resuspended in solutions of serum albumin (Sigma Chemicals, Poole, Dorset, England; bovine Cohn fraction V; 10–120 g/l Krebs' solution, pH 7.35). COP was 5–80 cmH₂O. Packed cell volume was 30–40%. In one study red cells were resuspended in whole human plasma, diluted by Krebs' solutions or concentrated by evaporation, and dialysed to isotonicity. The two extracorporeal circuits contained perfusates of different COP to

facilitate abrupt changes in intravascular COP. Timed samples of venous effluent established that COP in the vasculature of the preparation achieved 90% of its final value within 100 seconds of switching tap A. Two minutes were allowed before the trans-synovial flow change was analysed.

Oxygen consumption was optimal at perfusion rates of greater than 15 ml/min.³ Vasodilator and blocking agents prevented active changes in vascular resistance (isoprenaline 20 $\mu\text{g}/100 \text{ ml}$; phentolamine 0.75 mg/100 ml; papaverine 1 mg/100 ml).

COLLOID OSMOTIC PRESSURE OF PERFUSATE (π_p)

Venous plasma COP was measured with an electronic osmometer⁵ fitted with an Amicon PM 10 semipermeable membrane (cut off mol. wt 10 000). Several commercial samples of fraction V albumin gave COPs which did not conform to the much used polynomial of Landis and Pappenheimer.⁶ This may be of practical significance for other investigators; COP should be measured rather than calculated from concentration.

Results

EFFECT OF PLASMA COP ON TRANS-SYNOVIAL FLOW

Switching from a vascular perfusate of low COP to one of high COP caused a marked rise in rate of fluid absorption from the joint cavity at 18 cmH₂O (Fig. 2). This reversed on switching back to a low COP. The response was a graded, quantitative one

Fig. 2 Effect of perfusate colloid osmotic pressure (COP) on trans-synovial flow (calculated over 2 drops from drop record: 1 drop = 13.4 μ l). At H, perfusion was switched to the second extracorporeal circuit, primed with blood of higher COP. The dashed line between the measured values of COP represents the time course of COP as determined by rapid sampling in control studies. At L, perfusion was switched back to the original circuit (blood of lower COP).

(Figs 3 and 5). The relation between change in trans-synovial flow and change in plasma COP for 16 joints was well fitted by a straight line of slope 0.20 (SE 0.01) μ l/min/cmH₂O (least squares fit: $n=83$; correlation coefficient = 0.94; $p<0.001$). The regression line passed virtually through the origin (intercept = -0.41 μ l/min), indicating that the effects of increasing or decreasing COP were symmetrical (Fig. 5).

COMPARISON OF EFFICACY OF COP CHANGES AND BLOOD PRESSURE CHANGES

A rise in synovial capillary blood pressure reduced the rate of fluid absorption (Fig. 3).³ Are the effects of plasma COP (π_p) and capillary pressure (P_c) equal and opposite, or is there a difference in magnitude as well as direction? In five joints the response of trans-synovial flow to albumin COP was compared with the response to capillary hydraulic

Fig. 3 The effect of perfusate colloid (albumin) osmotic pressure on the rate of absorption of Krebs' solution from a joint cavity (●) compared with the effect of mean capillary blood pressure in the same joint (■). Mean capillary pressures were calculated from arterial pressure, venous pressure, and synovial pre- to postcapillary resistance ratio (3.3 for this joint). The effects were of course opposite in direction; but note also that the response to albumin COP (slope of dashed regression line 0.21 (SE 0.015) μ l/min/cmH₂O) was less than the response to capillary pressure (slope -0.29 (SE 0.015) μ l/min/cmH₂O) ($p<0.01$, t test). The ratio of the two slopes (the efficacy of the colloid) is 0.78 for this joint.

pressure, altered by step changes in arterial or venous pressure.³ Hydraulic pressure had a relatively greater effect on exchange than did an equal change in COP (Fig. 3). The sensitivity of trans-synovial flow to COP (slope $dQ_s/d\pi_p$) was less than its sensitivity to capillary hydraulic pressure (slope dQ_s/dP_C)—although of course of opposite sign. This difference was observed consistently in all five joints. The ratio of $dQ_s/d\pi_p$ to dQ_s/dP_C averaged 0.78 (SEM 0.06) ($n=5$), i.e., on average serum albumin exerted only 78% of its potential COP across the blood-joint interface—see 'Discussion'.

OSMOTIC TRANSIENTS GENERATED BY SMALL SOLUTES

Edlund reported that intravenous injection of hypertonic sucrose solution promotes fluid absorption from the joint cavity.⁷ We reinvestigated Edlund's intriguing observation by five hyperosmolar perfusions in four joints, after the effect of COP had been determined. The hyperosmolar fluids were 1 M glucose (three experiments), 1 M sucrose (one experiment), and 0.5 M NaCl (one experiment) in 20 g albumin/l Krebs' solution.

Upon switching from control perfusate (red cells in 20 g albumin/l Krebs' solution) to hyperosmolar perfusate, the trans-synovial absorption rate climbed rapidly and steeply, peaking at several times control level (Fig. 4). Absorption then de-

cayed rapidly, with an average half life ($t_{1/2}$) of 28 s for glucose transients (60 s sucrose; 17 s NaCl). The rapidity of decay ($1/t_{1/2}$) correlated with the solute diffusion coefficients, which are in the ratio 2.85:1.29:1.00 for NaCl:glucose:sucrose.⁸ Thus rapid outward diffusion of test solute into synovial interstitium and joint cavity was dissipating the osmotic gradient across the capillary wall.

Evidence that test solute accumulated in the extracapillary space was obtained upon switching back to isotonic perfusate. This caused a rapid decline in absorption rate to zero followed by a transient rise in intra-articular fluid pressure, showing that trans-synovial flow had reversed and fluid was being drawn osmotically into the joint cavity. The same phenomena, an initial osmotic transient then a reversed osmotic transient, were demonstrated *in vitro* by applying first a hyperosmolar glucose solution and then an isosmolar solution to the PM10 membrane of the osmometer.

COMPARISON OF OSMOTIC EFFICACY OF PROTEIN AND SMALL SOLUTES

Expressing osmolarity in cmH_2O and allowing for non-ideality in hypertonic solutions,⁸ the maximum increase in absorption rate per cmH_2O increase in arterial osmolarity ($dQ_{\max}/d\pi_a$) averaged $3.1 \times 10^{-3} \mu\text{l/min/cmH}_2\text{O}$ for glucose ($3.6 \times 10^{-3} \mu\text{l/min/cmH}_2\text{O}$ for sucrose; $4.7 \times 10^{-3} \mu\text{l/min/cmH}_2\text{O}$ for

Fig. 4 Effect of perfusion of synovial microcirculation with hyperosmolar glucose solution upon trans-synovial absorption of Krebs' solution (Q_s), calculated from drop record (1 drop = 13.4 μl). At H, perfusion was switched from red cells in 20 g albumin/l Krebs' solution to 20 g albumin plus 1 mol glucose/l Krebs' solution. Absorption rate increased transiently by over fourfold. The initial low hump may have been due to transient disturbance of perfusion pressure on switching circuits or to heterogeneity of synovial microvascular transit times, or both. On switching back to isotonic perfusate at I, drop fall ceased and absorption reversed to filtration into the joint cavity, as is evident from the rising intra-articular pressure (P_j). The point plotted for $-Q_s$ ($-41 \mu\text{l/min}$) was obtained from the initial rate of pressure increase ($0.85 \text{ cmH}_2\text{O/min}$) and elastance ($dP/dV = 20.6 \text{ cmH}_2\text{O/ml}$ at $18 \text{ cmH}_2\text{O}$).¹⁶

Fig. 5 Changes in trans-synovial flow produced by 83 changes in synovial microvascular COP in 16 knees at 18 cmH₂O intra-articular pressure (Δ). The dashed regression line through these data has a slope of 0.20 (SE 0.01) $\mu\text{l}/\text{min}/\text{cmH}_2\text{O}$. The effect of COP on trans-synovial flow at a lower intra-articular pressure (6 cmH₂O) was less marked ($\bullet$); the regression slope was 0.051 (SE 0.007) $\mu\text{l}/\text{min}/\text{cmH}_2\text{O}$ ($n=20$, five joints). The difference in effect is highly significant ($p<0.001$, t test).

NaCl). Thus even the peak osmotic efficacy of small solute was only 1/50th–1/100th that of albumin (see earlier). The ratio of $dQ_{\max}/d\pi_a$ (osmotic effect of small solute) to dQ_s/dP_C (effect of capillary blood pressure) in the same joint averaged 0.013 for glucose (0.010 for NaCl; 0.025 for sucrose).

OSMOTIC EFFECT OF INTRAVASCULAR COLLOID AT HIGH AND LOW INTRA-ARTICULAR PRESSURE (P_j)

The above experiments were carried out at $P_j=18$ cmH₂O, typical of a joint with a moderate effusion. The hydraulic permeability of the blood-joint barrier may change, however, with intra-articular pressure.^{9 10} Further perfusions were therefore carried out at a lower intra-articular pressure, $P_j=6$ cmH₂O, in five joints. The trans-synovial absorption rate was again a linear function of plasma COP (correlation coefficient 0.89; $n=20$; $p<0.001$) (Fig. 5). The slope of the relation, however, was much less steep—only 0.051 $\mu\text{l}/\text{min}/\text{cmH}_2\text{O}$ compared with 0.25 $\mu\text{l}/\text{min}/\text{cmH}_2\text{O}$ at $P_j=18$ cmH₂O in the same five joints ($p<0.001$, t test; see 'Discussion').

Discussion

The linear proportional relation between plasma COP and trans-synovial flow established unequivocally the applicability of Starling's hypothesis to the synovial system. A number of other interesting points also emerged.

OSMOTIC REFLECTION COEFFICIENT OF BLOOD-JOINT BARRIER TO ALBUMIN

Albumin exerted only 78% of its osmotic potential

across the blood-joint interface. The ratio of osmotic pressure across a test membrane to that across a perfect semipermeable membrane (here PM10) is by definition the 'osmotic reflection coefficient', σ .¹¹ Its value can range from 0 (no reflection of solute by pore) to 1 (total exclusion of solute by pore). This is one of the fundamental parameters describing a passive membrane, because it characterises molecular selectivity and equivalent pore size. The synovial data indicated that σ_{albumin} across the blood-joint barrier averaged 0.78 (SEM 0.06). A value of <1.0 is in keeping with the known slight permeability to albumin.^{4 12} Equivalent pore size can be evaluated from reflection coefficients. In terms of a homoporous neutral membrane model a σ of 0.78 for a solute of diffusion radius 3.6 nm (albumin) implies a pore radius of 5.5–6.3 nm.¹¹ A better physical representation of the blood-joint barrier, however, may be a fibre matrix model.¹³

The characterisation of reflection coefficients across the blood-joint barrier is of fundamental importance not only because of the clues provided about pathway dimensions but also because a fall in σ_{albumin} is probably crucial in the pathogenesis of inflammatory joint effusions.¹⁴

OSMOTIC EFFECT OF SMALL SOLUTES

The hyperosmolar perfusions confirmed Edlund's observation of enhanced fluid absorption⁷ but showed that the effects were not sustained. Indeed the rapid dissipation of the osmotic gradient across the capillary wall was dramatic evidence of the high permeability of the blood-joint barrier to small solutes. Nevertheless the osmotic reflection coefficient to small solutes was clearly greater than zero

A minimal value for σ is given by the ratio $dQ_{\max}/d\pi_a$ to dQ_s/dP_c , giving $\sigma_{\text{NaCl}} \geq 0.010$, $\sigma_{\text{glucose}} \geq 0.013$, $\sigma_{\text{sucrose}} \geq 0.025$. It is stressed, however, that these values are lower limits. Owing to rapid diffusion of solute out of the capillary, the mean osmotic gradient across the capillary wall was almost certainly less than the known arterial osmotic pressure by the time of peak flow (30–60 s), leading to underestimation of σ .

Although changes in plasma osmolality in vivo are much smaller than those used here, significant transient effects on synovial exchange could nevertheless arise. After haemorrhage, for example, plasma glucose is increased by accelerated hepatic glycogenolysis, raising plasma osmolality by up to 25 mmol (657 cmH₂O).¹⁵ For $\sigma_{\text{glucose}} \geq 0.013$ this means an increased osmotic pressure of ≥ 8.5 cmH₂O ($\geq 25\%$ of plasma COP) exerted transiently across the blood-joint barrier. Such transients may prove important to understanding short term fluctuations in synovial volume.

DEPENDENCE OF SYNOVIAL HYDRAULIC PERMEABILITY ON INTRA-ARTICULAR PRESSURE

The flow generated by unit change in plasma COP was about four times greater at 18 cmH₂O than at 6 cmH₂O intra-articular pressure. This was not owing to any breakdown in the semipermeable nature of the barrier because σ_{albumin} was high (0.78) at 18 cmH₂O. The flow generated by unit change in synovial blood pressure increased by a similar factor between 6 and 18 cmH₂O intra-articular pressure.¹⁰ Both effects can be explained by an increase in the hydraulic permeability of the synovial intima (second component of the barrier, in series with capillary wall) as intra-articular pressure rises—the ‘yield phenomenon’.¹⁰ A yielding of intimal resistance to flow at pathological joint pressures could greatly influence the kinetics of joint effusion formation and resolution.

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