Absorption of artificial effusions from synovial joints: an experimental study in rabbits

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(Received 22 November 1979; accepted 11 March 1980)

Summary

1. The absorption of fluid from the joint cavity was studied by measurement of the flow of Ringer solution or paraffin oil, from an infusion reservoir, into rabbit knee joints at constant pressures between 0 and 25 cm water.

2. Although no oil was absorbed across the synovium, oil flowed continuously into the joint cavity at constant intra-articular pressure. It was concluded that the joint capsule expanded with time (delayed compliance, viscous creep). Viscous creep of the capsule could explain, in part, the poor correlation observed clinically between effusion volume and pressure.

3. The rate of absorption of Ringer solution by the synovium was calculated by subtraction of the volumetric rate of creep from the volume flow of Ringer solution. The absorption rate increased as a linear function of intra-articular pressure up to 9 cm water but as a much steeper (six-times) function of pressure above 9 cm water (the ‘breaking point’ phenomenon). Since pressure increases upon joint flexion, flexion may minimize synovial fluid volume by promoting fluid absorption.

4. The absorption rate was unaffected by ligation of the lymphatic drainage of the joint, but was reduced to between 79 and 85% by intermittent interruption of blood flow to the joint. Fluid accumulated in connective tissue outside the synovium.

5. It is concluded that these artificial effusions are absorbed partly into the synovial microcirculation, in accordance with Starling’s hypothesis of fluid exchange, and partly into compliant connective tissue outside the joint capsule. The increased sensitivity of flow to pressure above 9 cm water is explained by a progressive reduction in synovial resistance to flow, and implies facilitation of absorption of joint effusions of pressures over 9 cm water.

Key words: effusions, joint, intra-articular hydrostatic pressure, synovial fluid.

Introduction

Large movements of fluid may occur between a synovial cavity and the periarticular tissues. This is frequently demonstrated in clinical practice by the formation or resolution of joint effusions, which are common complications in a wide range of diseases. Yet little is known about regulation of synovial fluid movement, even in normal joints, and the pathophysiology of joint effusions remains ill-understood. This paper attempts to explore some factors involved in the regulation of fluid movement across normal synovium. It is hoped thereby to define key factors which may form a basis for the investigation of pathological synovia.

The principal experimental evidence on the origin of synovial fluid derives from chemical analyses of the fluid, which normally resembles a plasma ultrafiltrate in protein and electrolyte composition (Bauer, Ropes & Waine, 1940). This implies that synovial fluid arises by ultrafiltration of plasma across the endothelium of synovial capillaries; hence synovial fluid formation or absorption can be described by Starling’s (1896) hypothesis, which states that the rate of fluid movement between plasma and tissues (in either direction) varies linearly with the hydrostatic and...
colloid osmotic pressures of extravascular fluid and capillary plasma. Starling’s hypothesis, if proven to be applicable to normal trans-synovial fluid movement, would provide a powerful quantitative basis for the investigation of states of disordered joint-fluid exchange. This paper reports experiments designed to test the applicability of Starling’s hypothesis to normal trans-synovial fluid movement, by investigating the effects of intra-articular pressure (i.e. extravascular fluid pressure, the most readily measured of the four ‘Starling forces’ in the joint) and of the synovial microcirculation upon the rate of absorption of simple, non-inflammatory artificial effusions. The work develops an approach initiated by Edlund (1949).

Methods
Experiments were carried out upon the knee (stifle) joints of albino rabbits (3–4 kg) anaesthetized with intravenous pentobarbitone/urethane solution (30 and 500 mg/kg respectively). The skin over the extended knee was incised. Two 21–22 gauge cannulae, with several lateral perforations, were inserted into the supra-patellar pouch via the medial and lateral aspects of the ligamentum patellae. One cannula was connected to a SE1150/WG pressure transducer (SE Laboratories, Feltham, Middlesex, U.K.) to record intra-articular hydrostatic pressure as described before (Levick 1979). The second cannula conducted fluid into the joint from an infusion reservoir whose height above the joint could be varied (Fig. 1). A photo-electric drop counter (drop size 10 μl) was interposed between joint and reservoir and recorded flow into the joint. Purse-string sutures were placed around the cannulation site to eliminate leakage.

Commencing with the infusion reservoir at joint level (0), the height of the reservoir was raised in vertical steps of 1–3 cm from 0 to 20–25 cm above the joint, thus causing a stepwise elevation of intra-articular pressure and creating an incremental artificial effusion within the joint cavity. Since intra-articular pressure depends upon the joint angle, and since the sensitivity of pressure to the angle increases as synovial fluid volume increases (Levick, 1979), the experiments were performed upon immobile joints at a constant angle. Joint angle was defined as that angle between the limbs of a pair of dividers, whose axis lay over the medial mid-articular point and whose limbs lay over the tibia and femur, 6 cm from the joint. An angle of 120–150° extension was adopted to maximize compliance and to facilitate cannulation. The angle of the limb was set by inextensible cords between the ankle and the bench. With each step increase in effusion volume, intra-articular pressure and flow into the joint were recorded for 15–25 min. Since flow into the joint cavity represented the sum of two different processes, namely trans-synovial flow (absorption) and joint

![Fig. 1. Diagram of the experimental preparation. Two cannulae are inserted into the suprapatellar pouch of the right knee of a supine anaesthetized rabbit. The differential pressure transducer is level with the joint. The reservoir containing the artificial effusate (Ringer solution or paraffin oil) lies in the vertical plane. A drop counter operated by interruption of an infrared beam records rate of absorption of fluid into the joint cavity.](image-url)
expansion, it was necessary to distinguish between these two effects. This was accomplished by comparison of the influx of two fluids, one of which was absorbed (Ringer–Locke solution adjusted to pH 7.4 by sodium bicarbonate) and the other of which was non-absorbable (light-grade liquid paraffin B.P.). The oil did not penetrate the synovium (see the Results section) and therefore oil flow into the joint represented the volumetric rate of expansion of the joint cavity. Ringer solution rather than a protein-containing solution was employed in order to eliminate the possible influence of oncotic pressure.

Pressure-absorption curves were determined in the manner described for both Ringer solution and paraffin oil, under three conditions: (a) after no further experimental interference (16 joints with Ringer solution, five joints with oil, from as many animals); (b) after interruption of the lymphatic drainage of the joint, identified by intra-articular injection of Evans-Blue-stained Ringer solution after passive joint movement. Dye appeared only in the two to four femoral lymph trunks. These were separated from the femoral blood vessels and ligated (six joints, six animals); (c) after cessation of synovial blood flow, which was produced by intermittent application of vascular clamps (bulldog clips) or snares (nylon tapes) to the abdominal aorta and inferior vena cava or to the femoral vascular bundle for periods of 10–15 min. Circulatory arrest was followed by at least an equal period of blood flow before repetition (19 interruptions of flow in three joints of three animals).

Results

Visco-elastic properties of tissues bounding the joint cavity

It was expected that a step increase of infusion pressure would cause paraffin oil to flow transiently into the joint as the periarticular tissues expanded elastically, and that flow would then cease because elastic equilibrium had been reached. However, this proved not to be the case (Fig. 2). The attainment of a Laplacian elastic equilibrium was indicated by the rapid attainment of a constant intra-articular pressure, yet oil continued to flow slowly into the joint cavity thereafter (37 observations in five joints of five animals). Post-mortem examination of joints, in which the oil had been deeply stained by Sudan Black, revealed that no oil had escaped from the joint cavity into the synovium. Thus expansion (strain) of the periarticular tissue was occurring in the absence of a change in the pressure (stress) to which the tissue was subjected; in other words, the tissues which form the walls of the joint cavity underwent time-dependent expansion, termed viscous creep or delayed compliance. The rate of viscous creep decayed exponentially with time, with a mean time constant of 53.0 min (SE ± 12.9 min, n = 37). The rate of viscous creep became greater as the volume of the joint increased (Fig. 3). The relationship between oil influx, at 15–20 min after a pressure step, and the steady-state pressure was fitted by a linear regression equation. Slopes ranged between 0.17 and 0.49 μl min⁻¹ cm⁻¹ water in the five joints.
Equation (1) summarizes the results for five joints,

\[ \dot{Q}_{ve} = (0.23 \pm 0.06) P + (0.4 \pm 0.6) \]  

where \( \dot{Q}_{ve} \) was rate of oil influx at 15–20 min, in \( \mu l/min \), and \( P \) was intra-articular pressure, in cm water (figures preceded by ± are standard errors of mean throughout). A significant rate of viscous creep at 15–20 min meant that the flow of Ringer solution into the joint had to be corrected by subtraction of oil inflow, at the same pressure, in order to calculate the rate of trans-synovial absorption of Ringer solution.

Effect of pressure upon the rate of absorption of fluid

The influx of fluid from the infusion reservoir at a given constant pressure was corrected by subtraction of the volumetric rate of creep (eqn. 1) to yield the rate at which the artificial effusion was absorbed across the synovium (\( \dot{Q} \)). The rate of absorption reached an almost steady state by 15–25 min (Fig. 2). The steady-state absorption rate increased as the volume and pressure of the effusion was increased (192 observations on 16 joints of 16 animals). Up to pressures of about 9 cm water, the absorption rate and pressure in a given joint could be related by a linear regression equation. In 16 joints the regression slopes ranged from 0.14 to 0.92 \( \mu l \) min \(^{-1} \) cm \(^{-1} \) water (correlation coefficients \( r > 0.9 \), probability \( P \) of null hypothesis < 0.01), and the mean regression coefficients were:

\[ \dot{Q} = (0.49 \pm 0.09) P + (1.4 \pm 0.2) \]  

At an average pressure of 9.2 cm water, however (range 6.1–15.3 cm water) the slope of the relationship increased by two to 12 times (average 5.7 times), as shown in Fig. 3. Above this critical pressure, the absorption rate continued to increase in a roughly linear fashion with effusion pressure and could again be described by a linear regression equation (\( r > 0.9, P < 0.01 \)). The regression slopes ranged from 1.25 to 4.55 \( \mu l \) min \(^{-1} \) cm \(^{-1} \) water (\( n = 16 \)) and the mean coefficients were:

\[ \dot{Q} = (2.81 \pm 0.30) P - (18.7 \pm 3.4) \]

The pressure at which the slope \( d\dot{Q}/dP \) increased markedly in connective tissue has been called the breaking point or pressure (McMaster, 1941; Edlund, 1949). Experiments in which the pressure was reduced to below 9 cm water after 'breaking' the synovium, showed that the low unbroken slope \( d\dot{Q}/dP \) was not readily restored in the short term, i.e. the phenomenon was not rapidly reversible (eight joints).

Radiographical studies were carried out upon five joints distended by radio-opaque iodoxyl solution (Uropac) at measured pressures. The radiographs showed that the low unbroken slope \( d\dot{Q}/dP \) was not readily restored in the short term, i.e. the phenomenon was not rapidly reversible (eight joints).

In order to elucidate the cause of the change in effusion absorption rate with pressure it was necessary to define the pathways across the synovium by which fluid absorption occurred. Three main possibilities appeared to merit consideration: flow into lymph vessels, flow into the synovial capillary bed and flow into the perisynovial interstitial space.

Role of the synovial lymphatic system

The contribution of the subsynovial lymphatic plexus to fluid absorption from the immobile knee was assessed by measurement of the pressure–flow relationship, in six joints in as many animals, after ligation of the femoral lymph trunks. Lymphatic ligation caused no significant change in the rate of fluid absorption, which ranged from 0.16 to 0.92 \( \mu l \) min \(^{-1} \) cm \(^{-1} \) water below breaking
point and from 1.89 to 5.3 $\mu l \ min^{-1} \ cm^{-1}$ water above breaking point. Breaking points were observed within the usual range of pressures (8.8–10.5 cm water) and the average regression coefficients describing the results were: below breaking point, $Q = (0.60 \pm 0.17)P + (0.1 \pm 0.3)$ and above breaking point, $Q = (2.90 \pm 0.64)P - (22.8 \pm 6.8)$. These slopes and intercepts were not significantly different from the control values. Post-mortem dissection confirmed that no lymphatic drainage of the Evans-Blue-stained fluid had occurred either distally into the leg or proximally beyond the femoral lymphatic ligatures. A small volume of blue fluid entered the lymph vessels between the joint and ligature, but since this volume was at most 0.16 cm$^3$ it was considered to be a negligible fraction of the total volume of fluid absorbed.

**Role of the synovial microcirculation in fluid absorption**

If the formation and absorption of joint fluid obey Starling's hypothesis, it follows that the interruption of synovial microcirculatory blood flow should reduce fluid exchange. The influence of synovial blood flow upon the rate of fluid absorption was therefore investigated. Absorption rate at constant pressure, between 4 and 20 cm water during 10–15 min of abdominal or femoral vascular clamping, was expressed as a percentage of mean absorption rate before and after interruption of bloodflow; in this way artifacts due to mechanical expansion of the joint were minimized. Intermittent vascular arrest reduced the rate of absorption of fluid in 17 out of 19 experiments on three joints of three animals (Figs. 4, 5), to an average 79% of the control absorption rate above the breaking pressure ($n = 13$, SD $\pm 4\%$, range 66–100%) and to 85% of the control absorption rate below the breaking pressure ($n = 6$, SD $\pm 5\%$, range 60–98%). The reduction in fluid absorption was preceded by a transient fall in pressure and rise in fluid absorption (Fig. 4), an effect attributable to increased capillary absorption of fluid as the capillary pressure fell (Mellander, 1960) and to vascular collapse.

It was noted that fluid absorption was not completely eliminated by circulatory arrest;
indeed a considerable rate of absorption persisted. The location of fluid absorbed, in the absence of a circulation, was determined by post-morten dissection of joints which had received Evans-Blue-stained fluid. The blue fluid accumulated in the form of a watery gel located in connective tissue planes outside the synovium, particularly in the dependent politeal fossa.

Discussion

These experiments were directed primarily at the assessment of the roles of intra-articular pressure and of blood flow in the absorption of fluid from a synovial joint cavity, but the oil control experiments led to the incidental discovery that the joint investment (synovium, collagenous capsule, extrasynovial connective tissue, fat and muscle) possessed not only the (long-recognized) property of elasticity but also viscosity, i.e. the tissues forming the walls of the joint cavity yielded or crept (strain) when subjected to a constant stress. Another possible explanation, namely that oil flowed into the joint because of a gradual change of joint angle, was discounted, since at 120–150° the joint was in a position of minimal sensitivity to angle (Levick, 1979) and no observable change in joint angle occurred. In view of the large number of other biological tissues in which visco–elastic properties have been demonstrated (Fung, 1972), this discovery was predictable, though joint investment visco–elasticity is thought not to have been described before. Visco–elasticity could prove to be of importance in the understanding of the pressure–volume relationships of clinical joint effusions, for viscoplastic creep is a time–progressive process and therefore a joint containing a constant volume of fluid slowly loses pressure (J. R. Levick unpublished observations). If the periarticular tissues of arthritic joints similarly possess visco–elasticity, this would in part explain the lack of correlation which has been noted between the pressure and volume of clinical effusions (Caughey & Bywaters, 1963; Jayson & Dixon, 1970a). A second important consequence is that measurements of joint compliance ($dV/dP$) have little meaning unless a third variable, time, is also considered; the influence of time has rarely been considered in past studies of joint compliance.

The principal conclusion of the present study was that intra-articular pressure exerted a major influence upon the rate of absorption of an artificially created effusion from the cavity of a healthy synovial joint; the higher the pressure, the greater the absorption rate. This effect of pressure may be of importance in regulating the volume of fluid in normal joints. A continuous filtration of fluid into immobile joint cavities has been demonstrated (Levick, 1979), which raises the question of how the, normally very small volume of, intra-articular fluid is preserved. It is suggested that during joint usage the changes in joint angle and in periarticular muscle tone cause increases in pressure in the rabbit joint, though not beyond breaking point (Levick, 1979), and that the intermittent elevation of pressure drives fluid out of the joint cavity to minimize the synovial fluid volume. A nicety of this system is that the sensitivity of pressure to joint angle increases markedly as the volume of fluid in the joint increases; a given increase of joint angle induces a greater increase in pressure and so creates a greater absorptive flow, i.e. a simple negative feedback system operates. In human joints, passive flexion may similarly elevate
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pressure and minimize the fluid volume; active motion can, however, produce subatmospheric pressures (Jayson & Dixon, 1970b), which should tend to increase synovial fluid volume.

The results presented also showed that the sensitivity of absorption rate to pressure (slope $dQ/dP$) at a given joint angle increased almost sixfold at around 9 cm water (breaking-point phenomenon). Thus both the formation and absorption of joint fluid may be expected to occur more rapidly above this pressure. This facilitation of fluid exchange above 9 cm water was shown not to be due to changes in the absorptive area nor to gross rupture of the synovial lining of the joint. Indeed in human knees, Jayson & Dixon (1970a) reported rupture only at pressures over 100 mmHg. It is deduced therefore that the facilitation of fluid exchange is caused by a breakdown of the resistance to flow in one of the pathways of fluid absorption.

The experiments to determine the pathways taken by the absorbed fluid allow deductions to be drawn as to the cause of the breaking-point phenomenon. Of the three pathways considered, that through the lymphatic system appeared to contribute little to the acute absorption of the artificial effusion. It must be stressed, however, that the lymphatic drainage of limbs and joints is well known to be ineffective in the absence of motion (Timbrell-Fisher, 1923); in the conscious animal lymphatic drainage is likely to contribute significantly to fluid balance. In the immobile joint, however, the absence of significant lymphatic absorption precluded a change in the nature of the lymphatic pathway as an explanation of the breaking point.

The experiments in which synovial blood flow was arrested demonstrated that the synovial microcirculation plays a role in the absorption of joint effusions, thus establishing a prerequisite for the application of Starling’s hypothesis to normal trans-synovial fluid exchange. Edlund (1949), however, reported that circulatory arrest had little effect on fluid absorption at 4–6 min into the knee joint. The value of this observation is reduced by failure to consider the effect of the considerable viscous creep which occurs at 4–6 min (Fig. 2). The endothelium of most systemic capillary beds offers constant resistance to transendothelial flow (Michel, Mason, Curry & Tooke, 1974). One may therefore regard a change in conductivity of this pathway as unlikely to be the cause of the breaking-point phenomenon.

The third pathway shown to be involved in fluid absorption leads across the synovial intima into extrasynovial interstitial spaces, which act as a quasi-infinite sink to accommodate large volumes of absorbed fluid (Fig. 6). It has been firmly established (McMaster, 1941; Guyton, Scheel & Murphree, 1966) that the conductivity of connective tissue is pressure dependent. Since synovium is a modified form of connective tissue, rather than a true epithelial membrane, it is suggested that its conductivity increases above 9 cm water to generate the breaking-point phenomenon. A simple mathematical analysis of the system showed that an abrupt increase in synovial conductivity (as proposed by Edlund, 1949) would not produce the experimentally observed curve but would produce a curve with a vertical step at the breaking point (Fig. 7a). Instead it is proposed that synovial conductivity first begins to increase at breaking pressure and thereafter increases as a continuous function of pressure, thus creating the curve observed experimentally (Fig. 7b). The mechanism of the increase in conductivity might be an increase in the width or number of patent intercellular channels, as pressure and tension rise within the synovium. No morphological data are at present available to test this hypothesis.
FIG. 7. Comparison of the theoretical effects of a sudden (a) or graded (b) increase in synovial hydraulic conductivity (slope $dQ/dP$) upon the pressure–absorption relationship. The curves are constructed from the model illustrated in Fig. 6. (a) Synovial conductivity ($K$), assumed to increase abruptly by fourfold from $K_1$ below breaking pressure ($P_b$) to $K_2$ at and above breaking pressure. Note the step on the curve and extrapolation to a positive intercept with the ordinate. (b) Synovial conductivity assumed to be constant ($K_e$) up to breaking pressure and thereafter to increase as a continuous function of pressure ($K_e \ldots K_3$). The theoretical curve now resembles closely the experimental results, displaying no step at breaking pressure and extrapolating to a negative intercept with the ordinate from above-breaking pressures.

These experiments remain a very incomplete analysis of the process of fluid absorption, even for normal rabbit joints, since the roles of joint motion and of osmotically active macromolecular solutes demand investigation. These studies may, however, provide a framework for the investigation of the effects of synovial inflammation upon fluid exchange. These experiments also reveal a phenomenon, the breaking point, whose existence in human joints is as yet uninvestigated.

Acknowledgments

The author is grateful to the Arthritis and Rheumatism Council of Great Britain for financial support for these researches.

References


