

Synovial perfusion and synovial fluid solutes

Peter A Simkin

Before embarking on a discussion of synovial perfusion, it seems appropriate first to ask why we should care about such an obscure and inaccessible variable as the blood flow within a joint. For this clinical rheumatologist, the answer goes back to the cardinal signs of inflammation. The redness and heat of this state relate directly to increased local blood supply, and these critical findings cannot be examined systematically without accurate quantification of flow. Further, the imbalances of vascular permeability and tissue clearance that lead to tissue swelling cannot be evaluated usefully without an understanding of perfusion. Thus, disorders of synovial blood flow lie at the heart of clinical arthritis. If we are to understand articular inflammation and to quantify our management of it, it seems desirable and necessary to measure the synovial blood flow.

Perfusion

A number of approaches are now available for assessing synovial perfusion. They have been usefully categorised into those that provide relative data and those that provide absolute values in units of flow such as ml/min.¹ Each of these approaches can contribute to our understanding of synovial physiology. In general, relative tools are of most value in dealing with questions that are acute and within subject—each subject can serve as his or her own 'control'. For instance, two studies have used different ways to increase the hydrostatic pressure in synovial effusions, but both relied on laser Doppler devices to measure the impact of pressure variation on synovial blood flow.^{2,3} Their concern was not with the absolute flow, but with the relative change, and the chosen methodology served this purpose well.

Absolute determinations of flow generally serve better for questions that are chronic or compare different individuals. Thus studies of synovial blood flow in one patient under different therapeutic regimens are better served by absolute methods, as are studies that compare the articular physiology of subjects with different forms of arthritis, or perhaps at different stages of one disease.

As mentioned, laser Doppler is now the most popular of the relative methods. Similar information can be derived from clearance constants and the older literature contains a wealth of information derived with that approach.⁴ Ultrasound and magnetic resonance imaging are additional techniques that offer promise, for example, in distinguishing between those swollen joints with high and those with low synovial flow. The absolute

methods used to date include arterial perfusion with radiolabelled microspheres (long regarded as the 'gold standard' of tissue blood flow measures), the articular clearance of radiolabelled markers, and continuous perfusion of the joint space. The most common methods will be briefly reviewed here with a special emphasis on clearance—the principal method used in my own work (table).

Laser Doppler devices are convenient to use and offer the great advantage of quantitative data in real time.⁵ Thus the examiner can identify the probe position that provides the most consistent data and can retain that position throughout the experiment. Doppler probes can be sterilised readily and can be introduced safely into human joints, where they involve no radiation exposure. These features make them attractive for many experimental concerns in arthritic patients. There are potential drawbacks, however. Chief among these is the selection of the tiny area of synovium to be studied and the adequate positioning of the probe over it. Laser Dopplers have been used most widely in studies of intact skin, where it is easy to ensure that the probe is vertical to the surface, is in continuous contact with that surface, and does not exert undue pressure on the underlying tissues. These concerns are much more difficult to assess when the same probe is introduced blindly into a suprapatellar pouch. It is also relevant to remember that the data output is quantified in volts. These numbers can be reasonably converted into flow units in skin, but the necessary validation for such conversions has not yet been done in the synovium, and seems likely to be difficult.

The microsphere method is quite different, in that it provides absolute data in ml min⁻¹/100 g of tissue.⁶ With this method, radiolabelled spheres of uniform size are injected acutely (ideally into the left atrium) and are then distributed throughout the arterial tree, where they embolise every perfused tissue. Perfusion is quantified by counting the spheres in a given tissue sample and converting that

Methods for evaluating synovial perfusion

	Doppler	Microspheres	Clearance
Units	Volts	ml min ⁻¹ /100 g	ml min ⁻¹ /joint
Sampling error	Possible	Possible	Unlikely
Human use	Yes	No	Yes
Moving joint	No	Yes	No
Instant reading	Yes	No	No
Distribution*	No	No	Yes
Flux data†	No	Limited	Yes
Positioning problems	Of probe	No	Of counter

*Potential problems with adequate intra-articular mixing and transport across the synovial barrier.

†Utility in quantifying blood borne transport of solutes to the joint.

number to units of flow. This approach offers impressive precision and a long, strong 'track record' of validation in other tissues. It is excellent for looking for short term effects. For example, it is clearly the best technique for evaluating effects of exercise because it alone can provide 'snap shot' evaluations *during* exercise for comparison with baseline flows measured at rest. When we used this method to look at flows in dogs, we found an impressive sevenfold increase in flow in wrist synovium with a lesser, threefold, increase in the knee.⁷ Joints, of course, must move in order to function and it is critically important for us to assess them in action rather than simply at rest.

The microsphere method, also, has its drawbacks. It is expensive and requires considerable technical expertise to assure uniform sphere injections which mix fully with the arterial blood. Because of the need for substantial specimens of tissue, the method is terminal and thus can never be used in humans. Finally, its results depend entirely on where the tissue samples are taken. This is a lesser problem in relatively homogenous tissues such as skeletal muscle; it is of real concern in synovium, however, because most synovial blood vessels are superficial: an intimal biopsy therefore has more vessels (and flow) per unit weight than one including much underlying adventitia. This problem becomes worse when such distinctions are blurred by infiltrating inflammatory cells. Regional variations between, for instance, aerolar and fatty synovium would also be expected. Thus the method provides attractive data in $\text{ml min}^{-1}/100 \text{ g}$, but the sceptical reader must always be careful to ask: '100 grams of what?'

An alternative use of radiotracer methodology involves intra-articular injection of a radiolabelled solute, with subsequent serial counts over the joint to establish its rate of removal or clearance constant. This is achieved most readily with a gamma ray emitting marker that can be followed easily by external counting. With several different isotopes and with a broad range of molecular sizes from ionic sodium up to IgM, such studies have been impressive for their consistent linear, first order kinetics which permit expression of the clearance constant in, for example, min^{-1} . These values are readily converted to the half lives that are more familiar to most clinicians. Expressed thus, clearance constants are relative values. They can be used reliably to compare different individuals or different studies in the same individual only if it is known that the respective distribution volumes of the solutes are the same. Unfortunately, those unique conditions are rarely met. The distribution volumes of labelled solutes can be measured, however, and their values in ml can be multiplied by the clearance constants in min^{-1} , to provide clearance rates in, for example, $\text{ml min}^{-1}/\text{knee}$.⁸ This simple measure converts a relative term into an absolute measurement that carries considerable power in evaluating issues of articular physiology. If the marker molecule equilibrates with

perfusing plasma, as radiolabelled iodide is believed to do, its clearance provides a measure of the synovial plasma flow to the entire knee.⁹ If, in contrast, the marker equilibrates with the lymph draining from the joint, as radiolabelled albumin is believed to do, its clearance provides a measurement of lymphatic outflow, again measured in $\text{ml min}^{-1}/\text{knee}$.¹⁰ The potential power of these two variables lies in their ability together to help quantify not only perfusion and drainage, but also microvascular permeability and the articular flux rates of individual solutes.

Given the usefulness of such information, it is important to examine the underlying assumptions. The linearity of the rate constant is a critical assumption that is strongly supported by a wealth of empirical observation. We have found occasional exceptions which may require re-examination but, in general, the clearance constants of all solutes can be expressed as single values. It is not reasonable, however, to consider the distribution volume as a single, constant value. In these experiments, the marker molecules are injected into the relatively small synovial fluid pool, passive motion is used to facilitate distribution of the marker throughout that pool, and the markers then begin to diffuse into the synovial tissue where the blood vessels and lymphatics lie. It is fair to generalise that none of these markers is cleared from the synovial space without first entering the interstitial space. Once there, the marker can promptly enter the nearest vessel or it can diffuse past the abundant superficial network into deeper, much less vascular aerolar tissues. Throughout this process, the distribution volume progressively increases. In the case of albumin, it seems clear that a limiting distribution volume is ultimately reached, demarcated by a boundary that we consider to be the joint 'capsule'. In human knees, the distribution volume was found not to change between 24, 48, and 72 hours, and we thus consider albumin the best marker of the articular interstitial space. It should be clear that this volume is not simply that of the synovial fluid pool, but is that of the entire intracapsular space. It should also be clear that this distribution volume applies not just to albumin, but also to synovially elaborated cytokines, to matrix macromolecules released from abnormal cartilage, and to other molecules of interest that we sometimes consider only in terms of their presence in the intrasynovial space.

In dogs¹¹ and in goats (unpublished), we have measured distribution volumes of free iodide and of albumin in contralateral joints and have found them to be comparable. We do not know, however, if iodide and other small markers might ultimately diffuse beyond the capsule into adjacent tissues. For this reason, we now consider the distribution volume of albumin to be the best measure of the intracapsular space and the volume to be used in calculating effective plasma flow in addition to lymphatic drainage from studied joints. Thus the clearance approach takes volumes determined *after* full distribution of albumin

throughout the intracapsular space and multiplies them by clearance constants that are established *during* that equilibration process. This is justifiable only because of the convenient fact that the clearance constant remains linear throughout. What that linearity implies is that small molecules in joints have comparable access to the microvasculature irrespective of whether they are within synovial fluid or in synovial adventitial tissues. The teleology of this arrangement is an interesting subject for speculation, but the value to the investigator lies in its great practical utility.

Synovial solutes

In general, the solutes of synovial fluid exist in a stable equilibrium with those of plasma. For smaller solutes like electrolytes, urea, urate, or creatinine, the synovial barrier is highly permeable and 'equilibrium' implies a full equilibration in which concentrations depart from equality only in response to the plasma protein Donnan forces and to the excluded volume effect of synovial fluid hyaluronan (fig 1).¹² Glucose concentrations in normal synovial fluid appear to vary in parallel with those of plasma, with transport into the joint facilitated by a specific system in synovial lining cells.¹³

This normal state is sometimes altered in chronic synovitis, especially that of infection or of rheumatoid arthritis, in which a 'circulatory-metabolic imbalance' may occur.¹⁴ In this state, the vascular supply fails to keep pace with increased metabolic activity and, in comparison with plasma, the synovial fluid reveals increased lactate concentration and carbon dioxide partial pressure, together with decreased pH, oxygen partial pressure, and glucose concentration.¹⁵ There is no doubt that synovial hyperplasia increases the articular metabolic demand. In 'imbalanced' rheumatoid joints, it also seems clear that there is a disproportionately small and functionally inadequate vascular response.⁹ In such joints, microinfarctions of synovium frequently result in 'rice bodies' of sloughed tissues within the effused joint space. These findings, together

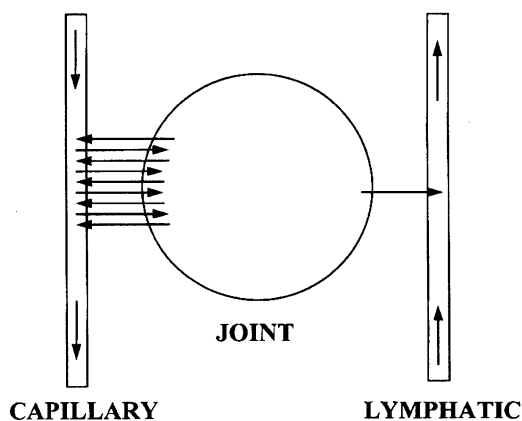


Figure 1 Articular transport of small molecules. The highly permeable fenestrae of synovial microvessels permit rapid, bidirectional exchange of small solutes between plasma and synovial fluid. Most small solutes that diffuse into the joint diffuse back out by the same path and lymphatics play only a trivial part in their clearance.

with the apparent paradox of decreased temperature in active rheumatoid joints, imply that some inflamed joints are significantly ischaemic. It is a matter of concern that this ischaemia may be unwittingly potentiated by prostaglandin depletion when physicians use non-steroidal anti-inflammatory drugs (NSAIDs) to treat the underlying rheumatoid disease.¹⁶

The largest literature on small solute transport across the synovial barrier deals with the concurrent kinetics of therapeutic agents in plasma and synovial fluid.¹⁷ Such studies are usually conducted by administering a test drug to a patient with a large knee effusion and then recording drug concentration over time in serial samples of plasma and synovial fluid (fig 2).¹⁸ This basic plan has been followed for essentially all therapeutic agents used in the treatment of infection or inflammation in human joints. Originally, the intent of this work was simply to demonstrate that drugs do achieve entry to target joints. By now, it should be clear that all drugs studied are able to achieve entry, and the appropriate questions now deal with the routes and rates of articular transport.

We have begun this process with an analysis of data from 10 different studies of articular NSAID traffic.¹⁹ We looked only at single dose studies with oral administration of the trial drug to patients with rheumatoid knee effusions. We assumed that the synovial blood flow and protein kinetics in these knees were like those we had evaluated in rheumatoid patients in Seattle and we subjected the data to a simple compartmental analysis. This approach allowed us to find the best fitting rates of drug entry into and exit from the average rheumatoid knee effusion.

The analysis indicated that an average of 23% of the NSAID perfusing each knee diffuses out of the vasculature. This value compares well to the expected extraction of free drugs and therefore demonstrates that these '99% protein bound' drugs are widely available to the local tissues. In fact, it could be that NSAIDs are 'albumin carried' in a delivery system analogous to that by which oxygen is 'haemoglobin carried' to peripheral tissues. The possibility that significant amounts of each drug might cross the barrier in a bound state was considered and rejected, however, on the basis of available data on albumin transport in comparable knee joints. The study also shows that the relatively slow increase in synovial fluid concentration reflects not any impairment of transport, but the large intra-articular volume that must be supplied by a relatively low plasma flow rate in order to reach concentrations in synovial fluid that equal those in plasma. These same considerations also explain the relatively slow removal of drugs from effused joints, and we found no support for the concept of specific articular 'concentration' of NSAIDs. Finally, the analysis suggested that essentially all of the agents evaluated have comparable access to the joint space. This last conclusion cannot be drawn definitively because it is based on the

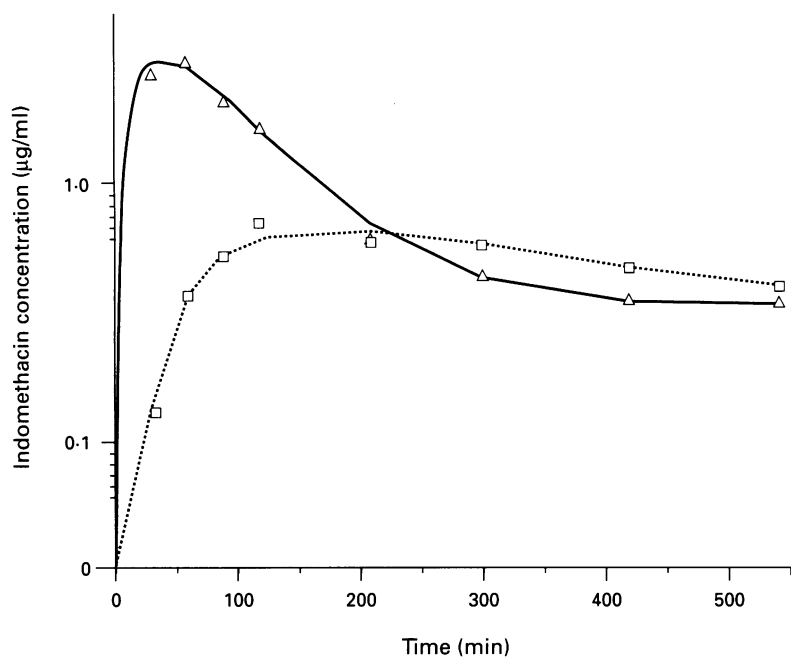


Figure 2 Mean concentrations of indomethacin in serum (Δ) and synovial fluid (\square) of rheumatoid patients after a single oral dose. The synovial fluid curve (dotted line) was fitted using a model of free, bidirectional diffusion across the synovium and assuming synovial vascular variables equivalent to those found in rheumatoid knees in Seattle. The data fit this model well as manifested by symmetrical, calculated clearances of 0.35 ml/min from plasma to knee and 0.37 ml/min from knee to plasma. (Recalculated from the data of Emori et al.¹⁸.)

assumption that each group of rheumatoid patients had synovial vascular kinetics comparable to those of the patients we studied in Seattle. For future studies of articular pharmacokinetics, however, it would be quite feasible to analyse the relevant vascular variables concurrently with the standard sequential determinations of drug concentration in serum and synovial fluid. Such experiments would individualise the extraction data to a level that has not been possible in previous studies of drug delivery to other connective tissue sites. This, then, is the approach that will be needed to determine if a few avidly bound therapeutic agents may be less accessible to the joint than are more loosely held drugs.

Plasma proteins comprise the final important class of synovial fluid solutes. It has been known for years that the total concentrations of intrasynovial proteins is invariably smaller than that of plasma proteins, that the concentration increases with articular inflammation, and that the synovial fluid/serum (SF/S) disparity increases with the size of the protein species.²⁰ Most data on molecular size rest on measurements of specific proteins by radial immunodiffusion in paired S and SF samples. These data have been interpreted as evidence of size-specific passive diffusion into the joint space opposed by size-independent, convective clearance of proteins through synovial tissue lymphatics (fig 3). Each of these mechanisms implies passive transport of proteins without local synthesis, release, or consumption of individual protein species. Exceptions to this rule exist, however, and have elicited considerable recent interest. In the past, the principal examples were lubricin, a locally produced lubricant within synovial

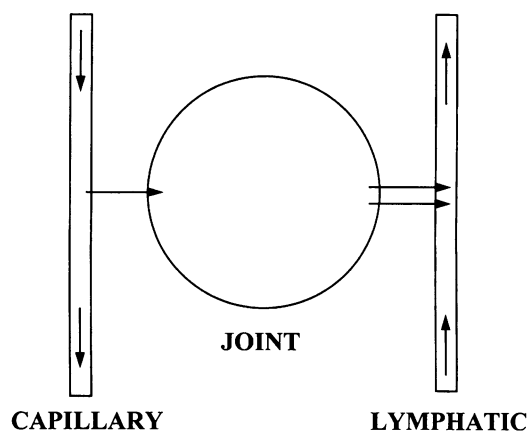


Figure 3 Articular transport of plasma proteins. The synovial microvessels permit small amounts of plasma proteins to diffuse into the joint and these proteins ultimately return to the circulation through lymphatics. If 100 μ l of plasma is cleared into the joint per minute while 200 μ l of synovial fluid is cleared back out, the SF:S concentration ratio for that protein is 0.5. As smaller proteins diffuse in more readily than large, while lymphatic clearance is independent of size, the SF:S ratio is inversely proportional to the molecular radius.

joints; immunoglobulins, which are produced in some rheumatoid joints; and complement, which can be locally consumed in immunologically mediated forms of synovitis. Of course, hyaluronan is another macromolecule that is produced within the joint and which will be reviewed in this symposium.^{21 22} Recently, the greatest interest has focused on two classes of molecules: cytokines and structural components of articular cartilage.²³ In both cases, an abundance of papers confirm synovial fluid concentrations greater than those in concurrently obtained samples of serum. Such data are appropriately interpreted as unequivocal evidence of intra-articular synthesis and release, as reviewed in this symposium.

The interest in cytokines and in cartilage 'markers' arises from their significance as evidence of the local inflammatory response and of disease impact on cartilage. To achieve their maximum potential use to the clinician, we should be able to use serial observations to draw inferences regarding the success or failure of therapeutic interventions. The principal limitation of the available data is that they are largely limited to measurements of SF concentration. In every case, such values reflect not release alone, but the balance between release and clearance from the joint. If macromolecular clearance was constant within and between individuals, we could readily forget about it, but such is not the case. In rheumatoid arthritis, for instance, the lymphatic drainage was about twice that in osteoarthritis when groups of patients with each disease were compared.¹⁰ Those findings require confirmation in other laboratories, but if they pertain generally, it would be necessary that a cartilage marker had twice the concentration in an osteoarthritic joint than that in rheumatoid disease in order to indicate a comparable level of intra-articular release. In another example of this principle, we might postulate that effective anti-inflammatory therapy would lead to a decreased rate of

lymphatic drainage. If this were so, an increasing concentration of SF marker molecules could herald an improvement in the overall well being of the joint with a concomitant decrease in marker release. These instances imply that comparisons between individuals and within individuals would be much more valuable if concentration data could be combined with clearance values to provide flux rates in absolute units such as mg/minute per knee.

The overwhelming majority of the proteins in SF are not unique to that site, but are also found in the perfusing plasma. The clinician who wishes to interpret their concentration often turns to the SF:S ratio. As previously mentioned, however, SF:S decreases with increasing molecular size and thus must be considered for each molecular species within the context of its dimensions. To examine this relationship further, we obtained 'normal' SF from the knees of patients dying without known joint disease and 'inflamed' SF from the swollen knees of patients in our arthritis clinic. Aliquots of these samples together with sera from the same individuals were size-fractionated by passing them over gel filtration columns. This simple approach permitted us to derive essentially continuous plots of mean values for SF:S across a broad range of molecular radii, for both normal and inflamed joints.²⁴

Both in normal and in inflamed knees, these data showed that small molecules were fully equilibrated between SF and S and the SF:S ratio was 1.0 or, in inflamed knees, sometimes greater. SF:S then decreased progressively as molecular size increased, reaching plateau values that were much smaller in normal than in inflamed joints. We interpreted these plateaus as points at which diffusion no longer contributed significantly to the SF concentration, and therefore assumed that the plateau values reflected the balance between a 'plasma leak' and lymphatic drainage. As both of these presumed mechanisms are convective, they would no longer be size-selective and should lead to plateaus such as those we observed. For illustrative purposes, we are re-evaluating these data within the context of available values for protein clearance from normal and diseased knees.

Analyses of the continuous SF:S values in concert with protein kinetic values provide substantial power in that they permit calculation of the presumed plasma leak, of the path permitting protein diffusion into the joint, and of the limiting dimension where smaller solutes begin to diffuse through fenestrae, as well as between endothelial cells. This approach should be applicable to individual joints in

human beings and in experimental animals. If this proves to be so, it will provide unprecedented characterisation in general, in addition to size-specific terms of the normal transport processes, of their alteration by disease, and of their response to therapeutic intervention.

Supported by NIH grant AR 32811.

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