Techniques of Microperfusion of Renal Tubules and Capillaries

NORMAN BANK and HAGOP S. AYNEDJIAN

Renal Service, Montefiore Hospital and Medical Center, Bronx, New York 10467

Received for publication 19 November 1971

During the past few years, we have tried several different methods of microperfusion of rat proximal convoluted tubules(1,2). Some of the advantages of microperfusion as an experimental tool are quite obvious. First, one can control the rate of flow down the tubule independent of changes in whole kidney glomerular filtration rate. Thus, the effects of tubular flow rate and peritubular flow rate can be dissociated. Second, one can control the composition of the perfusion fluid to suit the experimental situation. Thus, hyper- or hypoosmolar solutions can be injected and water movement across the epithelium can be studied. Finally, flux measurements in either direction can be studied with radioactive isotopes.

With regard to the perfusion pump described by Sonnenberg and Deetjen(3), we experienced the same difficulties as did these authors. In addition, because of the weight of the motor and the fact that the unit is mounted directly on the micromanipulator, we occasionally encountered difficulty controlling the movements of the pipet. Because of these problems, after an initial project(1), we subsequently have used a Sage pump and Hamilton syringe to control the flow of the perfusion fluid out of the micropipet. We find this system easier to use and generally reliable.

The main part this discussion pertains to the technical aspects of microperfusion, both intratubular and peritubular capillary. First, with regard to intratubular perfusion, we have tried several different methods. The first one, shown in Fig. 1, is similar in several respects to the technique described by Sonnenberg and Deetjen(3). Using sharply ground pipets, we introduce an oil-filled collecting pipet first into a randomly selected proximal convolution. In order to determine the direction of tubular fluid flow, we inject a few small droplets of colored oil and observe their movement and identify other convolutions of the same nephron. Once having done this, we introduce the perfusion pipet in the convolution just distal to the one with the collecting pipet, and start the perfusion. The per-
fusion solution contains a low concentration of lissamine green (0.1 g/100 ml) in order to outline all of the convolutions of the nephron. The next step in this sequence is the injection of a proximal oil block between the two pipets. We try to make this a rather long column. The collecting pipet is then withdrawn from the lumen and thrust repeatedly into the same convolution of the tubule in slightly different locations. The purpose of this is to provide enough holes to allow the fluid coming from the glomerulus to drain onto the surface of the kidney. One of the problems which this drainage introduces is that large amounts of surface fluid accumulate. If radioactive material is being infused intravenously, the surface fluid will obviously become contaminated with it. One must, therefore, be very careful to avoid aspirating any surface fluid into the collecting pipet. The last step in the sequence is to introduce a second oil block into the most distal convolution being perfused, so that the perfused segment of the tubule is completely isolated by two oil blocks. The distal oil block is important for two reasons: First, one can calibrate the rate of perfusion in vivo by having a non-reabsorbable solute in the perfusion fluid (i.e., inulin). We found that there is a difference between in vitro and in vivo calibration rates. Usually the rates are 10–15% lower in vivo than that determined in vitro and occasionally the variation is even greater. Thus, it is very risky to depend upon in vitro calibration and to assume in vivo perfusion rates. One cannot be certain of the perfusion rate unless it is actually being measured in each perfusion in vivo. Second, the distal oil block prevents back-flow contamination, which can be a problem unless one is collecting the perfused fluid at very low rates and allowing the rest to flow past the pipet. If one is interested in flux measurements, it is, of course, essential to have quantitative collections, i.e., to collect all of the fluid coming from the perfusion pipet, and a distal oil block is necessary to accomplish this. The problem with the distal oil block in this particular technique is that as soon as the distal oil column is injected, a closed space is created between the two oil blocks.

![Diagram of microperfusion technique](image)

Fig. 1. Technique for microperfusion of renal tubules. The collecting pipet is filled with Sudan black-stained castor oil. The cross-hatched areas represent the perfusion fluid.
two oil columns immediately start to move in opposite directions. If the perfusion rate is relatively slow, that is 10 nl/min or less, there is time to inject the distal block and start collecting fluid before the oil columns have moved very far. At faster rates of perfusion, however, the introduction of the distal oil block must be made much more rapidly. After it is in place, the collection is started and continued at a rate which maintains both oil blocks in a constant position. Occasionally, if the distal block is not injected fast enough, or if the collection rate does not keep pace with the perfusion rate, the proximal oil will be pushed out of the holes in the tubule and, of course, one cannot continue with that perfusion. That is one of the difficulties with this particular method.

In Fig. 2 is shown a variation which we have tried and found to work somewhat better(1). The sequence starts off in the same way by inserting the collecting pipet and identifying the convolutions of the nephron with a few small oil droplets. We then inject a very long column of oil which fills almost the entire proximal tubule. The collecting pipet is then withdrawn, a few holes are made by it in the same convolution, and the collecting pipet is then introduced into the most distal convolution that has been filled by the oil column. The perfusion pipet is next inserted into an oilfilled convolution just distal to the original site of oil injection. As the perfusion fluid flows into the tubule, it splits the oil, and at the same time we start collecting oil into the collecting pipet. The rate of collection of the oil is controlled and maintained at the same rate as the perfusion fluid is advancing. Eventually the perfusion fluid reaches the collecting pipet and enters it. This method has the distinct advantage of having the distal and

![Diagram of perfusion process](image)

Fig. 2. Modified technique for perfusion of renal tubules. The long oil column is split by the entering perfusion fluid. As the fluid enters, oil is continuously aspirated by the collecting pipet (Stage 4) until the perfusate reaches the collecting pipet.
proximal oil blocks in place at the time the perfusion is started. Because a pressure build-up is prevented, due to the withdrawal of oil into the collecting pipet, there is little or no tendency for the two oil blocks to move out of position. The timing of the collection of perfusate is started when the fluid reaches and enters the collecting pipet. A theoretical disadvantage of this technique is that the oil might have a damaging effect on the epithelium and thus cause abnormalities in transport or permeability. We have noted no obvious defects in transport, however.

Fig. 3 describes a third method of microperfusion developed by Mr. Aynedjian. A vascular star is first identified and the collecting pipet inserted into an end-proximal convolution adjacent to the star. An oil column is then injected and allowed to flow down into the loop of Henle. With careful observation of the surrounding area, one can see proximal convolutions of the same nephron dilate. At times it is possible to determine a retrograde sequence of dilation. One of these proximal convolutions is then selected, the perfusion pipet is inserted into it and the perfusion started. The collecting pipet is then withdrawn. As the perfusion proceeds, the excess fluid comes out onto the surface through the hole left by the collecting pipet. The collecting pipet is next introduced into a more proximal convolution and a proximal oil block injected. A few holes are made with the pipet as it is withdrawn. The same collecting pipet is then replaced in the original hole in the terminal convolution and the collection started. This method has the disadvantage that the collecting pipet has to be placed back into the same hole made originally; otherwise perfusion fluid might be lost onto the surface of the kidney and the collection would not be quantitative. The advantage is that a fixed distal oil block is assured since it descends into the loop of Henle and is not able to move further. When used carefully, this method can yield successful perfusions. It cannot be used in diuretic animals, since the

Fig. 3. Modified technique for perfusion of renal tubules. The distal oil block is injected first into a late segment of the proximal convoluted tubule located near a vascular “star.”
tubules are already dilated and one cannot identify those convolutions belonging to a given terminal segment.

These three methods are described in some detail because they all were developed with the two oil block system in mind. As stated above, we believe it is important to have both a proximal and distal oil block when perfusing tubules. The two main reasons are to allow in vivo calibration of the perfusion rate and to avoid contamination of the perfusate by retrograde flow of tubular fluid.

With regard to perfusion of peritubular capillaries (Fig. 4) several technical points should be stressed. First of all, the kidney must be very carefully immobilized. Since one of the pipets is in a blood vessel, movement of the kidney can cause tearing of the blood vessel and bleeding on the surface. Very sharp pipets are necessary because, as we carry out this technique, the two pipets are quite close to one another. Thus, once the first pipet is in place, the second pipet must be introduced without undue pressure, otherwise the first pipet will be dislodged. We start off by introducing the perfusion pipet into the center of a vascular star and perfusing from that point. Three reasons for this are: these large blood vessels are easier to puncture and one can use a large-size pipet tip, i.e., 8–10 μ, the direction of flow of perfusion fluid is the same as that of normal blood flow, and the filling of the branches of the star can be judged before choosing a tubular convolution for puncture. As shown in Fig. 4, the perfusion not infrequently surrounds some terminal convolutions more completely than others. By starting the perfusion first, one can be certain that the tubule from which the collection is

Fig. 4. Technique for microperfusion of peritubular capillaries and simultaneous collection of free-flow tubular fluid. The perfusion pipet is inserted into the center of a vascular “star.” The cross-hatched area represents the perfused capillaries. A marker (¹⁴C inulin) is added to the perfusion pipet in order to detect accidental puncture of the tubular lumen by the perfusion pipet.
made is actually being perfused optimally. We have used both gas pressure and a Sage pump to drive the perfusion solution. In our experience, the pipet tip diameter has a major influence on the rate of perfusion when gas pressure is used to drive the perfusion. The larger the tip size, the higher the rate of flow. Very large variations in flow rate can occur, unless one is careful about producing pipets of a uniform size. We do not know how precise or consistent the Sage pump is in capillary perfusion, since in vivo calibration is not possible, but it is probably better than the gas pressure system. After we have judged the perfusion of the capillaries visually, we insert the collecting pipet into the most distal convolution at the vascular star, inject an oil block, and start collection. In order to detect possible contamination of the tubular fluid with the perfusion fluid, due to an accidental puncture of the tubule with the perfusion pipet, we add a non-reabsorbable substance to the perfusion fluid. In the particular situation shown in Fig. 4, $^{14}$C-inulin was in the perfusion fluid and $^{3}$H-inulin was given intravenously. We then count the collected fluid for both $^{14}$C and $^{3}$H. The method for calculating contamination by this method is carried out by the following equations:

$$SNGFR = \frac{\text{Total } ^{3}\text{H in tubular sample/min collection}}{\text{plasma } ^{3}\text{H}}$$

1. $\text{Expected } ^{14}\text{C} = SNGFR \times \text{plasma } ^{14}\text{C}$.  

From the intravenously infused $^{3}$H-inulin, single nephron GFR (SNGFR) is calculated in the usual way. One can then determine the expected amount of $^{14}$C that should be contained in that collected sample from the SNGFR $\times$ the plasma level of $^{14}$C. Any tubular collection that contains more $^{14}$C than is estimated by this equation is assumed to be contaminated, due to entry of the perfusion pipet into the tubular lumen. We found in a series of 105 consecutive perfusions that accidental puncture of the tubular lumen occurred in about 15%, even though we could not detect contamination by discoloration of the collected fluid with lissamine green. We think, therefore, that this is an important control device to ensure that the perfusion pipet has not punctured the tubular lumen.

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