A Review of the Uses and Disadvantages of Various Renal Micropuncture Techniques

This essay will critically review some of the major uses of renal micropuncture techniques, focusing in particular on the stationary method, free-flow micropuncture and renal microperfusion, the latter including both in vivo and in vitro methods. The essay will conclude with a short review of other uses of micropuncture.

Wearn and Richards (1924) were the first people to use micropuncture successfully on the kidney. They successfully inserted a micropipette into the glomerulus of a single amphibian nephron and their results provided conclusive evidence that ultrafiltration of the blood occurred at this site. Walker, Bott, Oliver and MacDowell (1941) later extended the technique to the proximal and distal convoluted tubules. They demonstrated isosmotic reabsorption in the proximal convoluted tubule, and an above plasma concentration of chloride ions. This latter result implied that reabsorption in the kidney was not a simple passive affair. Gottschalk and Mylle (1958) micropunctured the loop of Henle providing conclusive evidence for Kuhn's theoretical countercurrent multiplier system, and Sakai, Jamison and Berliner (1965) micropunctured the collecting duct.

A problem with micropuncture techniques is that they require exceptionally fine and skilled micromanipulation to avoid puncturing the other side of the tubule. This is why the original experiments, up until Gottschalk (1956), were done on amphibian nephrons. The problem with mammalian nephrons was that they were too small, had an absence of glomeruli on the surface of the kidney, and tended to move due to respiratory movements. These problems were overcome by a combination of two improvements. Alexander and Nastuk (1953) developed the micropipette puller, which mechanically "pulled" capillary tubes over heat, instead of by hand. This produced micropipettes with much smaller diameters (8x10-6m approx.) allowing more accurate micromanipulation. Gottschalk (1956) developed a method whereby the kidney is isolated in a double cup, thus decreasing any movements due to the animals respiration.

All the above experiments used the free-flow micropuncture technique. A micropipette is inserted through the tubule wall and fluid enters the pipette spontaneously due to intratubular hydrostatic pressure. One problem with this technique is that if too much fluid is withdrawn from the tubule, either by negative pressure or simply by capillarity, tubular flow will change affecting tubular pressure, decreasing transit time and increasing the glomerular filtration rate. Analytical data from such samples is then of little use. To avoid this firstly, fluid should be allowed to enter only very slowly (therefore a little positive counter pressure should be applied to the pipette). Secondly tubular diameter should be closely monitored to avoid collapse. Thirdly the sample size should not be greater than 10-20% of the total volume flow past the puncture site. Finally intratubular pressure should be measured before and during sampling and kept constant.

Later experiments blocked the nephron with a lump of mercury allowing all the fluid passing the puncture site to be collected. This allowed the glomerular filtration rates of single nephrons to be calculated. Mercury was originally used to block the nephron, although a high pressure was required to inject it, and problems such as filling the whole nephron with mercury, or breaking pipette seals often arose. Kennedy's method of staining mineral oil provided a superior substitute.

The next technique to be developed, by Windhager and Schatzmann (1953), and later improved upon by Gertz (1963), was the stationary perfusion, also known as the "split-drop" technique. A droplet of oil was injected into the tubule, followed by the test solution which split the oil drop. Time lapse photography was used to measure the changes in the droplet length and thus demonstrate reabsorption or secretion along discrete tubule lengths. One problem is that the length of the oil droplet must be very accurate. It must be long enough to prevent fluid entering or escaping between the brush border, but sufficiently short to allow oil to move when the test droplet is shrinking. Furthermore castor oil must be used (paraffin oil provides incomplete sealing and may damage the brush border) which increases the luminal diameter from 20x10-6m to 30x10-6m. Therefore instead of a decrease in length the reabsorption may suck the sides of the tubule in. For several reasons, such as inaccuracy of the microphotographs or the effects of the brush border, a direct evaluation of the tubular diameter on volume reabsorption if difficult and so data can be conflicting. Another disadvantage is that the preparation is a static situation - won't flow itself have some effect on reabsorption?

Thus the technique of microperfusion was perfected by Burg and Orloff (1966). The flow of tubular urine is blocked proximal to the puncture site with oil and the rest of the nephron is perfused continuously with a test solution at a constant speed. Lower down the nephron is also blocked with oil to allow accurate calibration of the rate of perfusion in vivo, and to prevent backflow contamination. Samples of the perfusate are collected from single loops reappearing at the kidney surface and changes in the concentration of the perfusate are determined. This technique has several advantages. Firstly one can control the rate of flow down the tubule independent of changes in whole kidney glomerular filtration rate. Thus effects of tubular flow rate and peritubular flow rate can be dissociated. Secondly one can control the composition of the perfusion fluid to suit the experimental situation. Thus hyper-/hypo- osmolar 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. One problem is that due to the weight of the perfusion pump motor, and the fact that it has to be mounted directly on the micromanipulator, occasional difficulties can be encountered in controlling pipette movements. A more serious problem is that the two oil blocks have a tendency to drift apart. Therefore the distal oil block must be injected rapidly for if it isn't, or if the collection rate doesn't keep pace with the perfusion rate, then the proximal oil block will be pushed out of the holes in the tubule.

This latter problem can be overcome by filling the whole of the proximal convoluted tubule with oil. The perfusate is injected into the oil and the collecting pipette must draw up oil at the same rate as the perfusate enters. Once the perfusate enters the collecting

pipette perfusion can commence. An advantage of this technique is that the oil block is in place as soon as perfusion starts, so there is no pressure build-up and therefore the oil blocks do not move. Alternatively the oil block can be injects first into a late segment of the proximal convoluted tubule near a vascular "star". The proximal convoluted tubule swells retrogradely allowing its identification. The perfusate is then injected, followed by the proximal oil block and then the collecting pipette re-enters the tubule at the location of the distal oil block injection. One problem is that the collecting pipette has to be placed into the original hole, otherwise perfusion fluid might be lost onto the surface of the kidney and collection would not be quantitative. Another disadvantage is that the technique cannot be used on diuretic animals since their tubules are already dilated and so one cannot identify those convolutions belonging to a given terminal segment. The major 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.

This technique has also been adapted to in vitro methods. It has proved difficult to dissect single nephrons out of animals, and has been achieved only in rabbits and flounders. Furthermore the collecting ducts tend to split open, the distal convoluted tubule is too small to study (<1mm) and the loop of Henle is difficult to identify. Thus only the perfusion of the proximal convoluted tubules has been studied in vitro. Further problems have included leaks between the lumen and the bath. The seal was originally improved by advancing the inner pipette along the lumen length, although this ran the risk of damaging the tubule and it could not be used for convoluted tubules. Now Sylgard 184, a liquid dielectric is used. Further improvements included the "fluid-changing pipette" allowing a variety of perfusion fluids to be tested in a single tubule; also, whereas previously bath fluid could enter the perfusion pipette (diluting its contents) whilst the tubule was being cannulated, now the correct perfusion solution can be placed in the pipette after the tubule is attached. However, recent research has suggested that nephrons don't behave in vitro as they do in vivo, and the validity of this technique is being reexamined.

In conclusion some mention must be made of other uses of the above techniques which are beyond the scope of this essay. These include the determination of the hydrostatic pressure of tubular fluid and glomerular capillaries, measurement of electrical potential differences across tubular epithelium and across cell membranes, the determination of specific transepithelial resistance and the perfusion of blood capillaries. It is certainly no understatement to say that the technique of micropuncture has been the single most important event in the history of our understanding of renal physiology.