Localisation of Renal Function

D.I. Newble
B.Sc.

Abstract
An article based on a Dissertation read before the R.M.S. on 17th January, 1964.

Classical renal physiology as taught to the undergraduate during his medical course, regards the kidney as an entity with little attempt to relate function to the basic unit of the nephron. In most instances this is permissible as it gives a functional understanding of renal processes enabling the clinician to diagnose and treat conditions where this function is impaired, either from intrinsic or extrinsic causes. However, it is not sufficient today to regard complicated organs solely in this fashion. Thus the functions of the kidney, particularly that of ‘acid-base balance’ are briefly discussed at a more fundamental level.
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The first major advances in the localisation of renal function stem from the classical work of A. N. Richards in the 1920’s. He was the first to develop micropuncture techniques into renal physiology. He related the acidification process, in the frog, to the distal tubule. Walker et al (1946) later developed similar methods applicable to mammalian kidneys. Basically, the technique was to insert a micropipette into the renal substance and withdraw samples of the tubular fluid. The site of the puncture was marked by the injection of India ink and being accurately located by maceration and microscopic examination.

Further progress was made in 1957 when Malvin, Sullivan and Wilde described their “stop flow” analysis method. Pitts et al (1958) used this method to investigate tubular function in dogs. The ureters were catheterised and priming doses of creatinine PAH and phosphate were given. Infusions were maintained until stabilisation occurred. The catheters were then clamped for varying times ranging from 2 to 8 minutes. 20 to 40 one millilitre sample were collected automatically in vials fixed into a moving bar. Theoretically, clamping the ureter produces a rapid pressure build up in the tubules until the back pressure equals that of the glomerular filtration pressure. A stationary column of fluid is then in contact with the tubular epithelium which performs, in an exaggerated fashion, its normal functions. When the clamp is released, the fluid is forcibly ejected. The first samples obtained are those from the distal tubules and the later ones from the proximal tubules. The results showed that acidification, ammonia production and potassium-sodium exchange all reached peak values in the same samples, these being those from the distal tubules. Phosphate reabsorption occurred in the proximal tubule samples and was in no way related to the acidification process. Although this might appear conclusive there are many criticisms of the method. As samples were collected in air small pH changes could be missed. Also, the pelvis of the kidney acts as a mixing chamber, this effecting the later samples in particular. The method therefore provides valuable qualitative information but care must be taken in interpretation of the results.

One further technique has been used. Ulrich and Eigler (1958) managed to insert a polyethylene catheter into the collecting ducts of hamsters. They confirmed the long suspected fact that there is a large pH fall at
The first indication that these do not provide the complete answer came from Ellinger as early as 1940. He observed colour changes of an indicator passing along the tubule. In both the frog and the rat he found that acidification occurred specifically in the distal tubule only during a mild acidosis. If the urine was strongly acid then he found colour changes along the length of the nephron.

One of the most complete series of micro-puncture studies was performed by Gottschalk, Lassiter and Mylle in 1960. The fluid collected was scaled in the micropipettes which also acted as microelectodes. The pH was determined by potential changes in the fluid. All their equipment was equilibrated with air containing CO₂ at 27 mm Hg. Collections were obtained from non-diuretic animals and from those in a state of osmotic diuresis, both normally and during an ammonium chloride acidosis. In all cases there was a progressive acidification along both sections of the tubule. (Fig 1.) This is conclusive proof that, in rats at least, proximal tubules can acidify urine. Biochemical analysis has shown that equal amounts of carbonic anhydrase, the enzyme necessary for hydrogen ion exchange, are present in both sites.

![Fig. 1. Micro-puncture study illustrating progressive acidification along the nephron. (after Gottschalk et al. 1960)](image)

The pH of tubular fluid relative to plasma pH was measured at different parts of the tubule.

Obviously this state of knowledge is far from satisfactory but it might be profitable to attempt to summarise the mechanisms proposed at this time. About 80% of the glomerular filtrate is reabsorbed in the proximal tubules under what Smith called “obligatory reabsorption”. The evidence suggests that the bulk of the hydrogen ion exchange also occurs here.

The distal tubules are capable of the same processes and probably act as the fine adjustors of pH in the same way as they regulate the ‘facultative reabsorption’ of water. The collecting ducts can make very little contribution to overall sodium and bicarbonate reabsorption as the load presented is very small. Large pH changes could occur with a relatively low hydrogen ion secretion rate.

Having thus attempted to localise the processes in the occurring kidney the actual mode of transport of ions by the tubular cells must be discussed. The first recorded experiments on active transport in the kidney came from Wilbrandt in 1938. These he performed on Necturus which is an animal having conveniently large nephrons with long straight proximal tubules. He measured potential differences between the surface of the kidney and the lumen of the tubules using for electrodes micropipettes similar to those used by Richards. He obtained “transtubular potential” values of up to -12mV, negative inside the lumen. This he interpreted as being due to different ion permeabilities on the two sides of the cell.

Ussing et al (1951) demonstrated a potential difference across frogs’ skin arising as a consequence of active transport. He defined this as ion transport against an electro-chemical gradient. Perhaps more well known are the experiments of Hodglin et al (1952) where the electrical activity of nerves was shown to arise from the passage of Na and K ions across the cell membrane. Not unnaturally, workers turned to the kidney to study these processes as it is an organ where it is relatively easy to make electrical recordings and to determine ionic concentrations without substantially altering the physiological conditions.

Solomon (1957), utilising the specialised electrodes developed in nerve and muscle studies by Ling and Gerard, observed a bimodal distribution of potentials on random insertions into the tubules of rats. During the puncture, transient higher potentials were recorded indicating that the electrodes were passing through cells with a greater negativity than the lumen. The lower range was related to the proximal and the higher to the distal tubules.

Giebisch in a much fuller investigation using the proximal tubules of Necturus found a mean value of -72mV for the peritubular membrane potential (i.e. the P.D. between the peritubular fluid and the inside of the cell) and of -20mV for the transtubular potential. By difference,
the luminal membrane potential was about 52 mV negative inside the cell. Reductions in these potentials were produced by oxygen lack and by mercurial diuretics which have both been shown to reduce active transport processes. (Fig. 2.)

In investigations of the transtubular potential, direct microanalysis showed no concentration gradient between the peritubular and tubular fluids for Na, K and Cl ions. The potential difference is not, therefore, maintained by ionic concentration gradients. There must be one or more active mechanisms involved. Many workers believe that Na is the only ion actively transported while the other ions follow passively down the electro-chemical gradients set up. In Necturus, at least, there is probably some active K transport. The primary process can be regarded as a shift of positive ions from the lumen leaving it at a negative potential. This is a process requiring energy.

Microanalysis also shows that the Na concentration inside the cell is less than that in the tubular fluid. As shown above, the inside of the cell is at a more negative potential than the lumen so Na ions can passively enter the cell along an electrical and a chemical concentration gradient. However the opposite is true of the peritubular border so it is logical to assume that the active mechanism is situated here. This appears to be substantiated by electronmicroscopy where the mitochondria are shown to be almost exclusively situated on this border. There is some evidence to suggest that K uptake into the cell is linked to this "sodium pump" as the concentration of K inside is greater than would be expected if only passive forces were involved. (Fig. 3.)

This interpretation is obviously oversimplified. Refined techniques involving single nephron perfusion, measurement of ionic fluxes utilising radioactive isotopes have all been used, but the calculations involved in these methods are complex, and cannot be adequately discussed here. It would also be unwise to attempt clear interpretations and explanations at this stage of research.

Medicine is no longer an empirical art. Soon it will be inadequate to know simply what alterations in blood and urine biochemistry indicate. The basic changes occurring at cellular and sub-cellular levels must be understood. This article illustrates the limited advances made in one small field but perhaps indicates also the trend of research in the future.

REFERENCES

Ellinger. Quart. J. Exper. Physiol. 30. 1940.