ESSAYS ON APS CLASSIC PAPERS

Micropuncture: unlocking the secrets of renal function

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This essay looks at the historical significance of five APS classic papers that are freely available online:


The development of micropuncture by Wearn and Richards in 1924 (7) ranks as one of the greatest advances in renal physiology during the 20th century, along with the development of the isolated perfused tubule by Burg and colleagues in 1966 (1). It is fitting that the seminal papers reporting the initial development of each of these techniques were published in the *American Journal of Physiology* (1, 7). This essay will highlight some of the classic early micropuncture papers by Richards (Fig. 1) and his laboratory (3–7) published in the *American Journal of Physiology* between 1924 and 1941. These studies are remarkable for the technical skill required to develop the necessary techniques and novel scientific findings that resulted, most of which are as true today as they were when originally published.

Gottschalk (2) wrote in 1992 that Wearn and Richards’ 1924 paper (7) is one of the most important single contributions to renal physiology ever published. Wearn and Richards’ paper (7) describes the method for performing micropuncture in frogs. Equally important, it provides the first experimental evidence that a protein-free glomerular ultrafiltrate is separated from the bloodstream and the first evidence for tubular reabsorption (7)! To fully appreciate the significance of Wearn and Richards’ paper, one must recall that a major question at that time concerned the mechanisms involved in urine formation. Wearn and Richards’ introduction contains an elegant review of the experimental literature supporting glomerular filtration, tubular reabsorption, and tubular secretion. These were theoretical mechanisms, and their existence and their relative contribution to urine formation appear to have been actively debated at that time. Wearn came up with the idea of trying to puncture a glomerular capsule with a pipette and measure the composition of its fluid (2). Wearn and Richards measured protein, glucose, chloride, potassium, urea, and pH in blood, glomerular fluid, and bladder urine. Comparison of the composition of blood to glomerular filtrate proved that a protein-free watery fluid is separated from the blood as it passes through the glomerulus (7). The absence of sodium chloride or...
glomerular filtrate, proved that tubular reabsorption must take place (7). Wearn and Richards (7) concluded that the similarity of structure between the frog and mammalian glomerulus suggests that their observations in the frog are likely pertinent to the mammal, but they also suggested the need for developing a methodology for performing micropuncture in mammals.

The next step in the evolution of micropuncture was published by the Richards lab in the American Journal of Physiology in 1937 (3, 5). Richards and Walker developed methods for micropuncturing surface nephrons in Necturus and frogs and perfusing proximal or distal convoluted tubules in situ (3). Richards and Walker used an oil block to ensure that the collected fluid was from a specific portion of the nephron and not from back-leak from a more distal portion of the tubule (3), an approach that is still in use today. This approach was used by Walker and Hudson to study glucose reabsorption in the amphibian tubule (5). Walker and Hudson showed that glucose was reabsorbed from the proximal convoluted tubule, but not from the distal convoluted tubule, in both Necturus and frogs (5). Glucose reabsorption was reduced by increases in tubule flow rate or hyperglycemia and inhibited by phlorhizin (5). These findings are still true today.

The final contribution from the Richards lab came in 1941, when they succeeded in developing methods for performing micropuncture in mammalian kidney (4, 6). Micropuncturing a mammalian kidney was substantially more difficult than micropuncturing an amphibian kidney, but Richards had recognized as early as his original 1924 paper (7) that this needed to be done. Walker and colleagues (4) showed that mammalian glomeruli also produced a glomerular ultrafiltrate that was entirely or nearly free of protein. The mammalian proximal tubule reabsorbed all of the glucose and at least two-thirds of the fluid (4). Fluid reabsorption in the proximal tubule was isosmotic (4). However, the chloride concentration in the proximal tubule increased to a value 1.4-fold higher than plasma, indicating that bicarbonate was preferentially reabsorbed over chloride (4). All of these findings are as true today as when initially published in 1941. Finally, Walker and colleagues noted that the limited number of distal tubule samples that they were able to obtain were hyposmotic (4). Although they did not draw any conclusions from this finding due to the small number of samples, they did speculate that the site of water reabsorption was distal to the distal convoluted tubule (4), and we know today that osmotic water reabsorption does occur more distally, in the collecting duct.

After the 1941 publications, Richards became Chair of the Committee on Medical Research of the Office of Scientific Research and Development (2). The combination of Richards’ new duties and World War II resulted in no further micropuncture studies from his lab. In fact, no micropuncture studies were performed until the technique was revived in the late 1950s (2). However, this technique (3, 6, 7) and the isolated perfused tubule (1) remain the gold standards of renal physiology to this day. In the current era of genetically engineered mice, the need for detailed functional measurements by micropuncture and microperfusion is rapidly growing while the number of physiologists capable of performing these difficult techniques is rapidly shrinking. As physiologists relearn these classic techniques, a new generation will come to admire the pioneering work of Richards and colleagues, as reported in these classic papers from the American Journal of Physiology.

REFERENCES