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Mechanisms of Proteinuria

V. D. Nenov
Medical University of Varna, Bulgaria

Introduction

The current understanding of why protein appears in the urine in patients with renal diseases is based upon knowledge collected from observations dating back to past centuries, and up until our modern times of advanced scientific technology. The concepts which dominated during certain periods of time were built upon the current knowledge about renal physiology and the available experimental evidence, and because it was only indirect, also upon logical theoretical elaborations. From the evolution of these concepts we can see how technical advances can bring down established scientific theories and how inaccurate speculations about the cause for what has been observed can swing the whole scientific thinking into a wrong direction. We can also see how careful one should be when making such speculations.

A critical review of the controversial evolution of human understanding of what today is termed "nephrotic syndrome" by Cameron and Hicks (1) emphasizes how young is the scientific concept that the kidneys are the diseased organs which lose endogenous protein by leaking it into the urine, thus causing a hypoproteinemic edematous state. It all started with Richard Bright's (1789-1858) definition of the kidney as the cause of proteinuria and dropsy (generalized edema) who extended the idea of William Wells (1757-1817) that albumin in the urine was arising from blood plasma. Yet, even until the first quarter of the 20-th century it was believed that "'pure nephrosis' was a systemic disorder which arose from a disturbance of protein synthesis", while the abnormal protein was lost in the urine ("dyscratic proteinuria") (1). The first experimental evidence that under normal conditions protein-free urine forms out of the glomeruli into the tubular lumen was demonstrated using micropuncture methods by Wearn and Richards in 1924 (1, 2) and soon after that confirmed by White and Schmitt (3). Further studies using this technique directly pointed at the glomerulus as the site of origin of albuminuria and thus finally it was understood that albuminuria was a result of disturbed function of the glomerular capillary wall, which normally acts as a filter rejecting albumin.

Where exactly and how exactly the glomerular capillary filter rejects macromolecules is still a mystery today, with all the advances of modern science. It is now clear that the glomerular capillary wall is composed of three distinct layers: endothelium, basement membrane and epithelium. While the endothelium and the basement membrane can be found everywhere throughout the microvascular system, the epithelium is located only at the urinary aspect of glomerular capillary walls in the kidney.

Role of the glomerular epithelium

The glomerular epithelium consists of a single layer of specialized cells, podocytes, which connect to each other through interdigitations (foot processes). At the site of connection there is a highly organized structure, the slit diaphragm, which was first observed under electron microscopy by Rodewald and Karnovski (4). It is now known to be composed of several distinct proteins among which nephrin was isolated, its coding DNA sequenced and shown to be mutated in a hereditary disease of massive albuminuria, the Finnish type nephrotic syndrome (5). Since the pores of the slit diaphragm are about the size of an albumin molecule and since when the slit diaphragm is defective there is massive albuminuria, it is now assumed that the slit diaphragm is the site of rejection of macromolecules (6). Such assumption, however, can be made only if we ignore another possibility: that a defective slit diaphragm could just decrease the mechanical support at the urinary aspect of the glomerular capillary wall and thus render the basement membrane more prone to stretching, ruptures and protein leaking. The latter possibility, however, is not discussed by proponents of the slit diaphragm theory.

Endothelium

Endothelial cells form large openings, fenestrae, which are much larger than the size of an albumin molecule. It is therefore believed that the layer of endothelial cells lining all microvascular walls is not a significant obstacle to the filtration of macromolecules and therefore endothelial cells do not play a role in rejecting macromolecules.

The basement membrane

It is composed of a denser middle layer, lamina densa, and not as dense lamina rara interna and lamina rara externa. The ability of the native basement membrane to reject macromolecules is not clear today despite numerous studies using various different methods. The lamina rara interna is composed of a negatively charged glycocalyx which rejects electrically all negatively charged molecules, including the major plasma constituent, albumin. Large macromolecules do not cross the glomerular basement membrane as evidenced by ultrastructural studies using labeled albumin and/or ferritin or horseradish peroxidase (7, 8). Furthermore, some ultrastructural studies describe the basement membrane as a highly organized structure of defined uniform pores with a size diameter smaller than that of an albumin molecule (9). Similar ultrastructural studies have shown the appearance of "tunnels" through the basement membrane in the presence of albuminuria (9, 10). However, some investigators have suggested that the basement membrane is much more permeable for

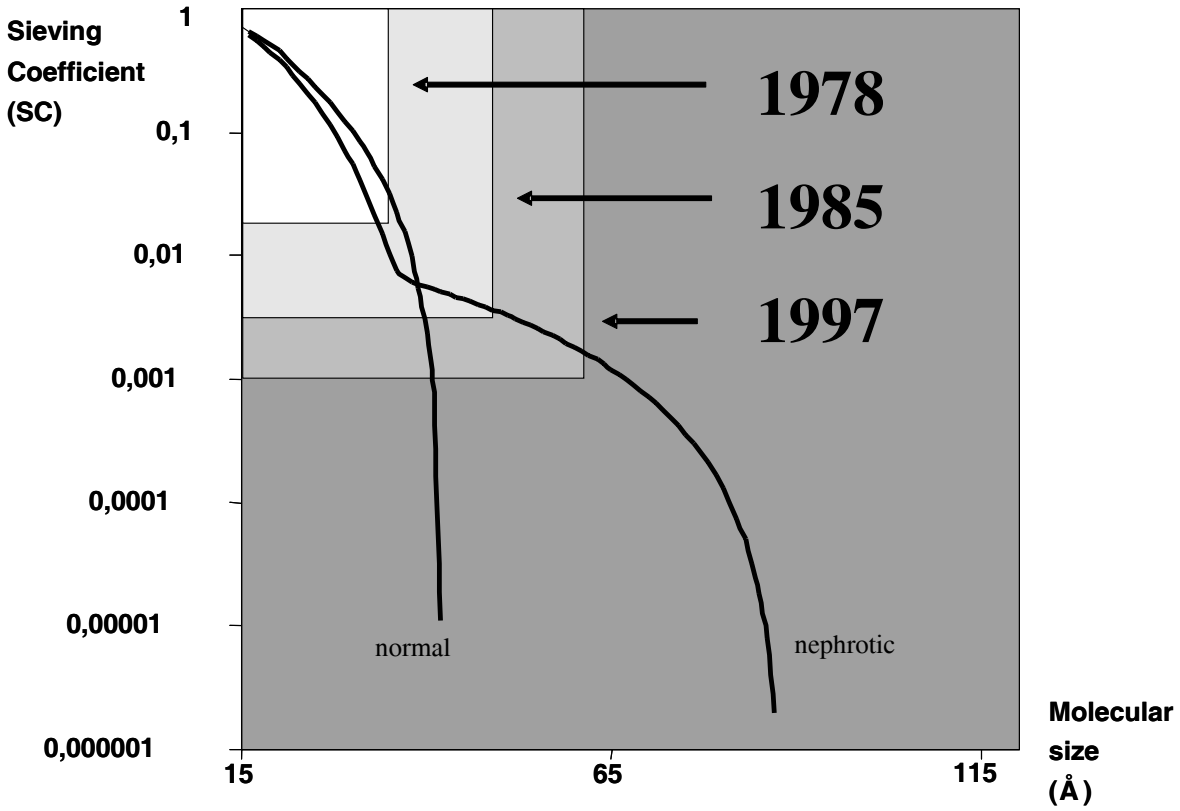
Correspondence to: Vesselin D. Nenov,, Medical University of Varna, 55 M Drinov St, 9002 Varna, Bulgaria
fax: ++359-52-305763; e-mail: vnenov@bantao.org

macromolecules than necessary to reject macromolecules alone to the extent the kidney does (11), and yet other investigators have suggested that albumin rejection at the level of the glomerular capillary wall is not as low and that tubular reabsorption is a major physiological mechanism contributing to the absence of albumin in the final urine (12).

Permselectivity studies

A great number of clearance studies using molecules of various sizes represents a major attempt at elucidating the mechanisms of macromolecular sieving in health and disease. In all of these studies the major output was a clearance sieving curve representing the relationship between the fractional clearance (in the range between 1.0 and 0.0) of a given substance and its molecular weight and/or size (fig. 1).

Figure 1. The glomerular sieving curve as seen during the last decades. Note how new technical advances opened further the window of the permselectivity curve, thus giving rise to new concepts about the mechanisms of proteinuria.



While most of these clearance studies utilized neutral polydisperse dextran as a clearance marker, which is neither reabsorbed nor secreted by the renal tubules, some more recent studies in humans also employed ficoll as a test molecule (13). In experimental animals a greater variety of markers has been applied, not only neutral but also charged polymers such as the highly negatively charged dextran sulfate (14) and the positively charged diethylaminoethyl- (DEAE-) dextran. Ever since clearance studies have been performed there has always been an attempt at interpretation of the results based on the current inderstanding and on mathematical speculations. The scientific understanding about glomerular permselectivity has changed during three major periods each initiated by a major technical breakthrough in the available technology.

The first proteinuric disorder studied by permselectivity curves was nephrotoxic serum nephritis in a rat experimental model. At the time of these first studies Chang and coworkers (14, 15) could measure dextran concentrations in the urine during intravenous infusion only up to about 45Å. This however turned out to be an insufficient upper sensitivity of the method, as proved by later permselectivity studies. Rats with nephrotoxic serum nephritis exhibited a reduction in the fractional clearances of neutral and positively (DEAE) charged dextrans with middle molecular weights (fig 1, the smallest window) in comparison with controls, which was contrary to an increase that should have mirrored the appearance of proteinuria. At the same time, an increase in the fractional clearances of negatively charged dextrans was interpreted as a sign that decreased negative charge of the glomerular filter was the cause of proteinuria in this experimental model. This was the beginning of the era of the charge hypothesis, an explanation for proteinuria which dominated

during the next at least 20 years. What is missing from most textbooks even today is the interrelation between charge and "apparent" size of a given molecule. In fact every molecule "sees" the charged glomerular pore with its own size, which is determined by its molecular diameter, but very importantly also by the size of its electrical field, determined by its charge. On the other hand, there is a purely haemodynamic effect of increased single-nephron glomerular filtration, almost invariably seen in all proteinuric disorders, which results in reduction of the fractional clearances of dextrans with middle molecular weights (between 20 and 40 kDa). The latter two mechanisms are sufficient by acting together to produce the above early experimental observations in the presence of a small number of large pores shunting protein to bypass the highly restrictive glomerular filter, and no alteration of glomerular charge would need to be involved. Therefore, to speculate by way of exclusion that altered glomerular charge was the mechanism of appearance of proteinuria was too early and not the right way to go as it turned out about one decade later.

The next technical advance occurred when dextran infusions were applied to humans and when lower concentrations in urine could be measured with the available technology. Then it was discovered that in patients with significant proteinuria, while the fractional clearances of dextrans with middle molecular weights were reduced, fractional clearances of the largest measurable dextrans (above 50Å) were increased (16). This led to a proteinuric sieving curve which crossed the normal sieving curve (fig 1, the second window). Apparently proteinuria was due to the appearance of a new population of pores with larger sizes, assumed at the time to be "shunt" pores, i.e. letting all larger molecules enter the urine without any restriction by their larger molecular size. This was a new theory about the mechanisms of proteinuria, quite different from the charge theory, but both continued to coexist at least for yet another decade. Knowing the crossover of the proteinuric curve over the normal sieving curve one could easily see why dextran sulfate clearances were increased in the early sieving studies: it was because the highly negatively charged dextran sulfate molecules behaved as their neutral counterparts with much larger molecular sizes due to the large sizes of their electrical fields, and hence their clearances reflected the increased clearances of uncharged molecules through the "shunt" pores. On the other hand, the reduction of clearances of molecules with middle molecular sizes (20-40Å) was confirmed in many more studies (16, 17), and while it was interpreted sometimes as reflecting decreased pore size, it could also be observed as a result of pure haemodynamic load of the kidneys (15).

The final technical advance in the field of permselectivity came when still larger molecular sizes, and lower concentrations with the introduction of radioactive labeling, could be investigated. Thus a new frontier in the permselectivity horizon was reached (fig 1, third window). Instead of a stable right-hand side, corresponding to presence of true "shunt" pores, a reduction of fractional clearances was observed with increasing molecular size in the largest sizes studied. This could no longer be explained by presence of true "shunt" pores, because in the latter case the right-hand side of the sieving curve should have been flat. Likewise, even if all "normal" pores were not of equal diameter (isoporous + shunt theory), but rather had a log-normal distribution of their diameters (lognormal + shunt theory) still the high-molecular end of the proteinuric sieving curve had to turn flat instead of steeply approaching zero. The tendency of macromolecular sieving coefficients (fractional clearances) to approach absolute zero at about double the size of the "normal pores" was confirmed by measuring clearances of endogenous proteins using methods with high sensitivity, and was best explained by a two-pore mathematical model (18). The two-pore theory assumes that the "shunt" pores are of definite size comparable but higher than the size of the "normal pores", about 110 to 115Å in a publication by Tencer and coworkers (18), allowing continued size-exclusion for molecules larger than the "normal" pore size, but limited up to the size of the "large" pores. Above this second size limit nothing of glomerular origin can be measured in the urine even in proteinuric disorders. The two-pore model was also applied to the data of Blouch and coworkers (13) by another group of investigators (19) and yielded a large pore radius of about 61Å, or 75-90Å from other similar studies (19). Because no flattening of the high molecular end of the proteinuric sieving curve can be observed with the latest technological advances, today it is clear than not shunt pores, but large pores which are about twice as large as the normal pores in the glomerular filter are the leaking pathway through which albumin and larger proteins leak into the urine in glomerular proteinuric disorders. It still remains unclear as to the exact physical location of these restrictive pores. Since the Finnish type nephrotic syndrome is caused by mutation of nephrin, a protein constituent of the slit diaphragm, this was considered as an indication that the slit diaphragm was the major site where glomerular sieving occurred (5). Earlier studies however indicated that the glomerular basement membrane was the barrier beyond which macromolecules from blood could not penetrate (7, 8). The latter question will remain to be answered by yet another technical advance, which is awaited. Preliminary results have shown that the systemic microvasculature may possess equal rejection to macromolecules as the glomerular filter, thus pointing at the basement membrane as the principal site of macromolecular sieving (20).

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