urinary proteins in health

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HISTORICAL

Proteinuria is said to have been noted for the first time by D. COTUGNO in 1770 although it was R. BRIGHT in 1827 who drew the proper conclusions from the phenomenom. Pathological proteinuria then became the subject of study by several workers. In 1878 C. VON LEUBE introduced the concept of a benign proteinuria. A few years later (in 1895) K. MÖRNER showed that normal human urine contained small quantities of protein. The figures he gave (22 to 78 mg/litre) are not far from the values most commonly accepted nowadays.

INTRODUCTION

Urinary proteins in health usually make up about one half of the macromolecules in urine. They are found in concentrations which are often too small to give a positive reaction to routine clinical tests. Owing to the extent of physiological variation and also to the variety of methods employed, it is difficult to define a range of normal values. RIGAS and HELLER[33] gave a mean value of total urinary protein output of 39.0mg/24 hrs, with a standard deviation of 5.7 mg. Several other figures often with little agreement have been quoted, such as a range of 20 to 75 mg/24 hrs[23], to a mean value of 103 mg/24hrs.[30] or even higher. About 65 proteins in normal urine have so far been described and at least half this number are thought to be plasma proteins. Most of the plasma proteins in urine originate by a process of glomerular filtration which is followed by tubular reabsorption. However it is possible that some plasma proteins may gain access to the urine in the more distal parts of the urinary tracts or in the genital secretions[4]. Some non-plasma urinary proteins are known to originate from the healthy nephron i.e. glomeruli and tubuli. Normal urine also contains proteins from the glands or shed cells of the urinary tract, and in the male seminal proteins arise from the genital tract especially from the prostate and bulbo-urethral glands[13].

FILTRATION-REABSORPTION PHYSIOLOGY

Most authorities agree that the normal glomerulus exhibits a small but significant permeability to proteins and that part of the filtered proteins are reabsorbed in the proximal convoluted tubules. The anatomical barriers, as seen under the electron microscope, which could act as filters to the macromolecules are: the endothelium, the basement membrane and the glomerular epithelium. So far there is no definite answer as to whether the basement membrane (or a component of it e.g. the lamina densa) or else the epithelial slit pore (or components of it e.g. the cell coats or the slit diaphragm) is the site for glomerular filtration of macromolecules[16]. Some workers consider the basement membrane to be the main filtration barrier[1, 18] while others favour the pore theory[19].

Calculations based on physiological data indicated that the glomerulus behaved as if it was a semi-permeable membrane containing cylindrical pores having a radius of 35 to 42 A°[19]. According to this theory, the protein molecules experienced steric hindrance at the entrance of the pore and viscous drag while moving through its lumen. More recently, experiments with artificial polymers have suggested that the glomerular membrane is heteroporous. ARTURSON et al[1] propose that the glomerular filter has a large majority of small pores of radius 20 to 28 A° and a minority of large pores of radius up to 80 A°, in the proportion, small to large, of 10,000 to 1. From this and other studies, it seems likely that the morphological structures responsible for the porous behaviour of the glomerular wall have variable pore sizes which are perhaps distributed around a mean pore size[18]. These results explain the progressive restriction of passage of macromolecules with increase in molecular size, together with the fact that some large proteins e.g. beta lipoprotein (mol. wt. 250,000) are not found in urine in health. However, mere molecular size is not the only factor involved in the selective behaviour of the glomerular membrane towards macromolecules. Thus it is known that proteins are cleared by the glomeruli at a much smaller rate than artificial polymers of approximataely the same molecular size. This has been suggested to be a purely physical phonemenom related to charge effects and a more rigid molecular structrue in the proteins[15].

The total quantity of plasma proteins filtered through the glomerular membrane in man is not known. It should be evident from the magnitude of the glomerular filtrate (170 litres/day) that a very small concentration of protein in the glomerular filtrate is more than enough to account for the normal daily urinary excretion of plasma proteins.

Reabsorption of part of the filtered plasma proteins occurs in the proximal convoluted tubules[4, 18] as has been shown by clearance, morphological and other studies. Some evidence on the subject of reabsorption is conflicting, but in health the process is thought to be selective, favouring the absorption of the smaller proteins. Pinocytosis has been suggested as a mechanism for reabsorption and it is thought that the tubular cells catabolise the protein, although return of native or partly catabolised protein to the bloodstream is not to be excluded. There is evidence that the kidney is the most important site for catabolism of many low molecular weight proteins reabosorbed by the tubules[4].

The existence of a reabsorption process for proteins implies that unless the reabsorption of

a particular protein has reached its maximum capacity (Tm) the urinary output of that protein will probably be zero. However, once this transport maximum or threshold has been exceeded the urinary output of the protein should increase linearly with the plasma concentration. Therefore, it is possible to relate the plasma concentration of a protein (P), the urinary concentration of the protein (U), the flow rate of urine per unit time (V) and the threshold for reabsorption (Tm). The glomerular clearance of the protein is given by the term:

$$\frac{U \times V}{P - Tm}$$

The ratio of the glomerular clearance of a protein to the concomitant glomerular filtration rate, i.e. G.C.P./G.F.R. is an index of glomerular permeability to the molecule. This fraction has been variously termsd "glomerular clearance ratio", "filterability", "permeability coefficient". or "sieving coefficient". The last term is the favoured one in the pore theory of protein filtration.

The clearance of proteins may be a useful index for studying renal function but several difficulties must be borne in mind[15]. Thus the existence of a tubular reabsorption mechanism about which little is known, the possibility of protein-protein interactions etc. complicate the issue. No direct relationship exists between protein clearance and molecular weight. Technical difficulties include the lack of suitable quantities of pure protein for "loading" experiments and the sophisticated nature of the quantitative immunochemical techniques. Methods employing enzyme clearances[9] offer some hope as simpler techniques.

BIOCHEMICAL TECHNIQUES

In the biochemical investigation of urinary proteins, the first step usually consists in concentrating the urine. Methods such as alcohol precipitation, evaporation, dialysis, ultrafiltration and lyophilization are used. The possibility of artifactual changes occurring during these procedures must be kept in mind when interpreting the results[25]. The concentrate is then analysed using one or more methods. Moving boundary electrophoresis was first used for this purpose by RIGAS and HELLER[33]. Electrophoresis may also be carried out on paper, cellulose acetate or starch gel. The first immunoelectrophoretic investigation of plasma proteins in normal urine was carried out by GRANT[12]. Other techniques used include gel or ion-exchange chromatography[27] and uttracentrifugation. Immunological techniques are commonly used for studying proteins originating from the urinary tract. Some qualitative and quantitative tests for urinary proteins have been recently evaluated[6].

PLASMA PROTEINS

Electrophoretic studies performed about 20 years ago suggested a resemblance between the protein patterns of normal urine and plasma[33]. The greater part of plasma proteins in the urine originate from the nephron as has been outlined above. However some plasma proteins in urine may orginate from the blood and the interstitial fluid in more distal parts of the urinary tract[4]. It is possible that some normal urinary proteins which may derive from the plasma are difficult to identify in it with the techniques currently available because of their very small concentration. The majority of urinary proteins of plasma origin have a molecular weight of less than 200,000 probably because of the molecular-sieve effect of the glomerular filtering membrane. It is difficult to classify plasma proteins in normal human urine and the problem is rendered still more complex by the fact that plasma protein fragments have also been identified. More comprehensive reviews than the following appear elsewhere[3,4].

Albumin (mol. wt. 69,000) is quantitatively the most important plasma protein in urine[28]. Results of urinary albumin excretion in health, as for many other plasma proteins, vary widely with the techniques used. Immunochemical techniques give results ranging from an average daily excretion of slightly more than 10mg.[4] to a range of concentrations from 0 to 66 mg/litre[6]. The Glomerular Clearance Ratio of albumin does not exceed 0.02% in average normal subjects[15].

Tryptophan rich serum pre-albumin (Thyroxine Binding Prealbumin) is found in very small quantities in normal urine [28]. It is a glycoprotein of low carbohydrate content and has a molecular weight of about 60,000.

Alpha-1 and alpha-2 globulins are the main plasma globulin components of urine in health. Alpha1 acid glycoprotein (orosomucoid) has a molecular weight of about 40,000 and a carbohydrate content of about 40% Its clearance is 3 times that of Albumin[4]. Alpha2HS glycoprotein is excreted in similar quantities and has a slightly higher molecular weight. Its clearance is 3.3 times that of Albumin[4] and this may be taken as evidence of the selective glomerular permeability favouring smaller molecules.

In normal urine only monomeric haptoglobin (an alpha-2 glycoprotein mol.wt. 170,000) from type 1-1 and 2-1 individuals has been observed. The fact that the polymeric haptoglobin from healty type 2-2 individauls has not been observed in urine[4] shows the size selectivity of the glomerular filter.

Caeruloplasmin, a Copper binding glycoprotein weight 150,000 and alpha-2 electrophoretic mobility is also found in very small quantities in normal urine. It demonstrates the difficulties of protein clearance as a renal function index since its clearance varies unpredictably from one individual to another[15]. This could be due to either genetically determined forms of differing molecular size (cf. haptoglobin) or else to protein-protein interactions in the plasma.

Other alpha globulins in normal human urine include antitrypsin, lipoprotein, Zn-glycoprotein and a microglobulin.

Beta globulins are generally excreted in very small quantities in normal urine. Thus, for example, only about one-third of a milligram each of transferrin and beta-2 glycoprotein I are excreted per day.

Several immunoglobulin components have been identified in normal human urine[3] and, as in the plasma, not all of them have the classical gamma globulin motility. Immunoglobulin G (mol. wt. 150,000) is found in the largest amounts, about 3 mg. being the normal daily average. In spite of its much larger molecular size, its renal clearance is about 0.8 that of albumin. This may be due to excretion in the urinary tract, relatively less tubular reabsorption or else formation of the macromolecule from easily filtrable polypeptide fragments[4]. Only about 1 mg of Immunoglobulin A is excreted per day but it is interesting to note that part of it may be secreted by epithelial cells[5]. Immunoglobulins M, D and E are either not found at all or else are excreted in extremely small quantities.

Most of the microimmunoglobulins of normal urine consist of free light chains i.e. the normal counterparts to Bence Jones protein[36]. Light chains isolated from normal urine have also been found to occur in polymeric forms[3]. Fragments of immunoglobulins in normal urine were first studied by FRANKLIN[11]. Urinary microimmunoglobulins closely related to the Fc fragment of IgG have been described[38]. Components of complement are found in very small quantities.

Immunochemical studies have shown the presence of small quantities of fibrinogen fragments in normal urine[4].

In health, urine is also known to contain small quantities of enzymes derived from the plasma, and these include lactate dehydrogenase, amylase, plasmin, trypsin, glutamate-pyruvate transaminase and others.

Protein hormones and their precursors have also been detected. These include insulin, proinsulin and hypophyseal gonadotropins.

Plasma proteins of small size, many of which are still unknown, probably form a large fraction of the proteins in normal urine. These proteins are often present in small concentrations in the plasma, but because of their size seem to be preferentially excreted in the urine[4].

NON PLASMA PROTEINS AND THEIR ORIGIN

In health, besides proteins entering the urine from the blood through the glomeruli, urine also contains (a) proteins arising from the renal cells themselves (b) proteins from the glands or shed cells of the urinary conducting and storage system (c) in the male, seminal proteins in trace quantities from the genital tract.

GRANT[13] showed the existance of numerous non plasma antigens in normal urine. A feature which seems to be common to all these proteins is that to some extent they all seem to contain carbohydrate moieties. Data concerning most of the non-plasma proteins in urine is fragmentary and many of the claims regarding their origin are speculative. BOURRILLON[7] claims that they make up to 20 to 25% of the non dialyzable constituents of normal urine and has separated them chromatographically into 17 fractions.

There is now no doubt that the healthy kidney contributes non-plasma proteins to urine. Recently it has been shown that soluble antigenic components of the glomerular basement membrane (GBM) are found in the urine of normal people[20]. There are at least two such antigens and they have low isoelectric points (between pH 1.7 and 3.8), a very high carbohydrate content and a post-albumin mobility on zone electrophoresis.

Probably the most renowned of the non-plasma urinary proteins is the glycoprotein of TAMM and HORSFALL[37]. The T and H glycoprotein is present in concentrations of up to 2.5 mg./100 ml. of normal human urine, and is an important fraction of the so called "uromucoid". Several authors[7, 14, 22] have confirmed that specific antibodies raised against the T and H glycoprotein are never precipated in blood plasma but aqueous extracts of renal parenchyma will combine with these antibodies giving a reaction of identity. Moreover, this glycoprotein can be identified in convoluted tubular cells by fluorescein labelled antiserum. The T and H glycoprotein has been extensively studied[22] and is one of the variety of soluble "mucoids" which by competing with the cellular receptors for certain viruses inhibit haemagglutination.

Other urinary proteins originate from the kidney, and these include enzymes such as carboxyl esterases, and one hormone — erythropoietin.

Some proteins could arise from the transitional epithelium of the pelves, ureters and bladder either from the surface lining or from shed cells. The origin of secretory immunoglobulin A is still speculative[5].

GRANT[13] found 4 trace components common to male urine, female urine and semen. These possibly arise from the urethra.

Components common only to male urine and identified immunologically in semen seem to be present only in small traces. Prostate and bulbourethal glands probably are the main contributors.

The female has urethral glands homologous to those of the male but no urinary proteins of non-plasma origin, peculiar to this sex have been identified so far.

Normal urine also contains several carbohydrate complexes such as blood group substances, glycopeptides etc. Glycopeptides form the major fraction of protein-related substances of normal urine[7].

PHYSIOLOGICAL VARIATIONS IN URINARY PROTEINS

At this stage it should be evident that all urine contains small quantities of protein. There-

fore, in the light of present knowledge, the term proteinuria should be taken to mean an excretion of urinary protein significantly greater than the values outlined above.

As all other physiological parameters, urinary protein excretion is subject to variation from one individual to another and also varies in the same individual from one physiological situation to another. The data given above correspond roughly to the urine from an average normal adult under basal conditions. The urinary proteins can vary with age, exercise, posture, pregnancy and possibly other factors such as sex, mental state, environment etc. In these changes from the "basal" state there is often an increase in the excretion of plasma proteins but this is not of significance in the lowering of the blood plasma protein level. However, clinically it may be difficult to distinguish on the basis of protein concentration in isolated speciments, whether a given proteinuria is pathological or else "functional" i.e. of physiological origin.

ENVIRONMENTAL FACTORS

Relatively little work has been carried out on geographical variations in urinary protein. It is known that low temperatures can produce a rise in plasma protein excretion but the mechanism of this phenomenom is uncertain.

EXERCISE

The proteinuria following mascular effort was observed for the first time by VON LEUBE in 1878. His results were subsequently confirmed by several workers.

During exercise, excretion of total urinary protein rises from a normal average of about 0.03 mg/min to as much as 2.00 mg/min. This rise is more impressive when expressed in terms of urinary protein concentration since urine flow diminishes during exercise. Thus it increases from 0.04 mg/ml. before exercise to as much as 5 mg/ml[29]. As in other functional proteinurias, the urinary proteins affected are the plasma proteins. The urinary colloids not detected in plasma do not account significantly for the rise of the urinary protein excretion which occurs during and after exercise.

Detailed studies have shown that following exercise there is particularly a significant rise in urinary levels of tryptophan rich prealbumin, albumin, alpha-1 acid glycoprotein, transferrin, immunoglobulin A and immunoglobulin G. The presence of higher molecular weight proteins in "exercise urine" might result from an increase in the glomerular permeability or else it is possible that tubular reabsorption had reached its maximum value for most of these plasma proteins. No direct relationship could be found between the molecular weight of a protein and the value of its renal clearance[28].

It is possible that in exercise release of epinephrine and norepinephrine produces a vasoconstriction of the renal glomerular arterioles slowing down renal plasma flow and glomerular filtration rate, thus allowing a better diffusion of plasma proteins through the glomerulus into Bowman's space[29]. In experimental animals renin has been known to induce proteinuria, and in rats this proteinuria seems to require the presence of certain corticosteroids and is also influenced by other hormones[26]. Whether hormonal factors are important in determining changes in urinary proteins in man during conditions of stress is unknown, but this still constitutes a possibility to be borne in mind.

POSTURE

During upright ambulation in healthy subjects the fractional daily protein excretion is high but this is usually not high enough to be detected by qualitative tests[34]. However in some healthy individuals the protein excretion on standing (orthostasis) may be quite marked. The proteinuria associated with posture has unfortunately been subjected to different diagnostic criteria and has been referred to by different names. Postural proteinuria can be broadly defined as a laboratory syndrome whose diagnosis requires the absence of qualitative proteinuria (i.e. protein excretion should be less than 0.03 mg./min.) during recumbency, and its presence during quiet upright ambulation or standing[34]. It is fairly com mon, equally found in both sexes, and commonly appears at the onset of puberty usually disappearing in the early twenties.

Some observers claim that all cases are related to lordosis, but others have found that lordosis plays no part in a majority of cases studied. It is generally held that the rise in urinary protein excretion is the result of some change in renal haemodynamics but there are two main schools of thought striving to explain the phenomenon. From the evidence gathered so far it would seem that both the postulated mechanisms do in fact play a part.

One school considers orthostatic proteinuria to be the result of the fall in venous return produced by venous pooling on assuming the upright posture. The resultant compensatory vasoconstriction affecting also the renal vessels would in some way determine an increase in plasma protein excretion. It may be of relevance to note that when experimental animals are kept upright proteinuria always follows, but division of renal nerves prevents this phenomenom from occurring[14].

According to the second school, on assuming the lordotic position the liver rotates forwards and downwards and compresses the inferior vena cava against the verterbrae. As a result there is a rise in pressure within the inferior vena cava and the renal veins, presumably producing passive venous congestion and hence a proteinuria. In some cases, the increased urinary excretion of protein comes from the left kidney only, and in this situation it is presumed that there is a rise in venous pressure in the kidney following compression of the left renal vein on the anterior convexity of the aorta.

On standing protein output was less than 1 mg./min. in 65% and more than 1 mg./min. in 35% of 350 cases of orthostatic proteinuria studied, but occasional very high excretion rates have been reported[14]. Different results of protein composition have been obtained. Some claim a strong predominance for albumin[14], while others found a rather poor selectivity of the glomerulus, with excretion of a large fraction of higher molecular weight globulins[35]. However, many of the results show the existence of concentration patterns for individual proteins that in general resemble those of normal urine.

PREGNANCY

In pregnancy[10], there is an increased excretion of those plasma proteins whose levels are increased in this physiological state. In particular, one has in mind the chorionic gonadotropin, a glycoprotein present in relatively large concentrations in the urine of pregnant women, first appearing in the second week after ovluation and persisting until about the fifteenth week of gestation.

One should note that the urine of sexually mature women during a period of about four days before ovluation contains a gonadotropic hormone, presumably of pituitary origin.

NEONATES

Normal new born infants may have higher levels of urinary protein during the first three days of life[32].

OTHER FACTORS

It has been claimed, especially in older writings, that ingestion of excessive quantities of protein may be followed by a delayed transient rise in urinary protein excretion.

Severe mental strain or emotion is also claimed to induce a transient proteinuria in some individuals.

CONCLUSION

In health, a wide variety of proteins are found in urine, ranging from the normal counterparts of Bence-Jones protein to fragments of the glomerular basement membrane. In fact, normal urine has been shown to contain most of the proteins which are present in the urine in various pathological conditions with variations only in the amounts excreted.

Many proteins experience filtration and reabsorption processes and the concept of clearance may, with caution, be applied to them also. However the behaviour of proteins is much more complex than that of small solutes and many proteins do not conform to what would be expected solely on the basis of molecular size. The site and nature of glomerular filtration of proteins are still controversial. The old "molecular-sieve" model is becoming more inadequate and it is hard to accept that the glomerular membrane behaves merely as an inert sieve. Physiological variations in functional pore size possibly associated with contractile properties in the podocyte have been postulated. Likewise, a great deal of work has yet to be done to elucidate the mechanism and selectivity of tubular reabsorption, and how far it is determined by plasma protein levels.

The importance of neural and hormonal factors in bringing about changes in urinary protein is not clear. Are the physiological changes in protein excretion secondary to haemodynamic changes or are there more subtle mechanisms acting directly on the nephron?

Relatively little is known about the nature, origin and function of the several non-plasma proteins in urine. Study of the Glomerular Basement Membrane antigenic fragments is yielding information suggesting their possible rôle as autoimmunogens involved in the causation of kidney disease. Not much is known about the functional significance of the glycoproteins produced by the urinary tract. There is insufficient explanation, for example, of the reasons for the complex chemical behaviour of the T and H glycoprotein, or of the nature and possible functions of urinary immunoglobulin A.

In future, these and other problems will probably be solved, and new ones posed. Until the normal patterns of urinary protein excretion are adequately explained, the full interpretation of the urinary protein patterns in disease will remain largely empirical.

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