PROTEINURIA

ETIOLOGY AND MANAGEMENT

Keep your enthusiasm, but let strict verification be its constant companion.

L. Pasteur

The history of our thinking about PROTEINURIA is a classic example of the errors that our elders and betters made by neglecting verification.

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September 25, 1975

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DEFINITIONS:

PROTEINURIA - All urine contains some protein of various types. At the present time, most authors accept up to 150 mg/24 hr as determined by standard methods.

ORTHOSTATIC PROTEINURIA - A reduction of the proteinuria while in the supine position to an amount on a time basis that is less than 150 mg/ 24 hr (e.g., 4 hour supine protein excreted should be less than 25 mg.)

TRANSIENT PROTEINURIA - The finding of abnormal amounts of protein in the urine on only some occasions. This may be true in patients with orthostatic proteinuria, hence "transient orthostatic proteinuria".

FIXED PROTEINURIA - Where abnormal amounts of protein are found in the urine; on each occasion it is examined regardless of the situation.

FIXED ORTHOSTATIC PROTEINURIA - The presence of abnormal amounts of protein in the urine every time it is examined providing the subject has been upright.

PERSISTENT PROTEINURIA - Another term for fixed; protein is present in abnormal amounts each time the urine is examined.

TAMM-HORSFALL PROTEIN - A mucoprotein produced by distal tubular cells. There are four molecules each with a molecular weight of 7,000,000 joined into rods 12,000 Angstroms long. It probably constitutes 70% of the non-serum protein excreted per 24 hours. It is an important constituent of urinary casts.

DALTON - A unit of mass, being one sixteenth of the mass of the oxygen atom, or approximately 1.65×10^{-24} gm. Used to describe molecular weight.

ANGSTROM (Å) - The unit of wavelength of electromagnetic and corpuscular radiations, equal to 10^{-7} mm.

SELECTIVE PROTEINURIA - When spillage is limited to protein of molecular weight 100,000 or less. This type of spillage is associated with a good response to steroids.

NON-SELECTIVE PROTEINURIA - Spillage of protein up to as high as 2,500,000 molecular weight. This is associated with NO response to steroid therapy.

PINOCYTOSIS - A non-specific process whereby proteins are reabsorbed from glomerular filtrate by proximal tubule cells.

Please note that molecular weight in protein chemistry cannot always be correlated with size. In this paper our standard reference is albumin at 69,000 daltons and a radius of 37 Å (1).

1. Hardwicke, J. Laboratory aspects of proteinuria in human disease. Clinical Neph. 3:37, 1975.

2. Drummond, Rennie I. Proteinuria. Med. Clin. of N.A. 55:213, 1971.

Normal Urine Protein:

The general accepted amount of protein expected per 24 hours in the urine is less than 150 mg. This is a combination of a number of proteins from various sites and every author gives different figures.

Serum protein: Albumin (20-50 mg)
Kidney cell breakdown products: (?)
Kidney produced protein - Tamm-Horsfall protein: (up to 50 mg)
Lower urinary tract secretions - prostate, etc.: (?)

Measurement of Urinary Proteins:

In most laboratories at the present time in the Dallas area either a modification of the Biuret method or a precipitation technique is used to measure 24-hour protein excretion. Each method described below has advantages and disadvantages. These factors must be taken into account in interpreting results depending on what aspects of proteinuria you are trying to evaluate; e.g., most are of little benefit in measuring low-grade tubular proteinuria except the Kjeldahl.

TABLE I

Quantitative Methods for Urinary Protein Determinations

METHOD	PRINCIPLE	COMMENTS
KJELDAHL	Measures nitrogen content of protein	The standard reference must remove non-protein nitrogen
BIURET	Copper complexes with peptide bonds on protein molecule	Inaccurate at low concentration, must precipitate high concentrations
PRECIPITATION Sulfosalicylic Acid Trichloroacetic Acid	Measure precipitate by lite or amount formed	Relatively inaccurate

3. Perce, A.J. Methods for the analysis of proteins in the urine. Nephron 13:93, 1974.

A simple basic review of the problems with the various methods utilized. An extensive bibliography for anyone who wants all the references.

In addition to the above methods most laboratories use dip sticks to determine a protein estimation. The basis for the test is the so-called "PROTEIN ERROR" of indicators. A strip of paper is impregnated with a dye e.g. tetrabromophenol blue and buffered to pH 3-3.5. At this pH the amino groups of the urine proteins react with the dye. The more amino groups or proteins present, the darker the (green-blue) color of the test strip.

The error with this method as compared to sulfosalicylic acid (SSA) for 1+ or less is as high as 50%. The more protein present, the less the discrepancy between the two methods. Generally dip sticks measure somewhere around 20 mg% of albumin. SSA can probably, as done in renal clinic, measure 10 mg%. The following table lists the method and ranges for hospitals in this area:

TABLE II

PROTEINURIA - DALLAS HOSPITALS

HOSPITAL	ACCEPTABLE N RANGE mg/24 hr	MEASUREMENT-METHOD Routine	24 hr
P.M.H.	150	D/S	Biuret/SSA Precip.
V.A.H.	150	D/S	3% T.C.A.
Presbyterian	150	D/S	SSA
St. Paul	50-100	D/S	1.5% SSA
Baylor	250	D/S	3% SSA
Methodist	20-80	D/S	5% T.C.A.
Renal Lab.	250	SSA	Biuret/Tsuchiya Precip.

*D/S = dip stick

Unfortunately there is considerable variability in both the methods and the normal range. It would, therefore, be important as patients are transferred back and forth to note where studies were done.

In addition to the variations from laboratory to laboratory and the problems with methods, there are also a number of substances that give false readings. These are listed in Table III as modified from Kark (6).

TABLE III

URINARY CONSTITUENTS THAT MAY GIVE FALSE POSITIVE AND FALSE NEGATIVE REACTIONS WITH VARIOUS TESTS FOR PROTEINURIA

CONSTITUENTS	TEST STICKS	SULFOSALICYLIC ACID	HEAT & ACETIC ACID OR NITRIC ACID RING TEST
Urine turbidity	No effect	May confuse reading	May confuse reading
X-ray contrast media	No effect	May cause false +	May cause false +
Tolbutamide metabolites	No effect	May cause false +	May cause false +
Penicillin (methicillin)	No effect	May cause false +	May cause false +
Para-aminosalicylic acid in urine containing preservative agents	No effect	May cause false +	May cause false +
Highly buffered alkaline urine	May cause false +	May cause false -	May cause false -

A random urine may give a variable reading depending on the urine concentration. If a very diluted urine is tested, the protein concentration may be below the range the screening tests can measure, yet the total amount present may be grossly abnormal; e.g., diabetes insipidus or pathological water drinkers. On the other hand, a very concentrated urine may give a positive test yet the amount of protein excreted may be normal. Table IV shows such a finding in a hypothetical case.

TABLE IV

RECOGNIZING SIGNIFICANT PROTEINURIA

TIME	VOL.	SP.GR.	PROTEIN MG%	PROTEIN TOTAL	READING
7AM-3PM	600	1.015	8	50	-
3PM-11PM	700	1.012	7	50	-
11PM-7AM	200	1.028	25	50	+
24 HR	1500	1.014	10	150	-

It is important to note the specific gravity or osmolality on urine specimens when testing for protein.

In summary, screening tests for protein give us a rough guide but really may only detect rather gross proteinuria. This is certainly true if the patient has a dilute urine. In any patient who is being worked up for a renal problem or to screen that patient properly to rule out renal disease, the following is required.

ONE, AND PREFERABLY 2, 24-HOUR URINES MUST BE MEASURED FOR PROTEIN IN A RELIABLE QUANTITATIVE LABORATORY. IN ADDITION, THE TOTAL CREATININE SHOULD BE MEASURED TO DETERMINE IF THE COLLECTION IS COMPLETE. THE ACCEPTABLE MINIMUM 24-HOUR CREATININE EXCRETION EXCEPT IN CACHETIC PATIENTS ARE:

MALE - 1000 MG PER 24 HR

FEMALE - 900 MG PER 24 HR

4. Joramo, T.A. Reagent strip and sulfosalicylic acid tests as screening methods for proteinuria. Am. J. Med. Tech. 38:298, 1972.

5. Thysell, H. A comparison between Albustix, Hema-combistix, labstix, the sulfosalicylic acid test, Hellers nitric acid test and a biuret method. Acta Med. Scand. 185:401, 1969.

A basic comparison.

5A. Larsson, S.O and Thysell, H. Are proteinuria tests reliable as screening methods for renal disease. Acta Med. Scand. 186:313, 1969.

The errors of the various methods are well demonstrated.

6. Kark, R. Proteinuria II. Hospital Practice June, 1971 (p. 59)

An excellent simple review of the overall subject.

Significance of Proteinuria

Not all proteinuria greater than 150 mg/24 hr is abnormal. Table V lists some benign causes of proteinuria that should be considered and ruled out before one assumes primary renal disease, e.g. orthostasis.

TABLE V

OTHER CAUSES OF PROTEINURIA

1. ORTHOSTASIS
2. LORDOSIS
3. POSTPRANDIAL
4. OBESITY
5. EFFORT (EXERCISE)
6. CIRCULATORY
   CHF
   CONSTRICTIVE
   PERICARDITIS
7. CEREBRAL TRAUMA
8. FEBRILE/INFECTION
9. PRESSOR TYPE

However, these are diagnosis of exclusion and should be considered in view of the current literature.

Orthostatic Proteinuria

Orthostatic proteinuria should always be ruled out particularly in adolescents. The best protocol is described on page 110 of reference (62). It must be stressed that urinary protein has to decrease to the normal range for the particular time period utilized. A decrease toward normal is not acceptable for the diagnosis. Orthostatic proteinuria is often thought of and even reported in the literature as less than 1 g/24 hr. This is misleading and the diagnosis should not be excluded because of spillage of large amounts of protein. In Hamburger's (62) series of 350 cases protein excretion was greater than 1.5 g/24 hr in 35% of subjects

studied. However, it is never associated with the nephrotic syndrome, and the urine sediment and renal function are always normal. When the above cirteria are used it would appear that the disease is benign (62,12), at least with 10 years of follow up. The data is displayed in Table VI from Thompson and Robinson paper (12). There appears to be a gradual decrease from fixed or transient orthostatic to negative without any evident impairment of renal function. A somewhat different conclusion is reached by King in reference (11) but it is not clear how carefully patients were selected.

TABLE VI

CURRENT PATTERN OF URINE PROTEIN EXCRETION IN 43 YOUNG MEN WHO HAD FIXED ORTHOSTATIC PROTEINURIA 10 YEARS EARLIER

PATTERN	1964		1969	
	NUMBER	PERCENT	NUMBER	PERCENT
PROTEINURIA	34	(83)	21	(49)
FIXED ORTHOSTATIC	19	(46)	7	(16)
TRANSIENT ORTHOSTATIC	12	(30)	5	(12)
PERSISTENT	1	(2)	2	(5)
VARIABLE	2	(5)	7	(16)
NEGATIVE	7	(17)	22*	(51)

*Includes two patients who were not evaluated in 1964.

The etiology of orthostatic proteinuria is unclear and at present their are at least three theories.

1. Increased venous pressure
2. Renal lymph passing into the urine at the renal pelvis.
3. A decrease in circulatory volume with renal vasoconstriction.

7. Lowgren, E. Studies on Benign Proteinuria. Acta Med. Scand. Suppl. 157, 1955.

Proposes the theory that protein is of lymphatic origin.

8. Slater, R.J. et al. Studies on human proteinuria by the mechanism of postural proteinuria. Pediatrics 190, August, 1960.

9. Bull, G.M. Postural proteinuria. Clin. Sci. 7:77, 1948.

The first detailed study on the subject and worth reviewing.

10. Greiner, T. and Henry, J.P. Mechanisms of postural proteinuria. J.A.M.A. 157:1373, 1955.

Proposes increased renal vasoconstriction secondary to a volume effect due to peripheral blood pooling.

11. King, E.S. Proteinuria in renal disease by preliminary observations on the clinical course of patients with orthostatic albuminuria. New York State J. Med. 59:825, 1959.

Suggests that orthostatic proteinuria progresses to renal failure, patient selection is not clear but does present opposite view to Robinson (12).

12. Thompson, A.L., Durrett, R.R. and Robinson, R.R. Fixed and reproducible orthostatic proteinuria results of a 10 year follow up evaluation. Ann. of Int. Med. 73:235, 1970.

Classic paper on the subject.

Lordotic Proteinuria

Probably similar mechanism to orthostasis above. Bull (9) has shown that 75% of adolescents will spill significant protein in the urine if they assume an extreme lordotic position for 30 minutes.

Post Prandial Proteinuria

A very rare form that can only be diagnosed by studying the subject after eating.

Obesity Proteinuria

Another rare cause that can only be ruled out by having the patient lose weight. The mechanism is assumed to be increased renal venous pressure.

13. Weisinger, J.R. et al. The nephrotic syndrome: A complication of massive obesity. Ann of Int. Med. 81:440, 1974.

Effort or Exercise Proteinuria

Initial reports (14) suggested that this entity was seen in young males who performed unaccustomed exercise of fairly moderate degree. It was reported to diminish and clear as the subject became conditioned. Taylor's study showed the urinary protein to return to normal in 1 hour. However, more recently Coy (15) has demonstrated that there are abnormal levels of protein in the urine for a 24 hour period following the effort even in conditioned participants.

Greater glomerular permeability secondary to increased blood acidity and/or renal ischemia have been postulated as mechanisms. The prolonged effect is explained as slow recovery or repair of the glomerular filter.

14. Taylor, A. Some characteristics of exercise proteinuria. Cl. Sci. 19:209, 1960.

15. Coy, R. D. and Rosandich, R.R. Proteinuria during the 24 hour period following exercise. J. Appl. Physiol. 15:592, 1960.

Interesting study that has not been followed up as far as I am aware.

Proteinuria with Circulatory Disturbances

The positive urine protein test in these states may in some cases be due to avid fluid retention and hence a measurable protein content in the remaining urine. In other cases low grade transient proteinuria is observed that disappears with correction of the underlying circulatory problem, e.g. constrictive pericarditis. Renal venous pressure changes have been considered as an etiology.

Proteinuria associated with Cerebral trauma

Intense (4+) proteinuria may be observed, however 24 hour studies are lacking. Stimulation of the renal sympathetic nervous system has been suggested as the etiology (62).

Febrile Proteinuria

In both bacterial and viral infection as high as 80% of the subjects have been shown to have significantly increased 24 hour urine protein (1 g/24 hr). Since studies show both a tubular and a glomerular type of pattern a transient lesion effecting both areas has been suggested. Biopsies have not been done to confirm the immunological etiology that has been proposed.

16. Jensen, H. and Henriksen, K. Proteinuria in non-renal infectious diseases. Acta. Med. Scand. 196:75, 1974.

Pressor Type Proteinuria

A large literature concerning this phenomena suggest the following. The increase in protein excretion is small and is dependent on the presence of corticosteroids. There is some evidence for a tubular as well as a glomerular abnormality with more recent evidence supporting the latter.

17. King, S.E. and Baldwin, D.S. Production of renal ischemia and proteinuria in man by the adrenal medullary hormones. Am. J. of Med. 217, Feb., 1956.

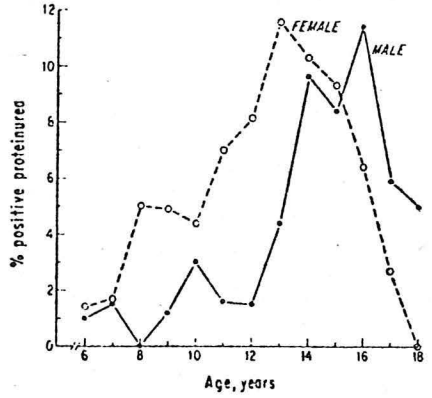
17A. Pessina, A.C. and Peart, W.S. Renin induced proteinuria and the effects of adrenalectomy. Proc. R. Soc. Lond B 180:43, 1972.

A good study with an extensive literature review.

Proteinuria as Related to Age

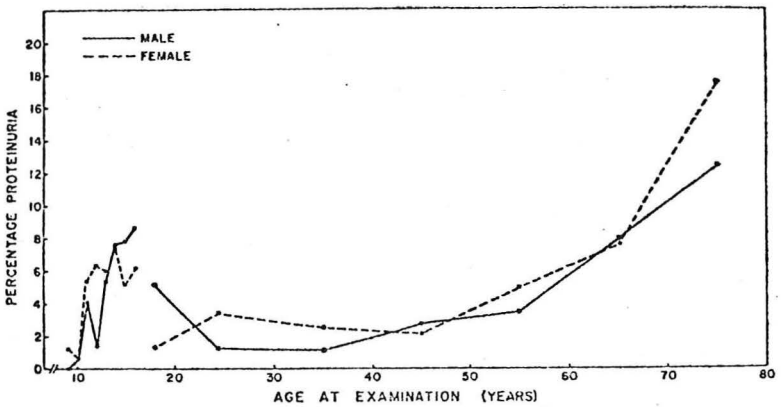
Several studies demonstrate that proteinuria is common in the first month of life presumably corrected as the kidney matures. The excretion then is low as shown in graphs 1 and 2 until adolescence when an increased incidence is observed. This transient rise is presumed to be due to orthostatic and lordotic proteinuria being more common at this age accompanying or following the growth spurt. There is then a return to base line incidence with females perhaps showing a higher occurance than males during the child bearing years. At age 50 a steady and continuous rise is noted. This rise is thought to be due to the increased findings of diabetes and amyloid in the older patients.

GRAPH I



% of Subjects Demonstrating Proteinuria as Related to Age

GRAPH II



% of Normal Subjects having Proteinuria at Various Ages

18. Freedman, L.R. et al. Proteinuria in Hiroshima and Nagasaki Japan. Yale J. Biol. and Med. 40:109, 1967.

19. Wagner, M.G. et al. Epidemiology of proteinuria. J. Ped. 73:825, 1968.

The Normal Glomerular Handling of Proteins

The classic studies of Pappenheimer on capillary membranes has been applied to the glomerulus and an impressive literature has outlined and defined the "pore theory". Many workers have demonstrated by the use of myeloperoxidase and other substances that, depending on molecular weight, one can estimate where in the GLOMERULAR CAPILLARY FILTER you will see substances deposited. Schematics of this filter system of pores is shown in Figures 3 and 4.

19A. Pappenheimer, J.R., Renkin, E.M. and Borrero, L.M. Filtration, diffusion and molecular sieving through peripheral capillary membranes. Am. J. Phys. 167:13, 1951.

Basic concepts of "pore theory" presented.

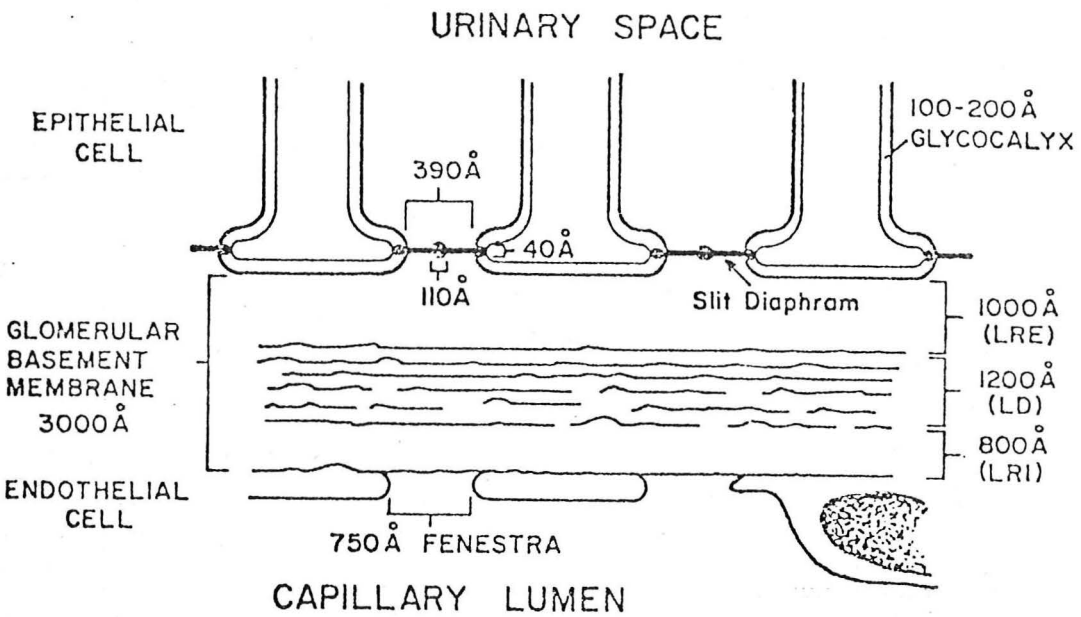
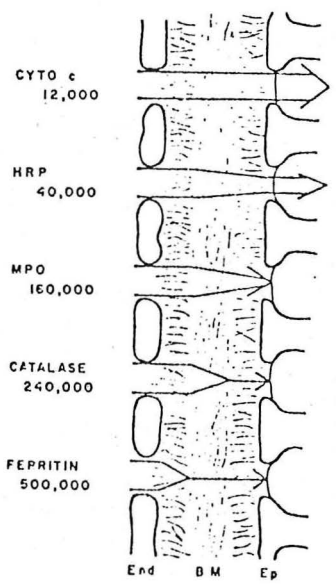


FIGURE 3

COURTESY OF R. GLASSOCK (12)



SCHEMATIC SHOWING WHERE VARIOUS SIZE MOLECULES APPEAR TO BE RETARDED.

FIGURE 4

The theory can be summarized as follows. The endothial pores or fenestrations are freely permeable and proteins then are able to pass through the basement membranes three layers at various rates depending on factors discussed later. PRotein up to 50-60,000 daltons can move through apparently limited only by the physical factors discussed below. From 65,000 to perhaps 200,000 daltons proteins pass through the basement membrane but are retarded at the epithelial slit pore. Proteins larger than 400,000 daltons are impeded by the lamina rara interna and the lamina densa as suggested in Figure 4. (63). There are two major questions that arise from these demonstrations:

First, how do proteins of various size pass through the layers of the basement membrane?

Second, what properties does the epithelial slit pore have to serve as the "final filter"?

The Basement Membrane (BM)

Only as a mathematical concept should we consider the BM as a series of various size pores. Biochemical studies have shown it to be composed of three layers or lamina. These layers are made up of collagen like glycopeptides composed of hydroxylysine, glycine, glucose, galactose and a non-collagen heteropolysaccharide glycopeptide rich in hydroxyproline. This latter substance contains galactose, fucose and sialic acid. Although collagen cannot be demonstrated, electron microscopy suggests a fine network of fibers in the lamina densa and to a lesser extent in the lamina rara interna and externa.

THe Epithelial Cell Layer

Next to the lamina rara externa is the epithelial cell body with its interdigitating network of processes extending over the BM. These "foot processes" are covered with a thin layer of a glycoprotein rich in sialic acid (GLYCOCALYX) which is highly negatively charged. The space between the foot process varies depending on the type of processing done on the material. Many authors believe that there is an ultra thin membrane covering the slit pore and that this is not artifactual but the ultimate fine filter to prevent middle size molecules 60-150,000 daltons from moving through. Last year Karnovsky (21) demonstrated bridges in the slit diaphram that may allow passage up to the approximate size of albumin (69,000).

Regardless if we accept the mathematical concept of pores to explain the movement of molecules or believe that the membrane is a series of thixotropic gels, certain additional physical factors effect protein movement. These properties of the protein molecule and/or the membrane explain why simple molecule size does not completely predict protein movement.

PROTEIN PROPERTIES THAT MAY EFFECT MOVEMENT THROUGH MEMBRANE

1. Size of the protein molecule - its relationship to pore diameter obviously controls movement in a major way.

2. Steric Hinderance - a fibrillar protein may not orient to the pore opening and passage will be decreased or hindered.

3. Viscous Drag - friction between protein molecule and stationary fluid layer on the pore wall will slow movement.

4. Electrical Hinderance - interaction between electrical charges of of the protein molecule and the pore wall.

5. Binding with larger proteins - e.g., hemoglobin binding with haptoglobin and not passing through.

In summary, while the movement of protein molecules through the basement membrane may be thought of as a series of pores and filters, it is probably not correct. Please consider such descriptions at present as conceptual.

It seems probable that there may be a completely different explanation for movement of such molecules. Just completed work by Brenner (23) and others (22) suggests that the loss of the highly negatively charged glycocalyx allows the passage of albumin and other proteins. In both human biopsy specimens and animal models the decrease or loss of sialic acid is concomitant with the onset of protein spillage.

19B. Glassock, R. 2nd Snowmass Nephrology Meeting, Personal Communications.

20. Maack, T.M., Sherman, R.L., Hernemann, H.O. PRoteinuria. Am. J. Med. 56:71, 1974.

An excellent basic review of protein movement in health and disease.

21. Rodewald, R., Karnovsky, M.J. Porous substructure of the glomerular slit diaphragm in the rat and mouse. J. Cell. Biol. 60:423, 1974.

Probably as advanced a proposal as is available at present if one believes the pore theories.

22. Blair, E.B. and Haas, J.E. Glomerular sialic acid and proteinuria in human renal disease. Lab. Invest. 28:477, 1973.

Presents an important concept that places more importance on charge and should direct work to such changes.

23. Brenner, B. Personal Communication, submitted to J. Clin. Invest.

24. Hulme, B. and Hardwicke, J. Human glomerular permeability to macromolecules in health and disease. Clin. Sci. 34:515, 1968.

A good basic review of the procedure and results.

Regardless of the exact mechanism by which proteins are handled by the glomerulus, certain facts are evident from inert molecule studies. Hardwicke (23) utilizing polyvinylpyrrolidone (PVP) has demonstrated movement is roughly correlated with size. However, one must recognize the difference between a charged protein molecular that can be reabsorbed and inert molecules of the same size. In Hardwicke's studies he showed PVP of 36 Å size had a clearance rate of 30-40% of creatinine whereas a similar sized albumin molecule has a clearance of less than 1% of creatinine clearance.

If one considers anything below 45,000 daltons as low molecular weight, evidence shows that such molecules, e.g. B_2 microglobin (11,800) or lysozyme (17,000) tend to be freely filtered. Yet, these non-inert molecules do not appear in the urine. Similar studies with substance over 50,000 daltons suggest that only a small amount, e.g. albumin (1-10 mg% estimated in proximal fluid) passes the glomerular filter and most of this is not found in the urine.

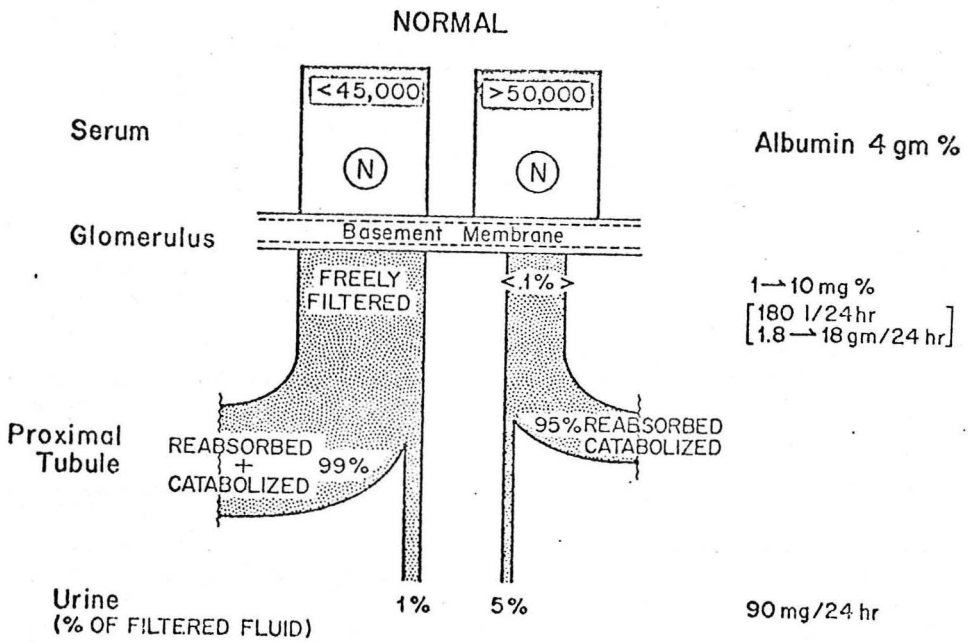


Figure 5-A

Figure 5-A is a schematic presentation of how the glomerulus appears to handle various size substances.

Low Molecular Weight Substances (<45,000)

These substances cross the glomerular filter in proportion to their molecular size and other physical factors mentioned earlier. Approximately 99% of these substances are reabsorbed in the proximal tubule and only 1% is seen in the urine.

Studies with such a substance, e.g., lysozyme (molecular weight 17,000) show that little if any of these substances are excreted or returned intact to the blood stream.

Therefore, low molecular weight substances are filtered and reabsorbed and probably catabolized in the normal nephron.

Intermediate and Larger Molecular Weight Substances (>50,000)

Albumin, as demonstrated in Figure 3, with a molecular weight of 69,000 is not filtered freely. Recent estimates suggest about 1 mg% of albumin is an accurate measurement in the earliest proximal tubule (25). Yet, only 5% of this small amount has been shown by Maunsbach (26) using ^{125}I albumin to reach the final urine. The labeled albumin was first observed in tubular cells in small spiral vacuoles formed by the pinocytotic pinching off of the cell surface membrane. Later this albumin was demonstrated to be in cytoplasmic bodies containing hydrolytic enzymes. There is no evidence to suggest that albumin is returned intact to the blood stream.

25. Strober, W. and Waldmann, T.A. The role of the kidney in the metabolism of plasma protein. Nephron 13:35, 1974.

An excellent and complete review of how the kidney handles various size proteins.

26. Maunsback, A.B. Absorption of ^{125}I labeled homologous albumin by rat kidney proximal tubule cells. J. Ultrastructure Research 15: 197, 1966.

Tubular Damage

Figure 5-B depicts the decrease in the reabsorption of small molecular weight substances. Large amounts are spilled in the urine yet serum levels are unchanged. There is some evidence that such tubular damage also decrease slightly albumin reabsorption. Cortney (27) has shown that there is still selectivity and this phenomena may depend on the amount of damage. If there is only slight, proximal tubular damage increased reabsorption down stream may take place and mask the problem. Table 7 is a partial list of causes of tubular proteinuria.

27. Cortney, M.A., Sawn, L.L. and Weiss, D.D. Renal tubular protein absorption in the rat. J. Clin. Invest. 49:1, 1970.

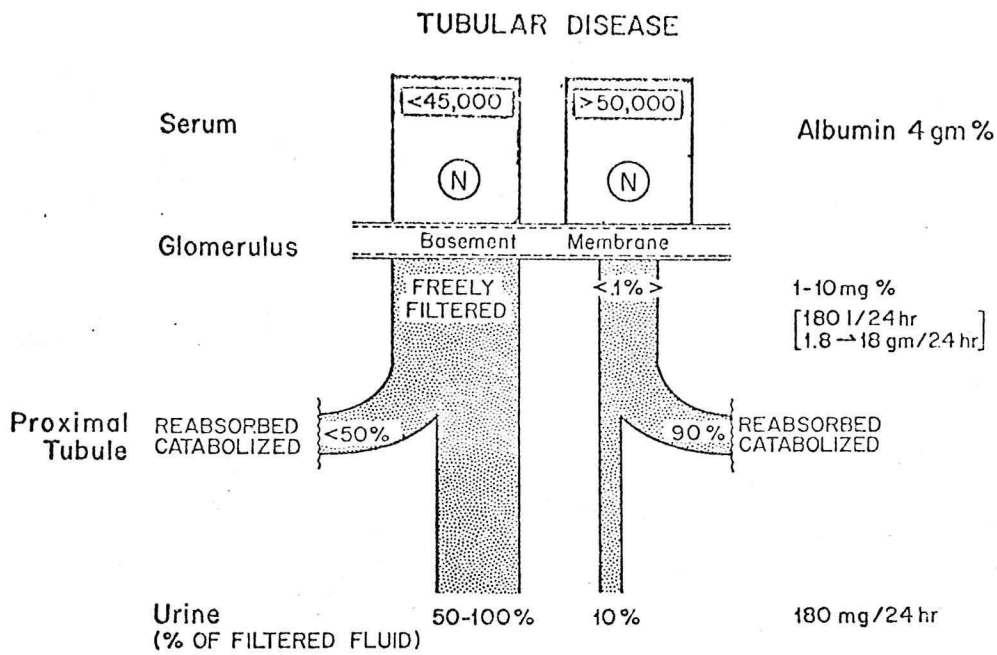


Figure 5-B

TABLE VII

TUBULAR PROTEINURIA

FRANCONI SYNDROME	POTASSIUM DEPLETION
CYSTINOSIS	"ACUTE TUBULAR NECROSIS"
CADMIUM POISONING - CHRONIC	PAROXYSMAL
RENAL ALLOGRAFT REJECTION	MYOGLOBINURIA*
WILSON'S DISEASE	ANALGESIC ABUSE
MYELOMA	BALKAN NEPHRITIS
GALACTOCEMIA	

ALMOST ANY CONDITION WITH A RENAL TUBULAR DEFECT

Glomerular Damage

Figure 6-A shows that increased filtration of protein regardless of etiology results in increased loss in the urine as well as increased endogenous catabolism. This is probably explained by exposure of more sites to greater concentration of albumin. It is estimated that normally the proximal tubule is responsible for 10% of albumin catabolism (28). In this situation it may increase to 20-40%. Since small molecular weight proteins are freely filtered anyway, tubular proteinuria is not significantly affected.

28. Katz, J., Ronnfield, Z. and Sellers, A. Role of the kidney in plasma albumin catabolism. Am. J. Physiol. 198:814, 1960.

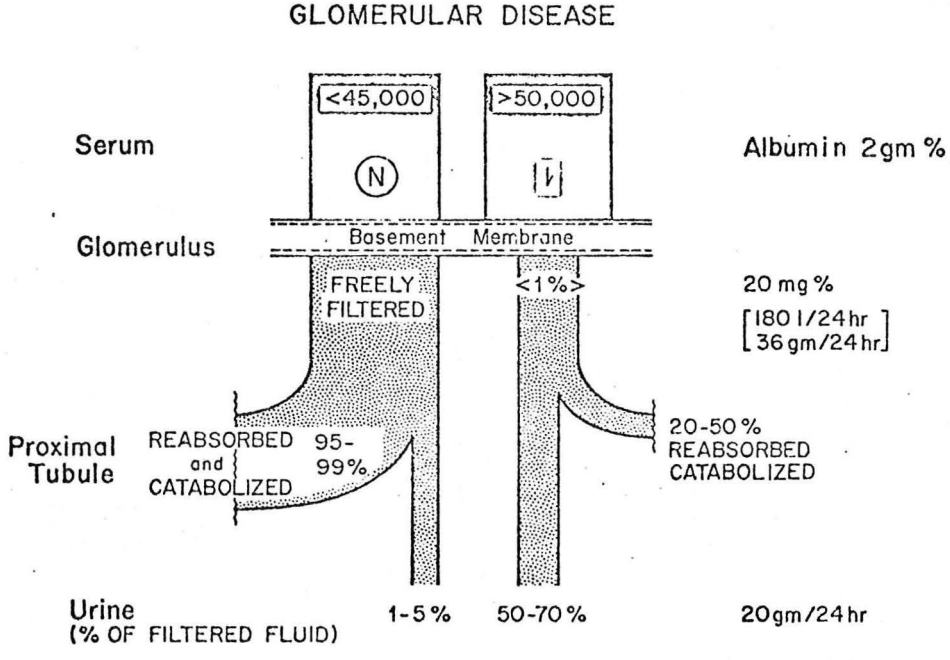


Figure 6-A

Chronic Renal Disease

In chronic renal failure, tubular as well as glomerular damage is usually present and eventually we will have a decrease in GFR. This will effect both tubular (small molecular weight) as well as glomerular proteinuria. There will be an increase in the serum levels of small molecular weight substances because filtration is decreased. Usually such diseases also have increased permeability in the remaining functioning glomeruli. Thus, early in the disease, larger molecular weight substances are lost from the serum and appear in the urine. As GFR falls further, the loss of albumin and other intermediate molecular weight substances decreases and serum levels may return to or toward normal.

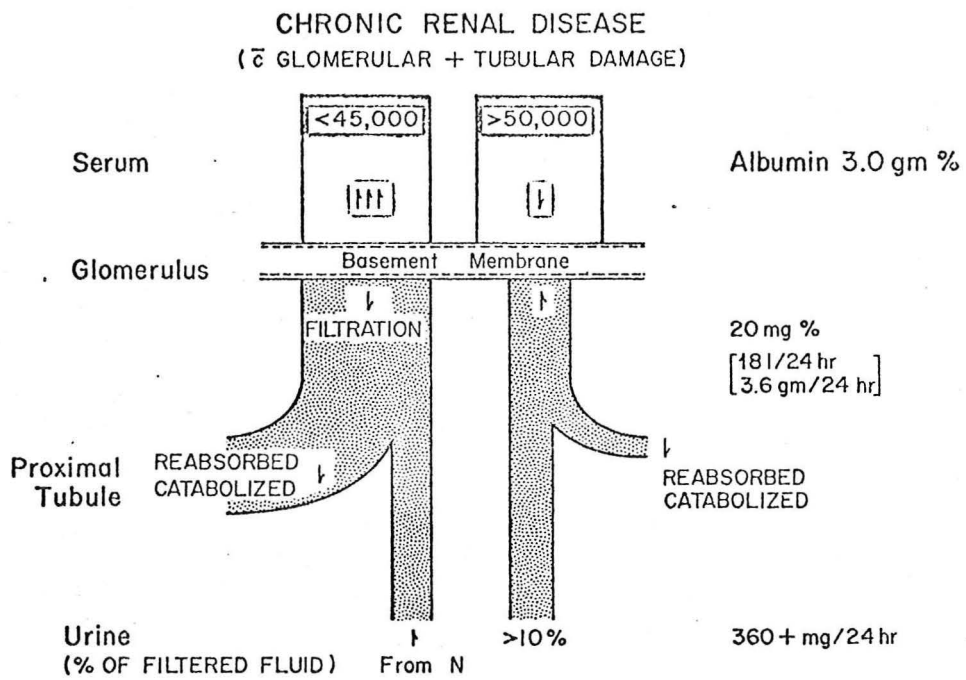


Figure 6-B

Summary

Depending mainly on size, proteins are selectively passed through the basement membrane. The proximal tubule selectively reabsorbs these substances. In tubular disease this process fails and large amounts of small molecular weight substances appear in the urine. This is a net loss of amino acids to the body since this was formerly reabsorbed and catabolized; i.e., serum levels are not effected providing synthesis is stable. In glomerular lesions large amounts of intermediate and large molecular weight substances are lost because the tubule while increasing its capacity cannot handle a very large load. Thus, the serum level of such proteins decrease if synthesis is stable. When nephrons being to fail and GFR decreases, both of the above processes take place. However, with the decreasing GFR less protein is lost through the kidney. This results in a marked increase, in serum levels of small moelcular weight substance and a normalization of larger proteins, e.g., albumin.

RENAL DISEASE AND PROTEINURIA:

The various types of proteinuria and the different mechanisms of spillage have been applied clinically to try to predict underlying diseases. One could summarize results by saying low molecular weight proteins only (tubular proteinuria) will predict a tubular lesion. However, since glomerular lesions are our major concern, these results are not very helpful. It had been hoped that Hulme and Hardwicke (24) work with various size proteins and inert molecules would allow a differentiation of the various glomerular lesions. Unfortunately seven years later this approach can be summarized as follows:

1. Selectively may change over the years when repeated in the same subjects.

2. A non-selective pattern probably rules out lipoid nephrosis.

29. Petrie, J.J.B., MacLean, P.R. and Robson, J.S. Protein selectivity in the assessment of patients with proteinuria. Aust. N.Z. J. Med. 3: 480, 1973.

A good review of the problems, results and disappointments with this method.

30. White, R.H.R., et al. The significance of variations in the selectivity of proteinuria. Clin. Neph. 3:42, 1975.

Although renal biopsy was an accepted tool, many centers were hesitant to biopsy patients with mild proteinuria. A general feeling based on no data and a few anecdotes was that mild protein did not progress to renal failure. In 1966 Muth (31) published a paper on the biopsy results in 51 people with mild intermittent proteinuria, diastolic pressure was below 90, and all had normal urine sediment and renal function by clearance. This paper even today has not received the proper recognition. The results showed that 31/50 (61%) had specific anatomical lesions, 4 (8%) had minimal non-specific lesions and 16 (31%) were normal. One year later Phillippi (32) published his results in 50 patients with persistent proteinuria. His criteria were not quite as selective as Muth's; e.g., he accepted a creatinine clearance of 80 cc/min as normal versus the 100 cc/min demanded by Muth. In addition, 17 of his 50 patients already had hypertension. The results, as might be expected, showed 32/50 (64%) had glomerulonephritis. An additional 7 patients had non-specific focal lesions. Thus 39 out of 50 (78%) had glomerular abnormalities. Table VII shows how much greater the chances were of renal disease on biopsy if hypertension was present in association with persistent or fixed proteinuria.

It seems fair to assume from these and other series that the severity and type of renal disease cannot be determined unless tissue is obtained.

TABLE VII

Histologic Diagnosis on Hypertensive Patients

Membranous Glomerulonephritis	8
Proliferative Glomerulonephritis	3
Mixed Glomerulonephritis	3
Normal	1
Arteriolarnephrosclerosis	1
Intestinal Nephritis	1
TOTAL	17

31. Muth, R. Asymptomatic mild intermittent proteinuria. Arch. Int. Med. 115:569, 1965.

Good evidence that asymptomatic proteinuria is not a benign entity.

32. Phillippi, P.J. et al. Persistent proteinuria in asymptomatic individuals. Military Med. 1311, 1966.

Good data but questionable selection of patients.

33. Black, D.A.K., Rose, G., and Brenner, D.B. Controlled trial of prednisone in adult patients with the liphrotic syndrome. Brit. Med. J. 3:421, 1970.

Well-controlled study, although steroid dose was probably too low, complications were high.

Renal Biopsy

At the present time renal biopsy is the only way to obtain tissue and diagnose the disease causing the proteinuria. Open renal biopsy includes the risk of an anesthetic and morbidity is much greater than with closed renal biopsy. Percutaneous renal biopsy is a relatively safe effective way to obtain tissue for diagnosis. Like all biopsy procedures, the more one does, the greater the expertise and the less the morbidity. At a teaching hospital this is always a problem as many people take part in the procedure. Mortality has been estimated by Welt in 21 such teaching institutions as .07% (6 in 8000).

TABLE VIII.

Complications With Renal Biopsy (Estimated)

Gross Hematuria	5-8%
Peri-renal Hematoma	.6%
Transfusions	.4%
A-V Fistulas	.05%
Nephrectomies	0-.05%

The references below discuss the indications and contraindications of this procedure.

34. Lordon, P.E. and Thompson, A.L., Jr. Precutaneous renal biopsy. Texas Med. 20:41, 1974.

A good recent review with references.

35. White, R.H.R. Renal biopsy. Arch. Dis. of Child. 38:260, 1963.

36. Kark, R.M. Renal biopsy. J.A.M.A. 205:220, 1968.

Detailed, includes Welt's questionnaire to 21 centers with over 8000 biopsy procedures.

Since most lesions associated with proteinuria are glomerular, I classified these in broad general terms as listed in Table IX.

The initial three groups are now fairly well defined and will be considered in some detail. However, it should be pointed out that one of these lesions really has only been separated out in the last five years and that was a result of biopsy data. The proliferative group is not at present as clearly delineated and is listed this way in an attempt to clarify general not specific points.

TABLE IX

Biopsy Classification of Glomerular Disease

1. Lipoid Nephrosis
2. Focal Glomerulosclerosis
3. Idiopathic Membranous Glomerulonephritis
4. Proliferative Glomerulonephritis
   - A. Acute Post Streptococcal
   - B. Membranoproliferative (Mesangiocapillary)
     - Hypocomplimentemic of West
     - Dense Deposit Disease
     - Lobular Glomerulonephritis
   - C. Focal Glomerular Involvement
     - Benign Hematuria
     - IgA - Berger's Disease
     - Other: Systemic Causes
   - D. Rapidly Progressive Glomerulonephritis

Lipoid Nephrosis (63) (NIL or minimal change disease)

This non-immunological process causes some unknown change in the glomerular basement membrane that allows some intermediate molecular weight proteins; e.g., albumin, to pass through (selective proteinuria). Lite microscopy is negative as are immunofluorescence studies. Electron microscopy only shows foot process fusion a secondary phenomena to protein leakage.

The response to steroid treatment is excellent within two to four weeks in most cases. A certain percentage (25-35%) are steroid dependent and require repeated steroid trials or the addition of cyclophosphamide to induce a prolonged remission. Variable reports on this additive effect plus the question of cyclophosphamide on reproduction raises considerable doubt about its use in a self-limited disease. Initial treatment should be diuretics and low salt diet. Only if edema cannot be controlled should steroid be used. It should be noted that until 1972 all reports on lipoid nephrosis tended to include the entity now separated out as focal

glomerular sclerosis. This makes interpretation of all data before 1973 very difficult since most patients diagnosed as lipoid who developed renal failure probably had another disease; i.e., focal glomerular sclerosis.

37. Cameron et al. The nephrotic syndrome in adults with "minimal change" glomerular lesion. Quart. J. Med. 43:461, 1974.

An example of the confusion regarding treatment and long-term effects when lipoid nephrosis is lumped with focal glomerular disease.

TABLE X

Lipoid Nephrosis

Lite Mic:	Neg.
E.M.:	Foot Process Fusion
Immuno:	Neg
Response to Treatment:	Excellent
Long-Term Prognosis:	Good

Focal Glomerular Sclerosis

In 1925 Fahr suggested the importance of this lesion in the German literature. It was not until 1957 that Rich (37) clearly delineated in 20 autopsies on patients with lipoid nephrosis that there was a lesion present in the juxtamedullary glomeruli. This was confirmed by Habib and others in the early 1970's.

Table XI outlines the salient features on biopsy in addition to the prognosis and response to treatment.

TABLE XI

Juxtamedullary Glomeruli Involved Initially

Lite Mic:	Earliest lesion near vascular pole Widening of mesangium Adhesions between Tuft & Bowman's capsule Tubular atrophy → interstitial fibrosis
E.M.:	Foot process fusion BM folding in involved areas
Immuno:	Small granular deposits of most types in the involved area
Response to Treatment:	Poor
Long Term Prognosis:	Generally poor (1-10 yrs)

A review of the recent series suggests possible clinical differences between lipoid and focal glomerulosclerosis as shown in Table XII. Unfortunately there is considerable overlap and early in the course of the disease there may be no difference clinically. We are planning to do selective protein studies (24) on all patients with lipoid nephrosis in the hope that selective proteinuria would confirm the diagnosis.

TABLE XII

CLINICAL COMPARISON

	LIPOID NEPHROSIS	FOCAL GLOMERULAR SCLEROSIS
SELECTIVE PROTEINURIA	HIGHLY SELECTIVE	NON SELECTIVE
HEMATURIA		
MICRO	50%	ALMOST ALL
GROSS	RARE	< 10% >
HYPERTENSION	RARE	50%
+RENAL FUNCTION	NO	UP TO 1/3
STEROIDS	90%	NONE
IMMUNOSUPPRESSIVES	GOOD	NONE
PROGNOSIS	EXCELLENT	POOR

37. Rich, A.R. A hitherto undescribed glomeruli in lipoid nephrons. Bull. Hopkins Hosp. 100:173, 1957.

An excellent paper unfortunately the observation was overlooked for almost 15 years.

38. Velosa, J.A., Donadio, J.V., Jr. and Holley, K.S. Focalsclerosing glomerulonephropathy. Mayo Clinic Proceedings 50:121, 1975.

A good complete recent review with references.

39. Habib, R., Gubber, M-C. Focal sclerosing glomerulonephritis. In Glomerulonephritis Part I. Ed. P. Kinkaid-Smith, et al. John Wiley and Sons, p. 263, 1973.

Emphasis on the disease in children.

40. Hoyer, J.R., Vernier, R.L. and Najarian, J.S. Recurrance of idiopathic nephrotic syndrome after renal transplantation. Lancet 2:343, 1972.

Another example of the confusion associated with lipoid nephritis until recently. A warning against transplantation in this group.

Idiopathic Membraneous Glomerulonephritis

Aside from its occurrence in systemic disease; e.g., S.L.E., this is a clearly demonstrated entity. Two ongoing combined studies at present show that 66% of adults with idiopathic nephrotic syndrome have a membranous lesion. Table XIII outlines the pertinent points associated with this disease. Figure 10 shows the 4 stages as outlined by Churg.

TABLE XIII

IDIOPATHIC MEMBRANOUS GLOMERULONEPHRITIS

GLOMERULI UNIFORMLY INVOLVED

LITE MIC: UNIFORM THICKENING OF GLOMERULAR CAPILLARY WALL UNACCOMPANIED BY AN INCREASE IN CELLS.

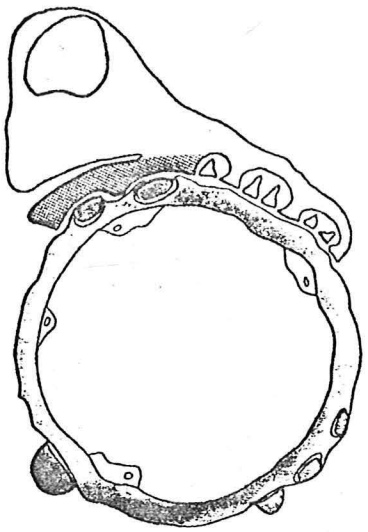
E.M.: DEPOSITS ON THE EPITHELIAL SIDE OF A NORMAL WIDTH BM. BM INCREASES IN WIDTH AND MAY REDUPLICATE.

IMMUNO: GRANULAR DEPOSITION OF MOST COMMONLY IgG AND COMPLEMENT, BUT OTHER SUBSTANCES MAY BE SEEN.

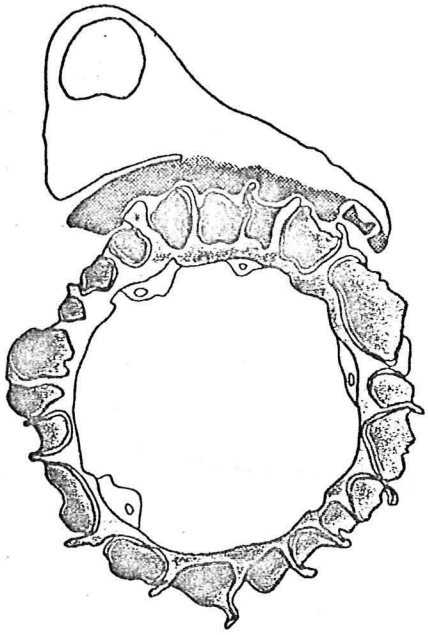
RESPONSE TO TREATMENT: POOR

LONG TERM PROGNOSIS: GENERALLY POOR (1-20+ YRS.)

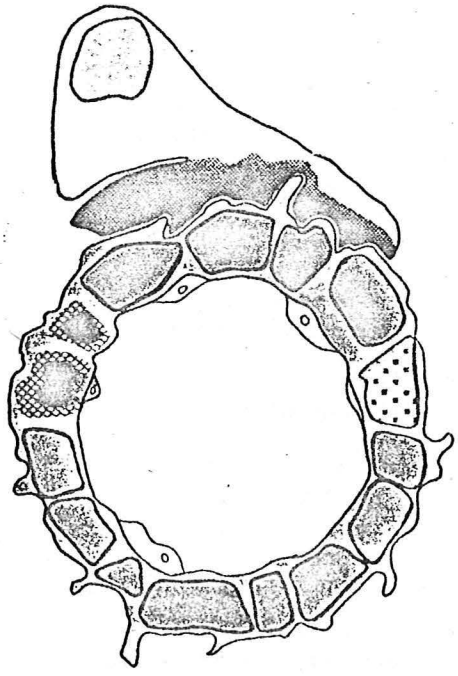
STAGES OF IDIOPATHIC MEMBRANOUS GLOMERULONEPHRITIS (Churg)



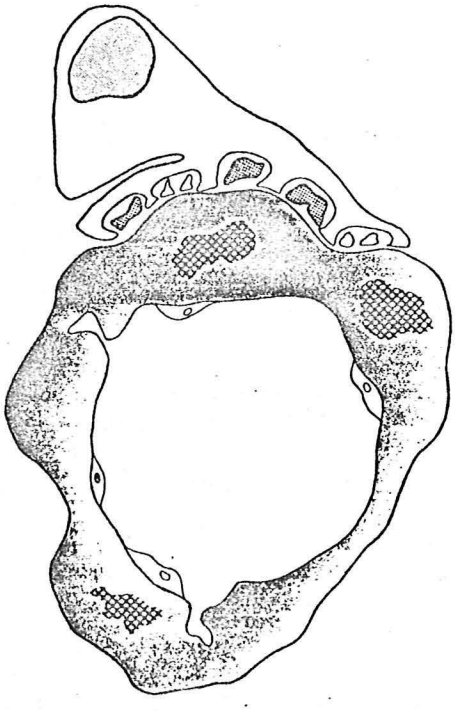
STAGE I



STAGE II



STAGE III



STAGE IV

Figure 10

Stage I (early): The subepithelial deposits are generally small, dense and sharply demarcated. The projections of the basement membrane (spikes) are inconspicuous or absent. At this stage it is difficult to diagnose on lite microscopy. May be reversible at this stage (42).

Stage II (fully developed: Deposits are large and often diffuse. They are separated by spikes having the same electron density as the basement membrane. The capillary wall is thick and the diagnosis can be made on lite microscopy.

Stage III (advanced): Deposits are being incorporated into basement membrane. Their density is variable. On lite microscopy the thickened capillary wall appears mottled.

Stage IV (late): The basement membrane is irregular and thick, deposits have almost disappeared.

The course is often slow, twenty years or more, but most cases progress. Rare remissions have been reported (42).

Renal failure generally begins with the onset of glomerular solidification. There appears to be a relationship between duration of illness and thickness of the capillary wall. There is also a positive correlation between the wall thickness and the BUN and creatinine elevation. There is no correlation between amount of proteinuria and thickness of the wall; in fact all patients do not develop the nephrotic syndrome with idiopathic membranous lesions.

41. Hayslitt, J.P. et al. Clinicopathological correlations in the nephrotic syndrome due to primary renal disease. Medicine 52:93, 1973.

Excellent review of one center's finding and their treatment results.

42. Forland, M. and Spargo, B.H. Clinicopatholgoical correlations in idiopathic nephrotic syndrome with membranous nephropathy. Nephron 6:498, 1969.

43. Gluck, M.C., Gallo, G., Lowenstien, J. and Baldwin, D.S. Membranous glomerulonephritis. Ann. Int. Med. 78:1, 1973.

Best spontaneous results in a selected group of patients.

Proliferative Glomerulonephritis

The term is used in its widest sense to cover an emerging group of diseases that at present are lumped together. This type of grouping will continue until we have learned enough to split them off separately.

Acute Post-Streptococcal Glomerulonephritis

The clinical picture confirmed by ASO rise and/or complement decrease must be proven by biopsy before one can be certain of the diagnosis. When the above criteria are met, three things become evident from a review of the literature.

1. Epidemic series do not show progression to renal failure (44).

2. Long-term follow ups have not been done in most cases and when they were carried out they were not done well.

3. Careful studies of biopsies may be suggesting two lesions, each with different prognosis (46).

The last point is going to require a careful review of our past experience and in the future special care to try and biopsy at a fixed time post-infection.

44. Perlman, L.V., et al. Post-streptococcal glomerulonephritis. A ten-year follow up of an epidemic. J.A.M.A. 194:63, 1965.

Strong evidence presented against progression to renal impairment following epidemic post-streptococcal glomerulonephritis.

45. Baldwin, D.S., et al. The long-term course of post-streptococcal glomerulonephritis. Ann. Int. Med. 80:342, 1974.

Best data for long-term follow up.

46. Richet, G., Chevet, D., and Morel-Maroger, L. Serial biopsies in diffus diffuse proliferative glomerulonephritis in adults. Glomerulonephritis, Part I. John Wiley and Sons, p. 363, 1973.

A very detailed presentation showing that even initially their 102 cases with follow up biopsies could be divided into two groups. The follow up suggests we must look at the number of polys present in the glomerulus and the duration of the disease more carefully.

MEMBRANOPROLIFERATIVE (MESANGIOCAPILLARY) GLOMERULONEPHRITIS

This is probably a number of different diseases that, at present, are grouped together. Since their presentation and to some extent prognosis is so similar, I have not discussed them separately. They may present clinically as mild proteinuria or with the nephrotic syndrome initially or later. There is microscopic hematuria and frequently a low serum complement. It should be

noted that not all patients have a decreased complement level and it is not always constant in patients that do. Therefore, at present, the etiology and significance of the depressed levels when present is not certain. Overall prognosis is poor and response to treatment is also generally poor.

Biopsy generally shows increased cellularity of the mesangium associated with irregular thickening of the capillary wall (hence the term mesangiocapillary. On immunofluorescence, the pattern is usually granular but linear staining has been found (48).

Recurrence of disease in renal transplants has been reported for some of these cases.

47. Berger, J., et al. Immunohistochemistry of glomerulonephritis In Advances in Nephrology, Chicago year book, 1971. Vol. 1, p. 11.

A report in English of their initial findings reported in French in 1963.

48. Habib, R., et al. Dense deposit disease: A variant of membranoproliferative glomerulonephritis. Kidney Int. 1:204, 1975.

A strong plea for the classification of dense deposit disease as a separate entity.

49. Galle, P., Hinglois, N., and Crosnier, J. Recurrence of an original glomerular lesion in three renal allografts. Transplant Proc. 3: 368, 1971.

50. West, C.D., et al. The natural history of membranoproliferative glomerulonephritis In Glomerulonephritis, Part 1, John Wiley and Sons, 1973. p. 531.

51. Lobular Glomerulonephritis In Heptinstall's Diseases of the Kidney. R. Little, Brown and Co., 2nd Ed., 1974. Vol. 1, p. 425.

The author explains why this type of biopsy now fits under membranoproliferative yet stresses some slight differences.

TABLE XIII

PROLIFERATIVE GLOMERULONEPHRITIS

1. ACUTE POST-STREPTOCOCCAL
2. MEMBRANOPROLIFERATIVE (MESANGIOCAPILLARY)
   - HYPOCOMPLEMENTEMIC OF WEST
   - DENSE DEPOSIT DISEASE
   - LOBULAR GLOMERULONEPHRITIS
3. FOCAL GLOMERULAR INVOLVEMENT
   - BENIGN HEMATURIA
   - IgA - BERGER'S DISEASE
   - SYSTEMIC:
     - COLLAGEN VASCULAR - SLE, PERIARTERITIS
     - HENOCH-SCHÖNLEIN SYNDROME
     - GOODPASTURE'S
     - HEREDITARY NEPHRITIS
4. RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

FOCAL GLOMERULAR INVOLVEMENT

I have used the term involvement because some of the members of this group may be very benign. By lite microscopy only certain glomeruli may be effected and the effected glomeruli may only show involvement in one or two lobules (segmental change).

Benign Hematuria

The recurrence of gross hematuria associatedwith infections usually upper respiratory marks this syndrome. The hematuria lasts 2-5 days and is often associated with low grade (<1 gm) proteinuria. Follow up studies

show microscopic hematuria between episodes but usually no progression to renal failure. It is thought to be due to a variety of etiological factors operating through a common pathogenic mechanism.

52. Vernier, R.L., Risnick, J.S., Mower, M.S. Recurrent hematuria and focal glomerulonephritis. Kidney Int. 7:224, 1975.

A good review with some basic data.

IgA or Berger Disease

The clinical presentation is similar to benign hematuria except biopsy shows IgA in the mesangium in most glomeruli. Lite microscopy shows a focal proliferative glomerulonephritis. On E.M. electron dense deposits are seen in the mesangium (IgA).

Hypertension and even the nephrotic syndrome can develop in perhaps 30% over a prolonged period. Approximately 20% of all cases go on to renal failure and there is at present no way to predict prognosis.

53. Berger, J., et al. Recurrence of mesangial deposition of IgA after renal transplant. Kidney Int. 7:232, 1975.

A somewhat biased description of the disease. Good references.

Systemic Diseases

These diseases often present as a focal lesion with varying amounts of proteinuria. SLE, periarteritis and Henoch-Schonlein's can present with necrosis present on biopsy. Recent reports suggest, at least in lupus erythematosis, the focal lesions can progress rapidly to a membranous or membranoproliferative lesion.

Focal Embolic Nephritis was the first situation where focal glomerular disease was described. It is still not clear whether there is true embolic phenomenon or just an immune reaction or both causing the lesion.

Goodpasture's Disease, when seen early on biopsy, is a focal lesion. Unfortunately in most cases it rapidly progresses to crescent formation and the full blown picture of lung and kidney involvement.

Hereditary Nephrotic (Alports Syndrome), when biopsied early, may only show a focal lesion. The clinical picture can be exactly like benign hematuria with microscopic hematuria and mild proteinuria before deafness or renal failure appears. At present the etiology is not known.

RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

This is a heterogenous group of diseases with probably a limited form of expression in the kidney. The presentation, progression and recovery are all variable. There is suggestive evidence that a post-streptococcal etiology has a better long-term prognosis and this has been our experience. Most of the above focal lesions can progress and express themselves in this manner. Immunofluorescence studies show granular, fine granular or linear deposits. Results with transplant are variable and unpredictable because in many cases proper and careful studies have not been done with the patient's own kidneys before transplant was attempted.

Treatment is variable and usually not responsive except in Australia (65).

TREATMENT

I have listed several references below of various new forms of treatment. However, until we are sure what disease entity we are treating, we cannot determine what role our agents are playing. At this time, I feel that there is little evidence that steroids influence the long-term course of most renal disease. The excellent two-part article by Schwartz (54) pointing out the lack of controls has still not been answered. At the present time I am only aware of one combined controlled study that is still functioning and these results although preliminary, would suggest an early response to short-term steroid dosage. The explanation of these results at present is still not available.

54. Skinner, M.D., and Schwartz, R.S. Immunosuppressive therapy. New Eng. J. Med. 287:221 and 281, 1972.

An excellent review showing the lack of controls in treating many diseases including renal.

55. Cade, R.J., et al. The effect of long-term, high-dose heparin treatment on the course of chronic proliferative glomerulonephritis. Nephron 8:67, 1971.

Very impressive results in a difficult group to treat.

56. Greifer, I. The therapeutic value of steroids in the nephrotic syndrome. Trans. Proc. 7:103, 1975.

57. Bolton, W.K., Spargo, B.A., Lewis, E.J. Chronic autologous immune complex glomerulopathy. Effect of cyproheptodine (Periactin). J. Lab. Clin. Med. 83:695, 1974.

A report on a new agent to prevent disease. Interesting literature review.

58. Barratt, T.M., et al. Cyclophosphamide treatment in steroid sensitive nephrotic syndrome of childhood. Lancet (7898) 1:55, 1975.

An example of the confusion when controls are not used and a proper tissue diagnosis is not made and followed.

59. Balsløv, J.T., et al. Cytostatic treatment of glomerular disease I and II. Acta Med. Scand. 193:483, 1973.

The effect of Azothioprine and a review of particularly the European literature.

60. Block, D.A.K., Vernier, R.L. and Relman, A.S. The use of steroids in treating the nephrotic syndrome In Controversies in Int. Med. II. Saunders and Co., 1974. Chapter 25, p. 651.

Unfortunately adult results are compared with children. In the summary strong support is given for steroids.

General References

61. Proteins in Normal and Pathological Urines. Manuel, Y. et al. Univ. Park Press. 1970.

All the well-known authors but you have to dig for the information.

62. Nephrology. Hamburger, J. et al. Saunders and Co., 1966.

Still the best basic reference for detailed evaluation of any problem in nephrology. The section on orthostatic proteinuria is outstanding.

63. Pathology of the Kidney. Heptinstall, R. et al. Little, Brown and Co., 2nd Edition, 1974.

A superb text for pathological information with excellent supporting clinical material.

64. Kidneys, Ureters and Urinary Bladder. Vol. 6, The Ciba Collection. Netter, F. and a collaborative group of well-known nephrologists.

The drawings, while schematic, often give the best representation of a problem or disease. Excellent teaching material and slides.

65. Glomerulonephritis: Morphology, Morphology History and Treatment. Kincaid-Smith, P. et al. JOhn Wiley and Son, Inc., 1973.

A good reference book with each subject covered by 3-6 different groups citing their experience.