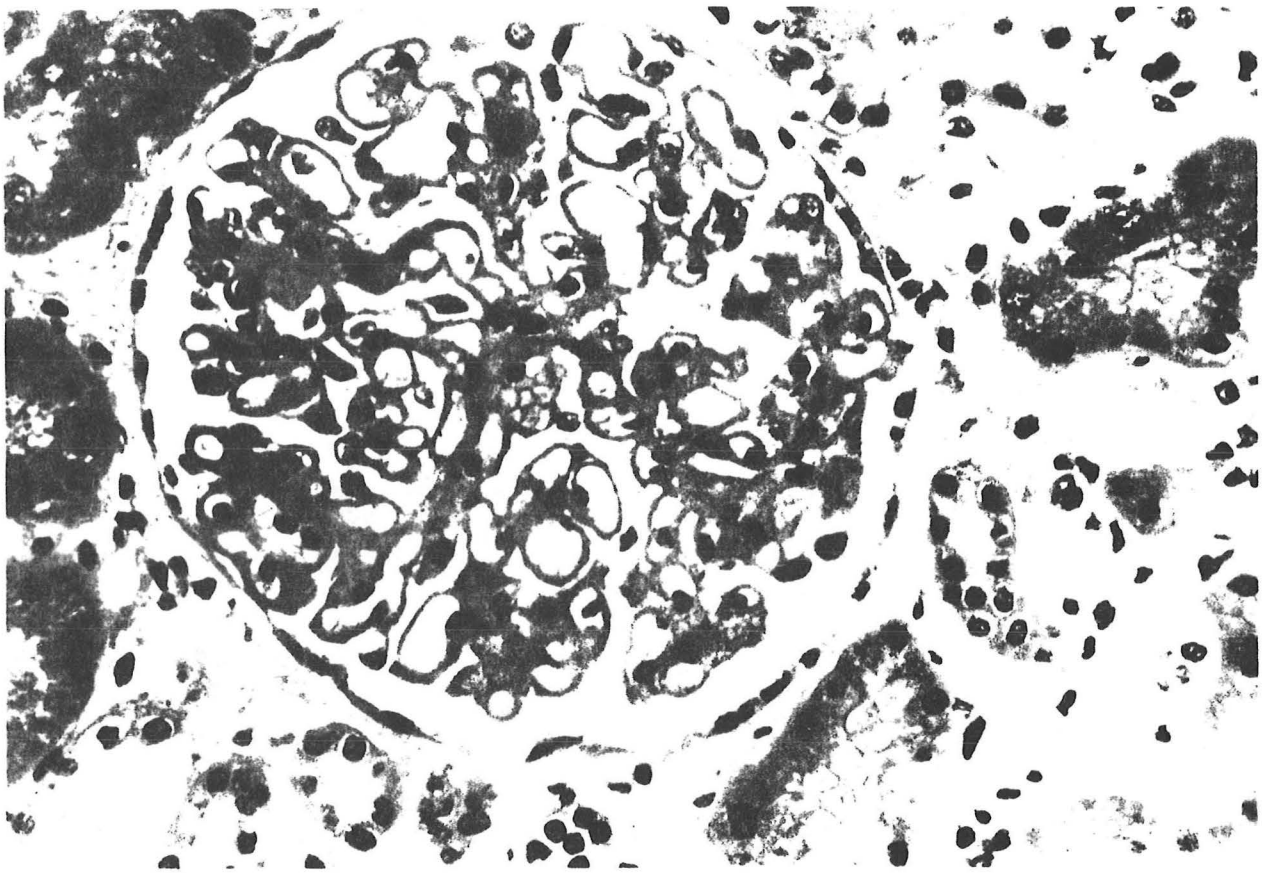
Renal

IDIOPATHIC MEMBRANOUS GLOMERULOPATHY



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Medical Grand Rounds

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INTRODUCTION

Idiopathic membranous glomerulopathy is a well characterized clinical and pathologic disorder that has earned its title primarily from anatomic considerations. The disorder is "idiopathic" in that a precise pathogenetic mechanism has yet to be well defined. The disorder I will review must also be unassociated in the strictest sense with other well-characterized systemic diseases. It is "membranous" in that the anatomic deviance from normal is best viewed at the level of the glomerular basement membrane. This variety of intrinsic renal disease is called a "glomerulopathy" to clearly demonstrate the absence of easily observable renal glomerular inflammation. More than 50% of the cases of nephrotic syndrome in adults in the United States are consequent to membranous GN, thus enhancing its importance for the internist in practice. That membranous glomerulopathy has generated interest beyond the narrow scope of the subspecialist is related to its service as a clinically relevant model of immunologically mediated human disease. The genesis of this review stems from the ferment of new information on the immune mechanisms which may underlie membranous glomerulopathy as well as the raging controversy concerning a more optimistic therapeutic approach to patients afflicted with this disorder. The new data from the clinic as well as that supplied from laboratory study of animal models has spawned a broad revision of our understanding of immunorenal disease which deserves careful analysis, while the potential for successful therapeutic intervention demands wide dissemination. This review will characterize the classic pathologic features which give rise to the notion that membranous glomerulopathy is a unique disease with an immune pathogenesis and will characterize the clinical features of membranous glomerulopathy with particular reference to the stability of diagnosis and natural history of disease. This understanding of the natural history of MGN will introduce the problem of therapeusis in this disease and allow for a careful review of the possibility of a more optimistic therapeutic approach generated from the large inter-hospital cooperative study recently concluded in the United States. Having drawn the inference that MGN is an immunologically mediated disorder, this review will pause and redevelop the classic Dixon hypothesis of immune mediation of renal disease raising questions from the explosion of new information based on animal and human data. The review will introduce the novel concept of <u>in situ</u> immune complex formation and its importance in the pathogenesis of immune renal disease. Finally, the review will attempt to formulate some holistic understanding of the pathogenesis of human membranous glomerulopathy.

<u>Pathologic Features of Idiopathic Membranous Glomerulopathy</u>

Because idiopathic membranous glomerulopathy derives its name from the morbid anatomy common to a group of patients, one can <u>a priori</u> assume that the pathologic features of the disorder are well described and are distinctive. L.T. Bell was the first to observe membranous transformation of the glomerulus in several patients with a nephrotic syndrome in what was felt to be lipoid nephrosis. In his classic monograph on lipoid nephrosis of 1929, Bell described three separate patients whose kidneys were swollen and lipid-laden on gross anatomy but

who did not have normal appearing glomeruli. In contrast, he found thickening of the basement membrane with almost complete obliteration of the glomerular capillary loop in at least one of the three patients studied.

(C) Renal Lesions Of Uncertain Type

"The glomerular lesions are very prominent.... the glomeruli are large and there is marked thickening of the basement membrane with narrowing of the capillaries. In case 1X the thickening of the basement membrane is so extreme that the capillaries are almost obliterated. The clinical picture.....is that of lipoid nephrosis, viz severe albuminuria, edema, normal blood pressure, and no nitrogen retention.

C.T. Bell Am. J. Pathol. 5:587, 1929

A careful description of the renal abnormality of this separable group of patients awaited routine application of the renal biopsy using standard and special histochemical stains of thin (<2 μ) sections of tissue prepared for light microscopy, electron microscopy, and immunofluorescence. By light microscopy, all glomeruli have uniformly thickened capillary walls with no or minimal cellular proliferation or inflammatory reaction in the usual case (Figure 1). Thus, the appellation diffuse, generalized glomerulopathy is correctly applied to idiopathic membranous GN. Cases felt to be more severe may exhibit coalescence of the membranes and solidification of the glomerular tuft that can be shown by special connective tissue stains to be the result of deposition of material into the basement membrane rather than expansion of matrix material alone. All special stains of matrix material demonstrate that the "deposits" have little histochemical properties in common with the basement membrane itself and can be uniquely identified with positive and negative staining techniques. Traditionally, a periodic acid-methenamine silver stain demonstrates in many cases finger-like projections of the basement membrane beyond negatively staining deposit material accounting for a phenomenon called a "spike".

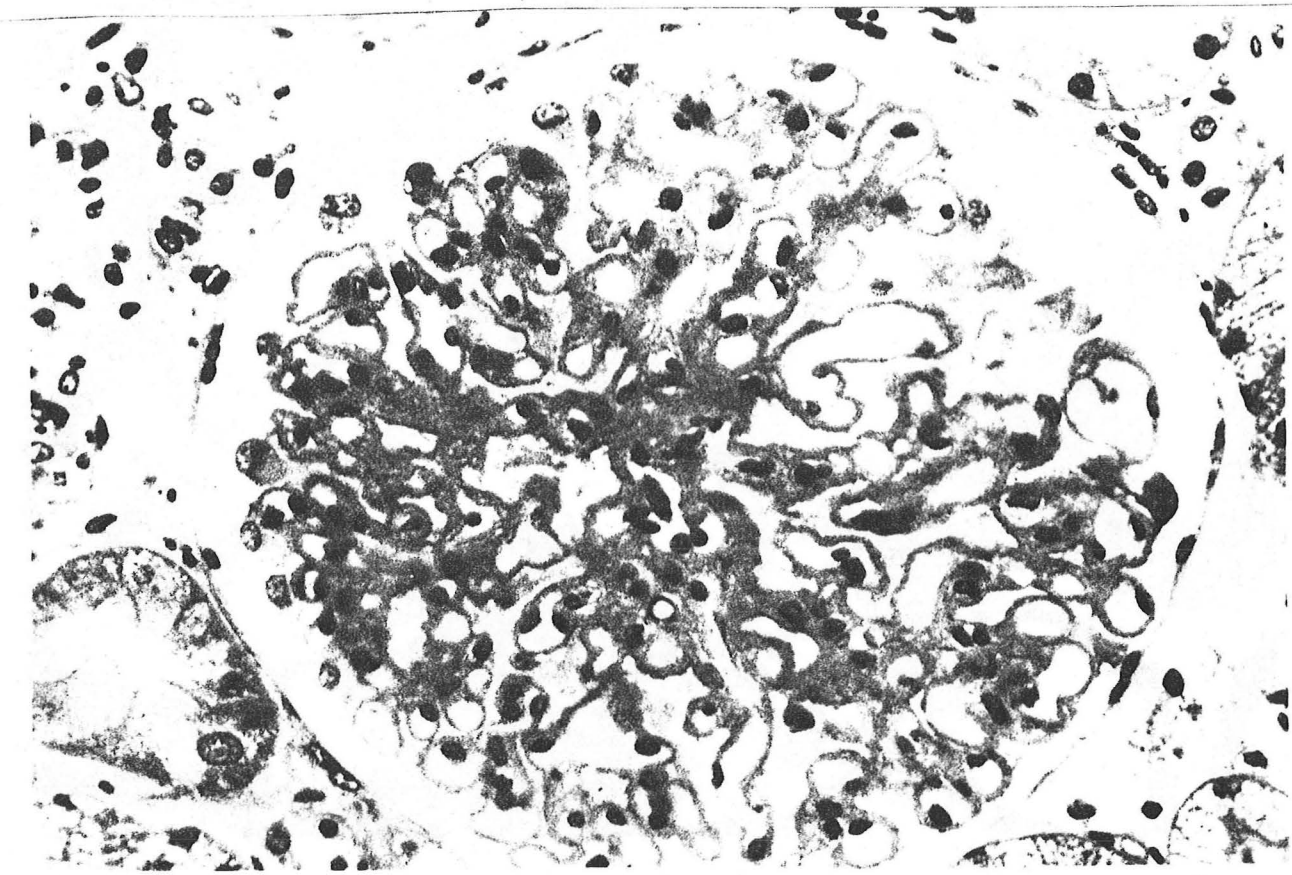


Figure 1 Light microscopic micrograph of a renal biopsy with N & E stain.

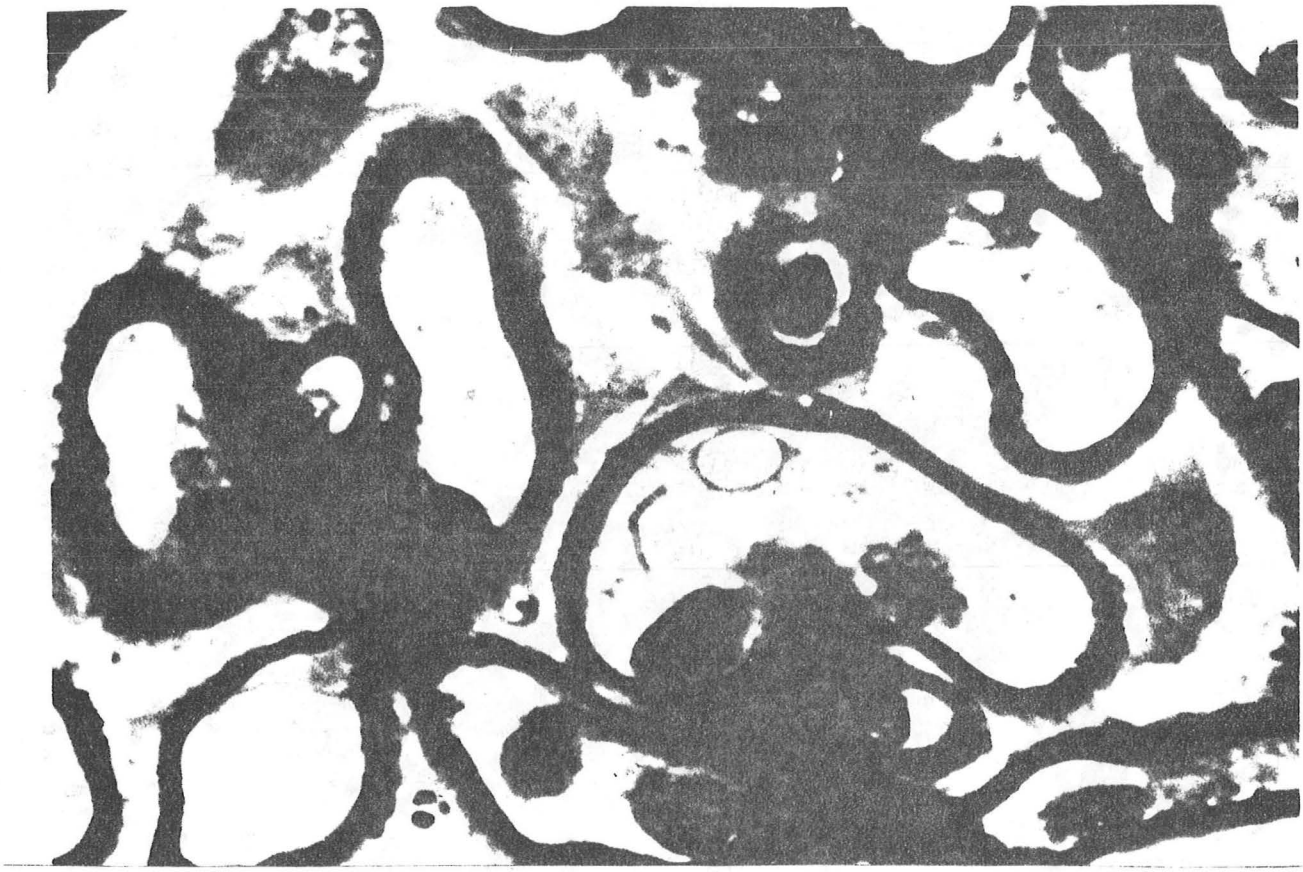


Figure 2 Special histochemical stains of MGN renal biopsy. Note the "spikes" of basement membrane material protruding from the epithelial side of the capillary loop.

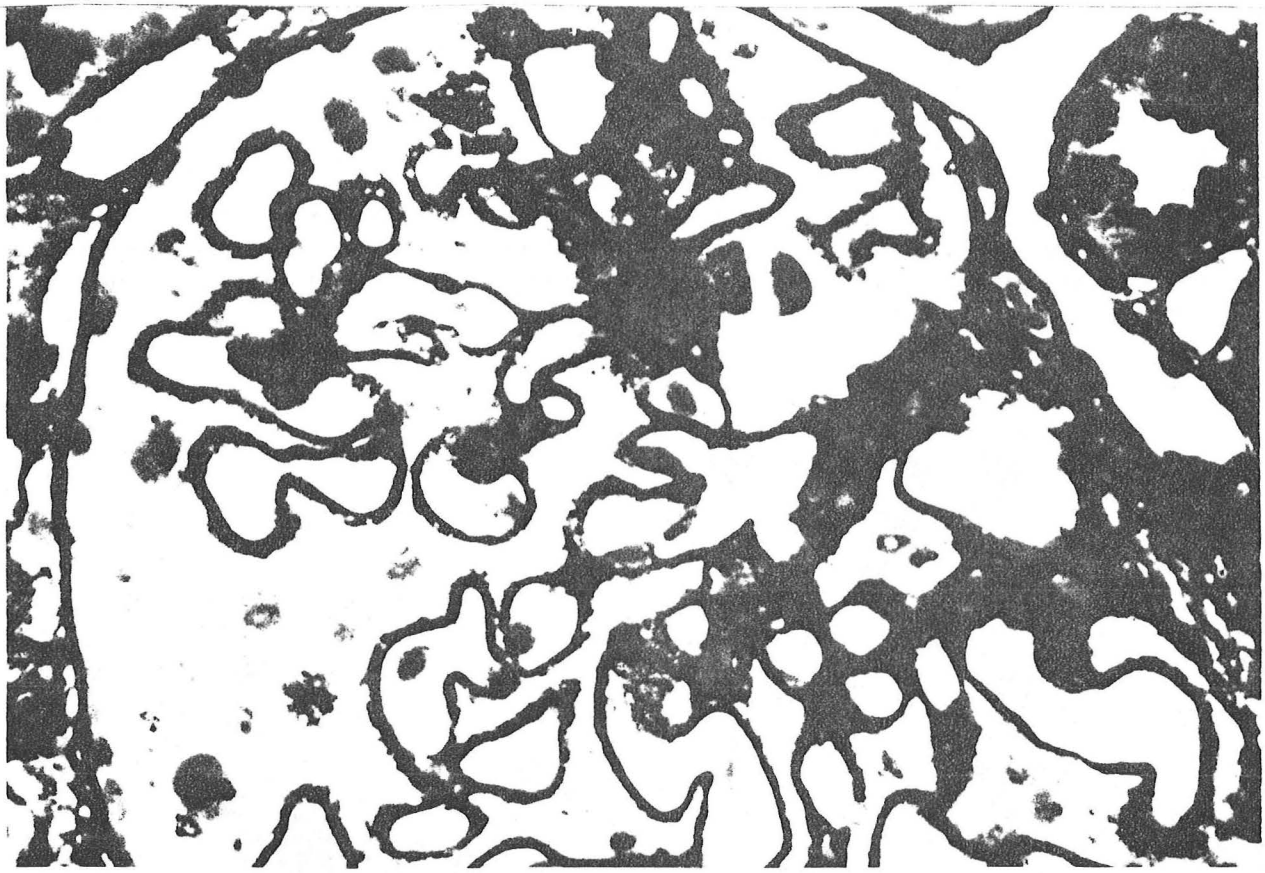


Figure 3 Special histochemical stain of MGN renal biopsy. Note the "spikes" of basement membrane material protruding from the epithelial side of the capillary loop.

This easily characterized morphologic picture was observed in biopsy material in patients all of whom had excessive proteinuria, most of whom had the nephrotic syndrome, as discussed below, with generally bland urinalysis so it was reasonable to carve out a special clinicopathologic entity based on the described morphologic derangments.

The electron microscopic evaluation of the biopsy material greatly extended the description of MGN providing initial attempts at pathogenetic explanation of the disease, means for "early" diagnosis, and a theory of progression of disease. Corresponding to the deposit material found lodged in the thickened basement membrane material by special histochemical stains with light microscopy are found electron dense deposits localized beneath the visceral epithelial cells which line the basement membrane separating it from Bowman's urinary space (Figure 4A). In some biopsied patients with nephrotic syndrome, the light microscopic examination may be nearly normal which would have led to the diagnosis of a nil change nephropathy but for the subepithelial deposit material identified by the electron microscope. Ehrenreich and Churg have described stages of the disorder by analyzing the position of the deposit in the basement membrane. In stage I, as shown in Figure 4B,

the electron dense deposits are found protruding from the epithelial side of the basement membrane. Stage II is defined by the finding of deposits imbedded in the membrane with protrusion of the matrix material on either side of the deposit to form the spikes identified by silver stain. When the deposits are surrounded by the basement membrane, type III changes are defined. In type IV the basement membrane is thickened but little deposit material can be identified, rather there is fracture of the membrane with loosened areas felt to represent dissolution of deposit once present.

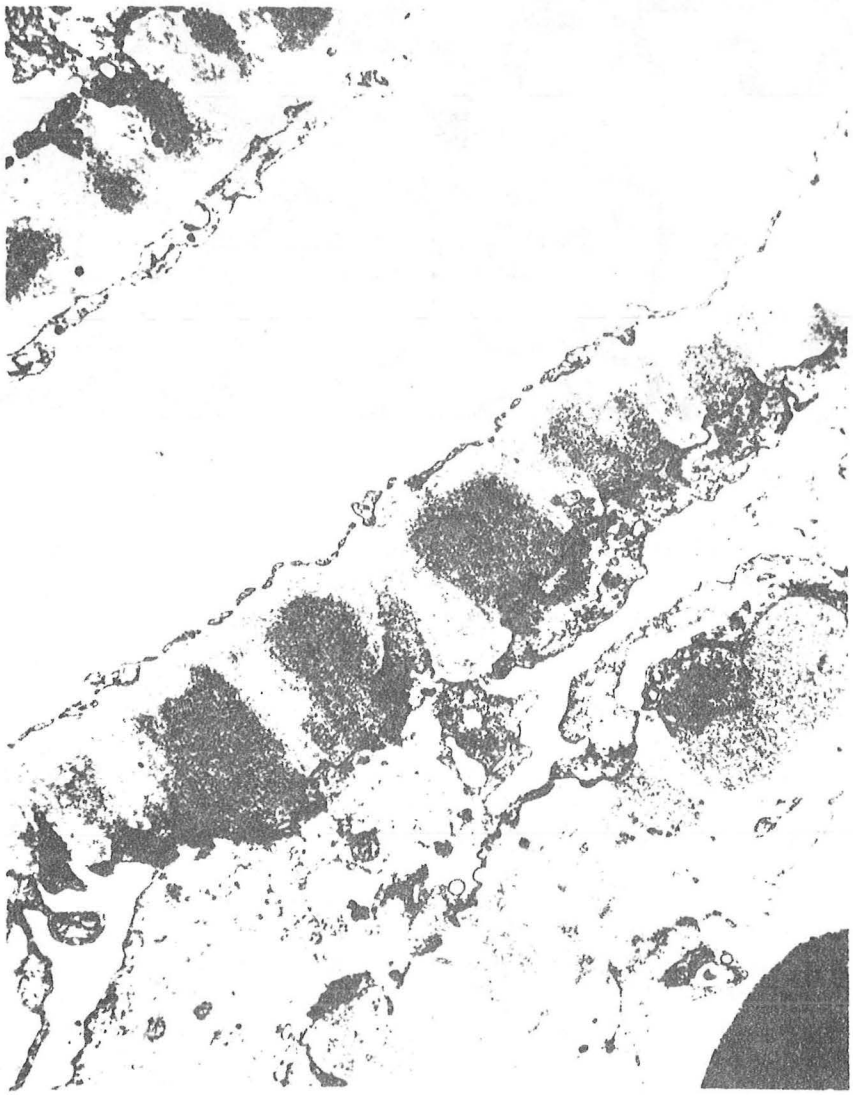


Figure 4A EM micrograph demonstrating elecron dense deposits in GBM.

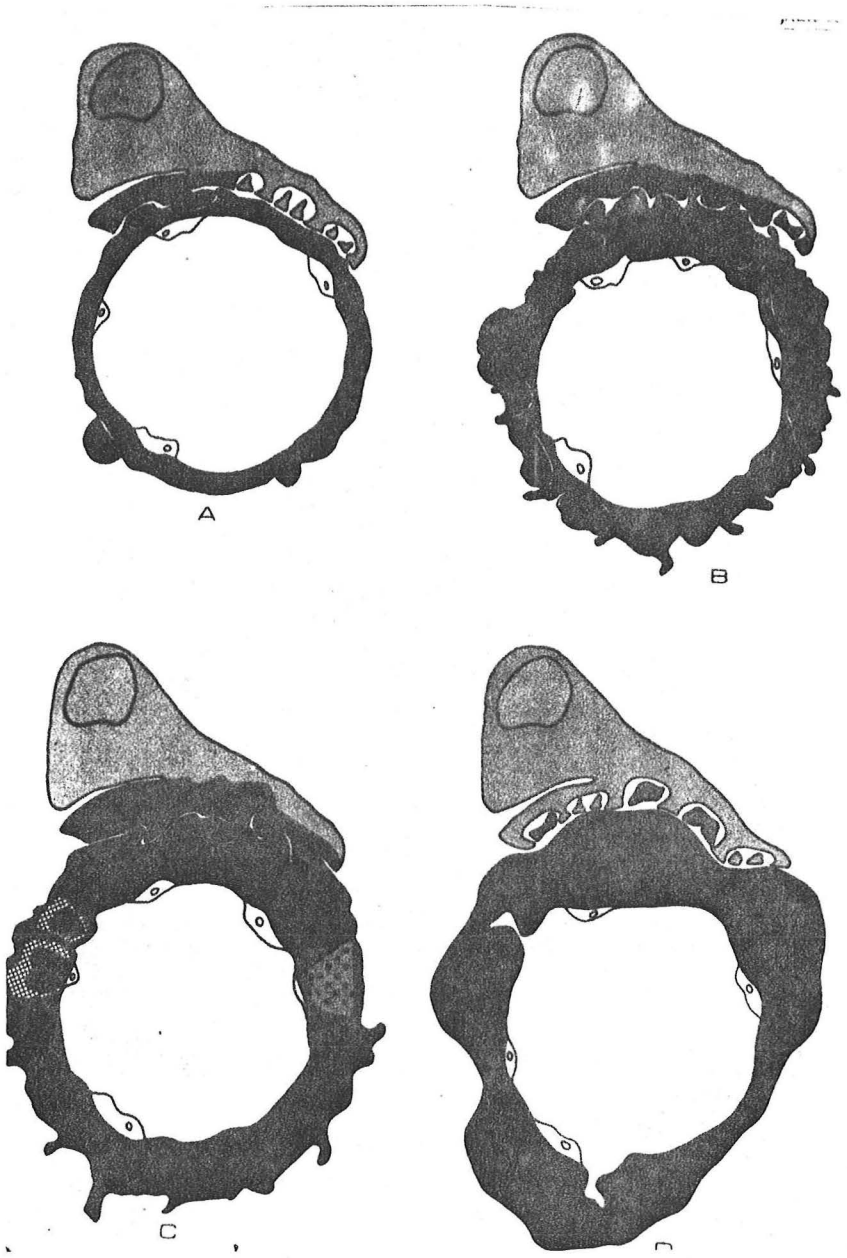
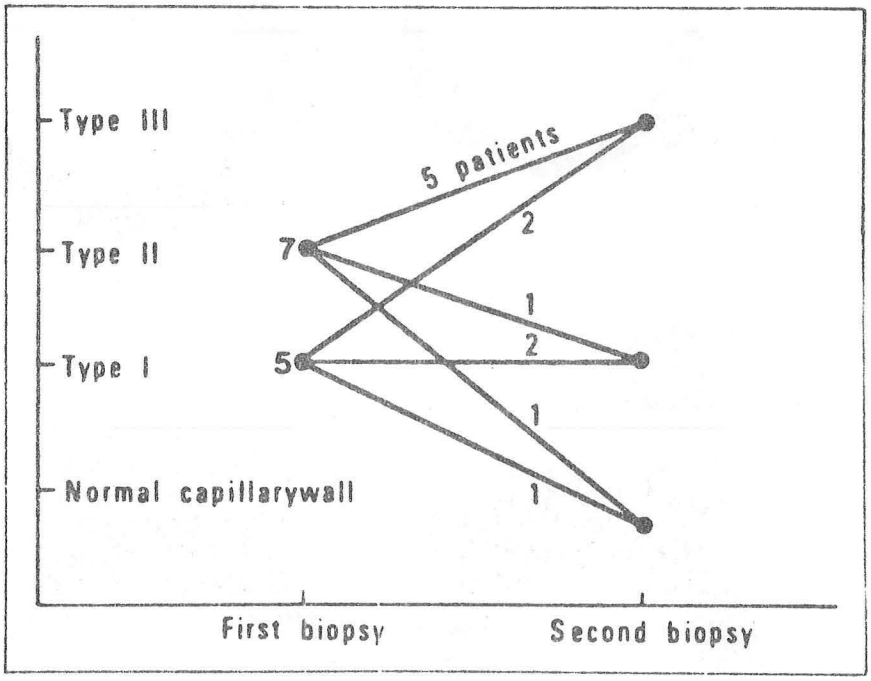


Figure 4B Four pathologic stages of MGN based on placement and amount of electron dense deposit. Stage I is pattern A, Stage II = B, Stage III = C, Stage IV = D.

Little hard evidence exists to support the view that any given patient actually progresses through the four stages of Ehrenreich and Churg. On the other hand, two pieces of softer evidence have supported the utility of this pathologic classification. Firstly, Noel and his associates applied a similar classification to 116 untreated patients with idiopathic membranous glomerulopathy studied in Paris. The time

between diagnosis and biopsy was considerably shorter in the patients found to have type I lesions with more frequent spontaneous improvements or complete remission in what is felt to be a more benign picture of the disease pathologically. End stage renal failure was never encountered in this "mild, early" group but accrued with equal frequency in the purportedly more severe morphologic types. Secondly, twelve cases have had two biopsies.



Noel, et al. Am. J. Med., 1979

Figure 5 The pathologic fate of twelve second biopsies in a group of patients with MGN left untreated.

Of these twelve, two biopsies appeared normal in patients now in remission whose first biopsy had mild lesions. 10/12 biopsies remained membranous with progression to the pattern of more serious involvement in seven. No repeat biopsy could be assigned to a separable variety of glomerulonephritis on morphologic grounds. In seven repeat biopsies performed during clinical remission there was noted regression of clearly defined deposit material from the previous biopsy and a movement to a type IV pathologic classification with thickened membranes and no observable deposit material. This finding supported the view that pathologic type IV membranous GN represented a reparative phase of the illness.

Immunofluorescent studies are of interest primarily for what they may reveal of the pathogenesis of idiopathic MGN.

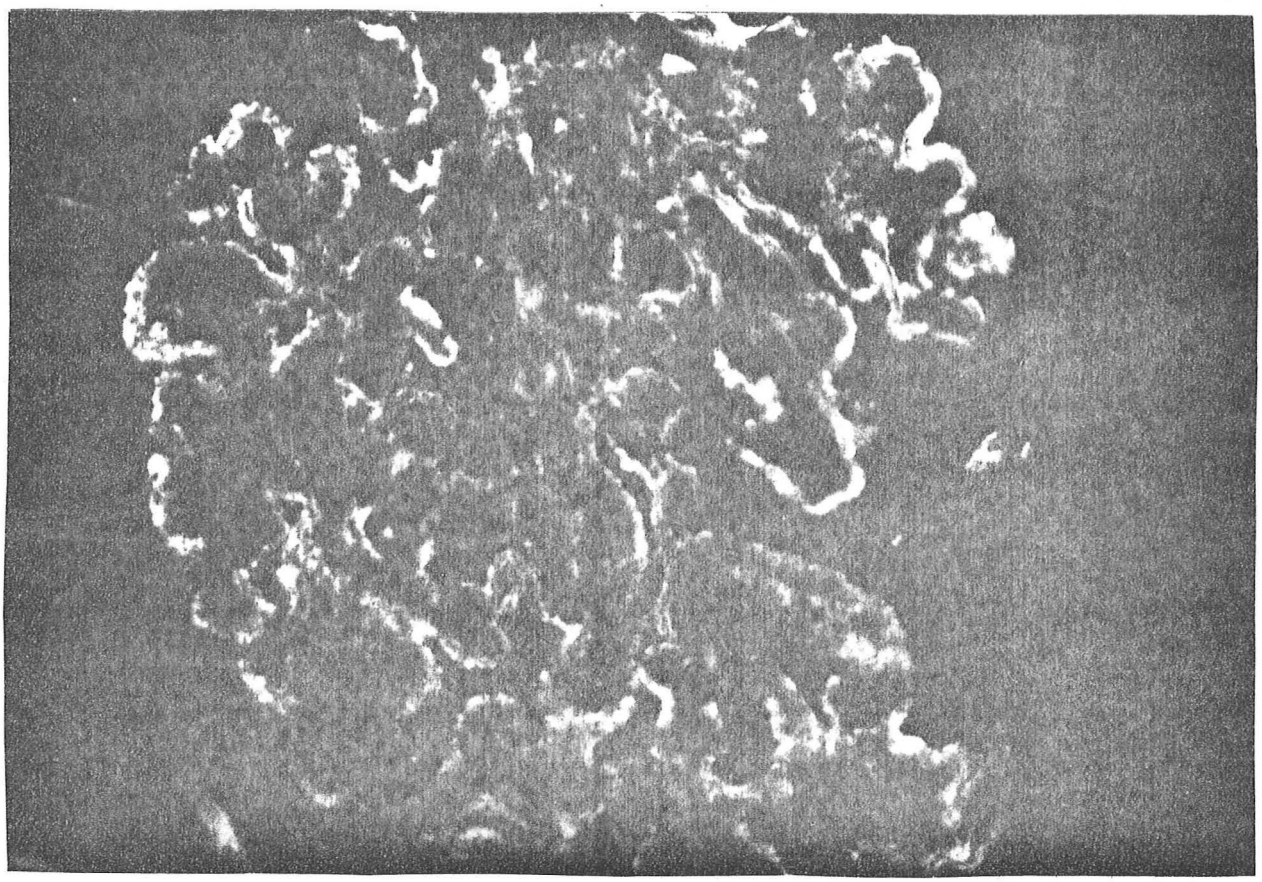


Figure 6 Granular staining of fluorescent antibody indentifying IgG deposted in GBM of renal biopsy from a patient with MGN.

Classically there is a diffuse, granular staining of deposit material by fluorescently labeled antisera to IgG. The third component of complement can be found in the deposit material as well, although less often. This "lumpy-bumpy" fluorescent staining pattern for immunoglobulin and complement has been the primary evidence for assigning an immunologically mediated mechanism to the pathogenesis of idiopathic MGN and consigning, probably incorrectly as we will see later, a similar immune complex-mediated mechanism to MGN felt to be at play for experimental models of glomerulonephritis worked out by Dixon and colleagues.

Medical disorders defined purely by pathological alterations discerned morphologically can stand as unique, clinically relevant diseases only if the pathologic description is reasonably unique to a separable and clinically definable group of patients and if such pathologic description is reasonably stable, bearing some relationship to the natural history of a given group of patients. That is to say, the pathologic description of tissue must "breed true" and carry prognostic import. The classic monographs of Pollak and associates, Rosen, Noel, et al, and Forland and Spargo elevate pathologically defined membranous glomerulopathy to such a unique clinical entity. Serial renal biopsies, as discussed above, indicate a picture which is morphologically stable

or progressive within the ambit of membranous changes depicted by Erhenreich and Churg. If care is taken to rule out systemic diseases which are associated with secondary renal involvement which pathologically may have membranous features (listed in Table I), Rosen has found that "no other renal disease evolves into it, nor does it become transformed into another type of glomerulonephritis".

TABLE I

SYSTEMIC DISEASE AND MEMBRANOUS GN

1. SLE
2. Diabetes Mellitus
3. Hepatitis
4. Penicillamine Nephropathy
5. Cancer
6. Hg SS

Pollak has shown that the degree of basement membrane thickening observed morphologically is associated with duration of clinical illness and that the more severely involved biopsy material presages the more dismal clinical outcomes. Thus a distinct clinical entity can be defined by pathologic description of tissue from a group of patients with a common clinical picture--the pathology is stable and "breeds true". This review will return to a more detailed description of the natural history of the idiopathic membranous patient in context of an analysis of the findings concerning potential successful therapeutic intervention but will now analyze the clinical features of this disease.

The Clinical Features Of Idiopathic Membranous Glomerulopathy

Just as the disease called idiopathic membranous glomerulopathy has a distinct morbid anatomy, so is the clinical presentation equally distinct. The clinical features which characterize membranous glomerulopathy are summarized in Table II.

TABLE II

CLINICAL FEATURES OF MEMBRANOUS GN

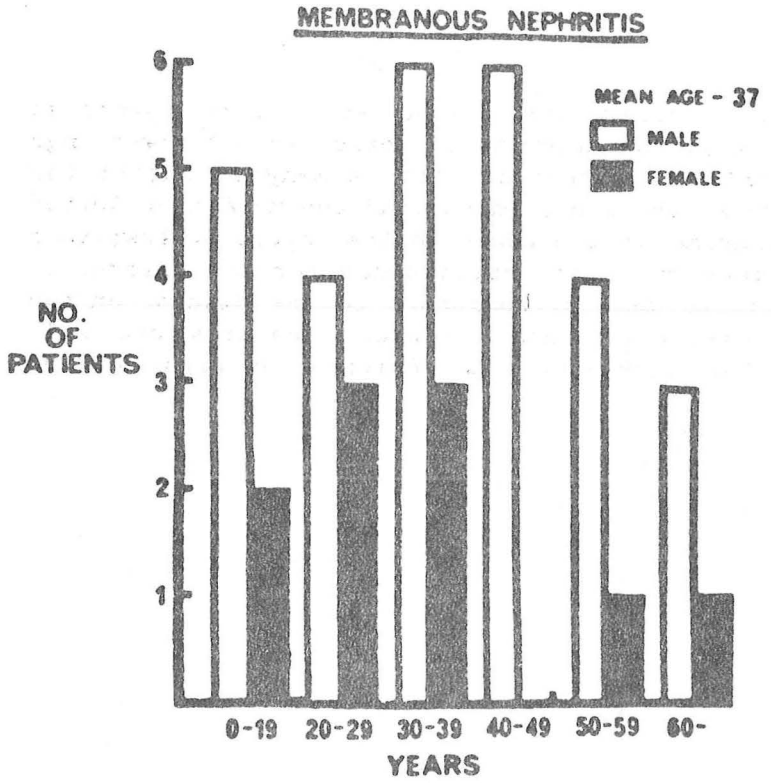
1. Proteinuria - all
2. Nephrotic syndrome - >80%
3. Peak age 3rd to 5th decade
4. Most common cause of nephrotic syndrome in the adult - 50%
5. Less common cause of nephrotic syndrome in children (10%) and more benign
6. Male to female ratio - 3:2

Although the disease occurs in children (the age of the youngest reported case being 2.5 years), the majority of series report a mean age in the third to fifth decade. MGN accounts for as many as 25% of the cases of nephrotic syndrome in the adult in England and more than 50% of the cases of nephrotic syndrome in the adult in the United States; thus in terms of incidence becomes an important disorder for the internist. There is a slight but definite male predominence to the disease on the order of 3:2. Several aspects of these clinical features are well described in reviews in the literature as depicted in Figures 7-9.

	Nephrotic syndrome	Non-nephrotic proteinuria
Number of patients	41	7
Males	31	5
Females	10	2
Age, yr		
Males		
Range	18-70	20-47
Mean	49.7	35.6
Females		
Range	26-68	44-62
Mean	47	53
Follow-up, mo		
Range	7-222	24-164
Mean	55	105

Erwin et al. Mayo Clinic Proc., 1973

Figure 7 Clinical description of 48 patients with MGN collected at The Mayo Clinic.



Laver & Kincaid-Smith, 1972

Figure 8 The male predominance and age of onset in Australia.

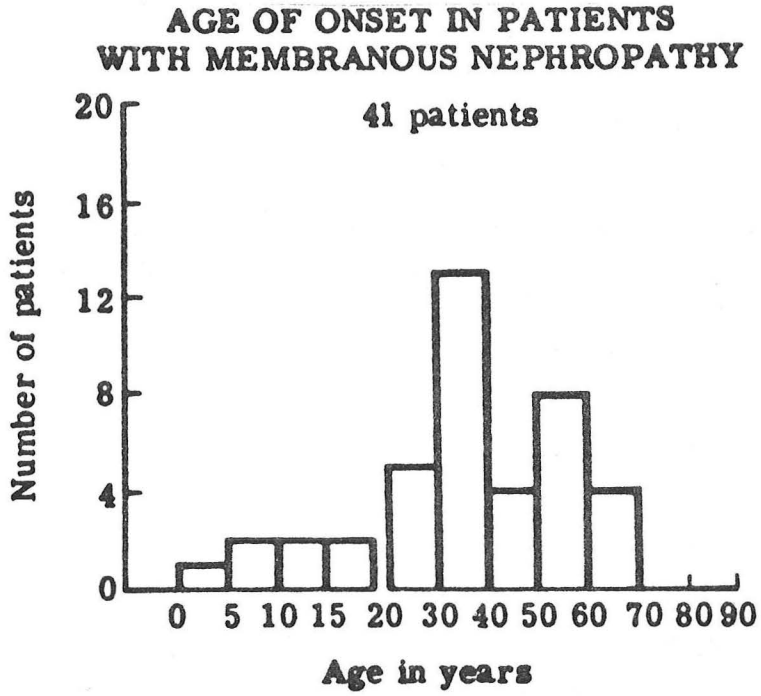


Figure 9: The peak age of incidence of MGN in England demonstrating that MGN appears to be a similar disease in at least three continents.

The hallmark of the disease is the insidious development of proteinuria discovered in most cases by clinical appearance of edema without recognizable antecedence. These patients, in contrast to others with varying forms of glomerular lesions, cannot date their illness to a viral or bacterial infection or to an episode of gross hematuria. In all patients there is a non-selective excretion of protein into the urine which may be of massive proportion. In as many as 80 to 85% of the cases of membranous GN, the patient fulfills the criteria for the diagnosis of the nephrotic syndrome. The nephrotic syndrome, as classically defined, consists of a triad as shown in Figure 10: 1) albuminuria; 2) hypoalbuminemia; 3) edema (Figure 10).

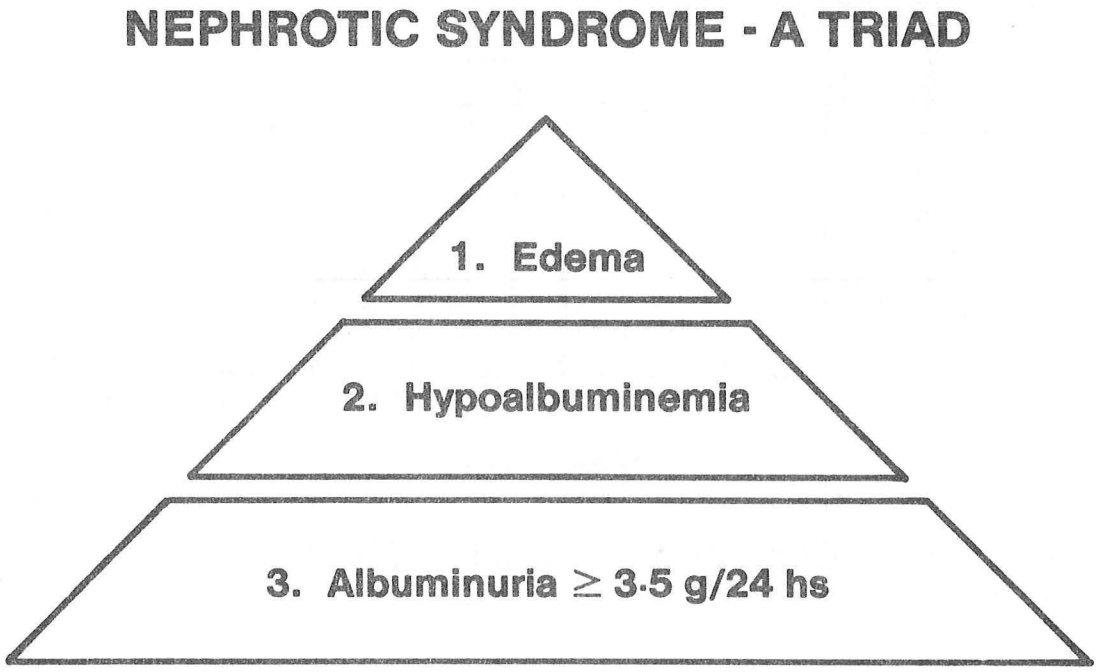


Figure 10 The pyramidal view of the diagnostic triad which constitutes the nephrotic syndrome.

I like to think of the nephrotic syndrome as a pyramid. The primary defect involves the failure of the glomerular filtration barrier to impede the passage of albumin into the urine. Albuminuria is thus the base of the metaphorical pyramid upon which the other features of the nephrotic syndrome will be built. Excess urinary albumin leads in part to hypoalbuminemia which contributes to clinical edema. The formation of hypoalbuminemia is a result of several derangements as shown in Figure 11.

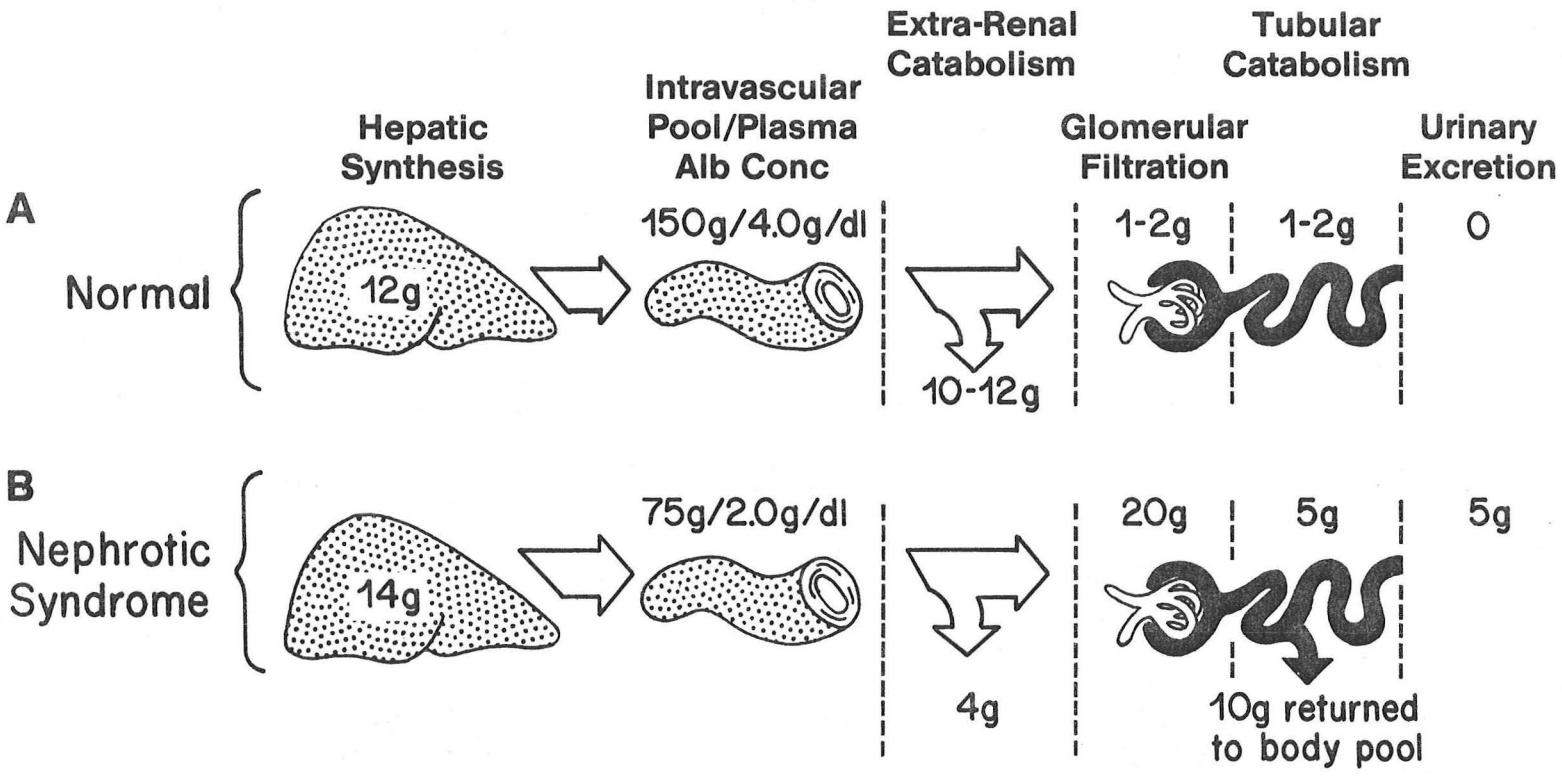


Figure 11 An artist's conception of the creation of hypoalbuminemia in nephrotic syndrome.

Firstly, obviously, there is albumin spillage into the final urine. It can be shown, however, that urinary loss of albumin cannot itself produce low plasma concentrations of albumin as the liver capacity to enhance protein synthesis can make up for most of the loss of protein into the urine. In pure nephrotic syndrome, hepatic albumin synthesis is indeed increased, albeit less than one might have expected for reasons as yet unclear. If urinary losses are inadequate to explain the observed plasma defect in albumin concentration, a second defect in albumin homeostasis must be postulated. Calculations from rat models and actual human clearance studies have demonstrated substantial filtration of albumin into the tubular fluid at least 20-fold above normal.

Indeed it can be shown that 10 to 60 gm of albumin may appear in Bowman's space. It is reasonable to assume that renal catabolism of this albumin appearing in gross excess in the filtrate is the second defect in albumin balance which generates hypoalbuminemia. Several pieces of evidence support this view. Strober and Waldmann in their review show that the proximal tubule has the capacity to catabolize several proteins including albumin while Maunsback morphologically showed proximal tubule uptake of albumin material. Interestingly, nephrectomy in experimentally induced nephrotic syndrome can restore the serum albumin level. The majority of recent studies in this field support the classic view of the importance of the renal tubule in the establishment and maintenance of hypoalbuminemia in nephrotic syndrome.

The abnormal loss of albumin into the urine generates the increased urinary protein loss and tubular catabolism which produces the next level of support in the pyramid of nephrotic syndrome. The diminished serum albumin sets in motion a series of adjustments in the forces that control plasma volume culminating in the third feature of nephrotic syndrome, edema as depicted in Figure 12.

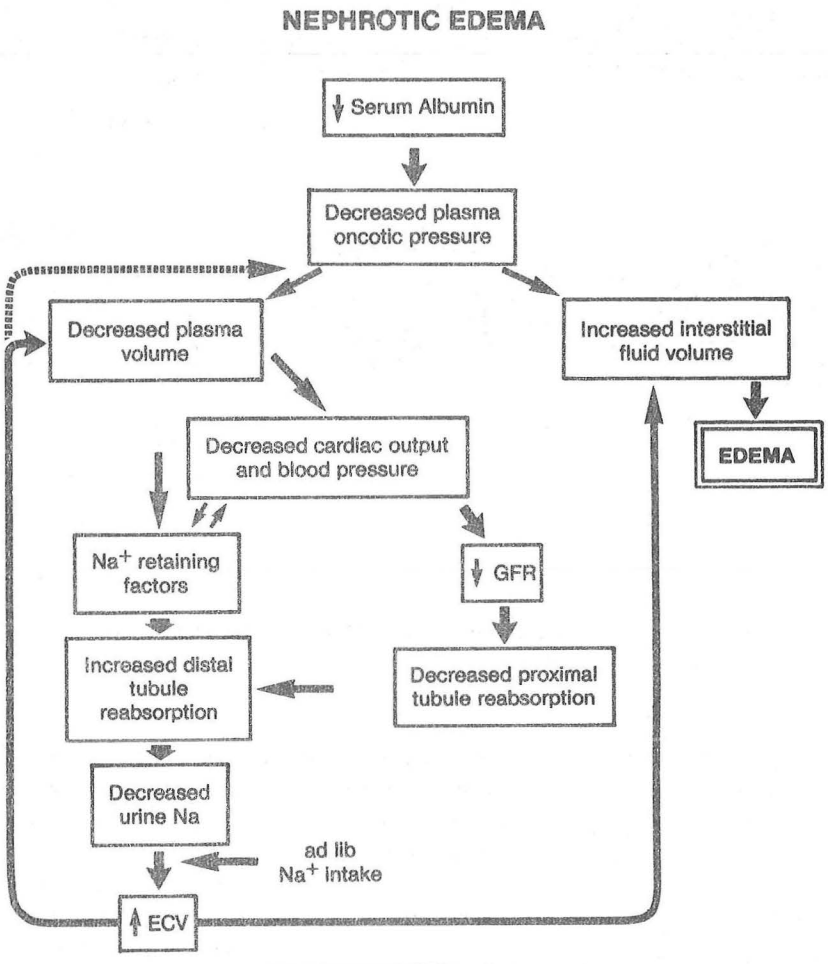


Figure 12 The mechanism of edema foramtion in nephrotic syndrome.

Diminution of the serum albumin reduces the plasma oncotic pressure altering Starling forces to favor volume shifts to the interstitial fluid compartment and edema formation while reducing intravascular volume. Several salt retaining factors operative presumptively at the distal renal tubule are stimulated directly by the contracted plasma volume and indirectly by reduction in cardiac output and blood pressure. The resultant fall in glomerular filtration rate reduces the filtered load of salt which in the face of a reduction in peritubular protein concentration and diminished filtration fraction leads to increased delivery of salt out of the proximal tubule to the more distal sites of salt transport which have been maximally stimulated by the contracted volume. The net result is a decrease in urinary sodium excretion and a retention of sodium and water. If a patient has free access to sodium, the increased sodium retention expands the extracellular volume which attempts to overcome the decreased plasma volume and may actually further reduce the plasma oncotic pressure by dilution enhancing the propensity for edema formation. The net result is that the added volume from avid renal salt retention is delivered into the edema fluid.

The genesis of major clinical difficulty for the patient with MGN, then is the "leaking" glomerulus which no longer excludes protein from the urinary filtrate. The normal glomerular capillary wall excludes albumin by virtue of intrinsic sieving properties based on its physico-chemical construction. The exquisite ultrastructural analyses using specifically charged probes initiated by Merrill and Farquhar and extended by Venkatachalam, Reinnecke and Cotran have pointed to the importance morphologically of the epithelial slit pore as the protein filtration barrier.

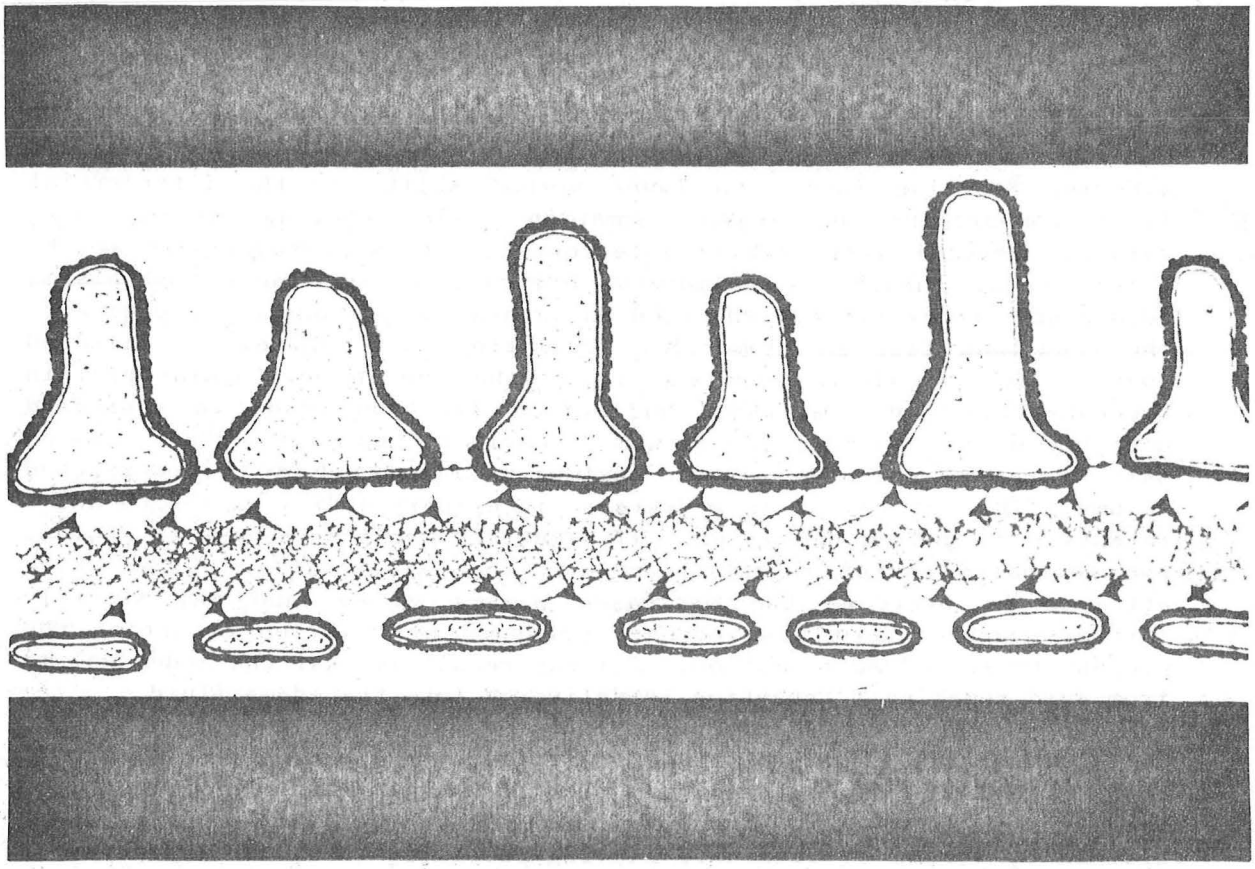


Figure 13 Artist's view of the GBM with epithelial slit pore and coat of sialoprotein on the hadocyte highlighted.

The best model of the capillary membrane is one of a model with fixed pores of specific size which bear a net negative charge felt to the be the result, in part, of sialic acid residues and discrete proteins called glycosoaminoglycans or GAGS. This model predicts that the membrane will exclude particles by size and charge as delineated in Table III.

TABLE III

SIEVING PROPERTIES OF THE GLOMERULAR FILTRATION BARRIER

1. Molecular size selectivity
2. Charge selectivity
3. Influence of Starling's hemodynamic forces

That hemodynamic forces which include renal blood flow ($\dot{Q}$) and the hydraulic pressure gradient across the capillary bed should also have important effects on protein movements can be mathematically predicted and experimentally demonstrated. The physiologic explanation of this latter complex matter is beyond the scope of this review. Predictions concerning the importance of molecular size and charge have also been experimentally confirmed by the elegant studies of Deen, Brenner and their associates using measurement of carefully characterized index molecules of known size and charge in plasma and in Bowman's space by glomerular micropuncture. Figure 14 is a stylized example of the results of this form of experiment from a review of one of these studies.

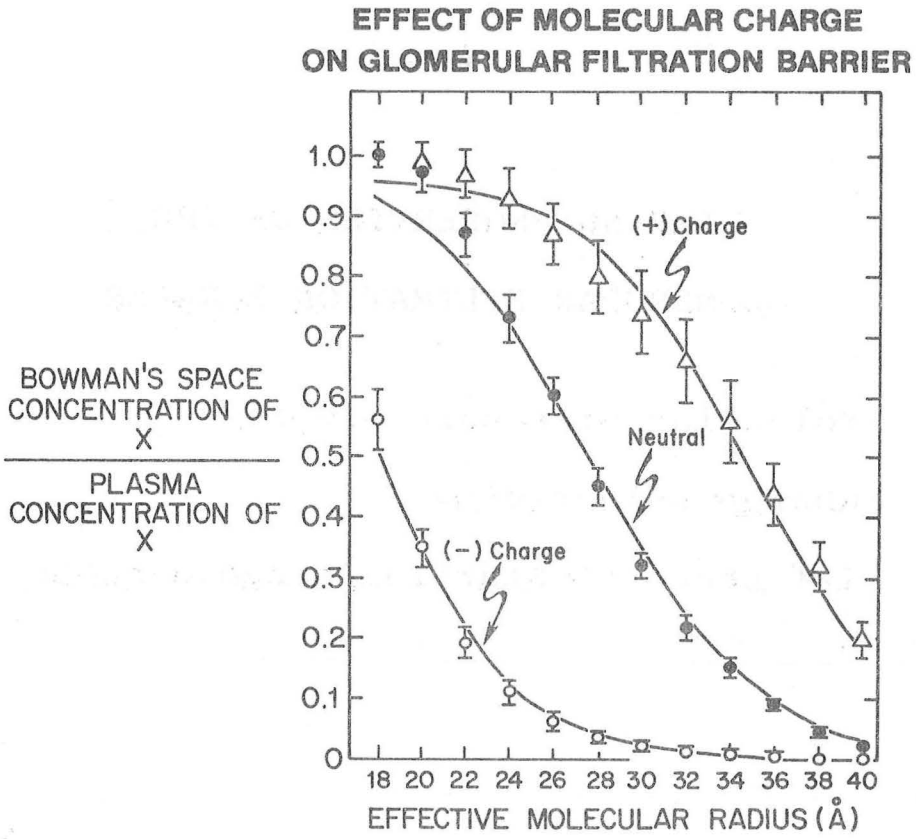


Figure 14 Relation of charge and molecular size to glomerular filtration of well characterized probes.

Here fractional excretion of a given solute, X, is evaluated as a function of size and charge. Clearance is graphed on the ordinate with the scale in terms of the ratio of the concentration of X in Bowman's space to the concentration of X in the plasma. If a molecule freely moves across the glomerulus, the concentration in Bowman's space at equilibrium will be equal to that in plasma, giving a ratio of 1.0. Complete exclusion of X by the membrane means that the Bowman space concentration will be zero as will be the concentration ratio. To examine the effect of size, one should concentrate on the series of ratios measured after provision of neutral molecular probes of given size to the experimental animal. Note that below a Stokes-Einstein radius of 26 Å one achieves a ratio very close to 1.0. This pore size is the smallest hypothetical pore size envisioned for the model membrane. As depicted by the model, charge considerations are equally important as size with greater exclusion for any given size for more negatively charged molecular species lending credence to the crucial role of the negative charge of the membrane itself in determining filtration characteristics. Confirmatory of the role of the membrane negative charge is the important opposite effect on the movement of the positively charged molecular species as shown in the figure.

The fate of the glomerular capillary basement membrane has recently been examined in patients with altered glomerular anatomy, including those with various glomerulopathies. Twenty-eight patients with glomerulonephritis were divided into two groups by Myers and colleagues by the nature of the selectivity of the proteinuria observed clinically. In group I (n = 13) the glomerulus permitted the passage of exclusively negatively charged albumin (isoelectric point around 5.2) with an effective molecular radius of 36 Å, while it passed a wide range of proteins including the neutral "large" (~ 55 Å) molecule IgG in group II (n=15). Electrofocusing of urinary protein from group I patients demonstrated the presence only of small negatively charged materials, while large molecules of all net charges were found in the urine of group II. These data permitted a model of pathologic alteration in the basement membrane produced by various forms of glomerulonephritis, as shown in Figures 15A, 15B and 15C.

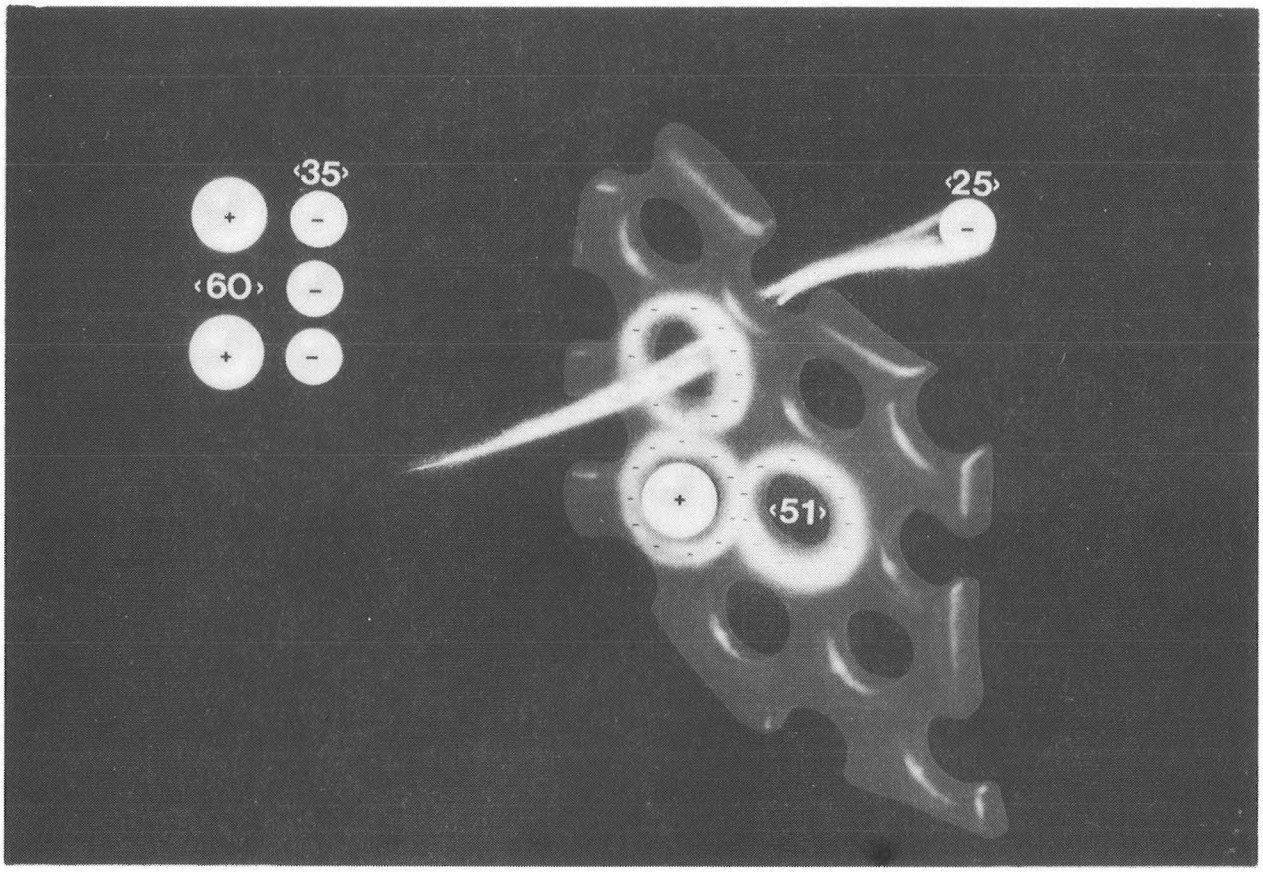


Figure 15A Diagram of a model of the glomerular basement membrane emphasizing the importance of pore size and net negative charge.

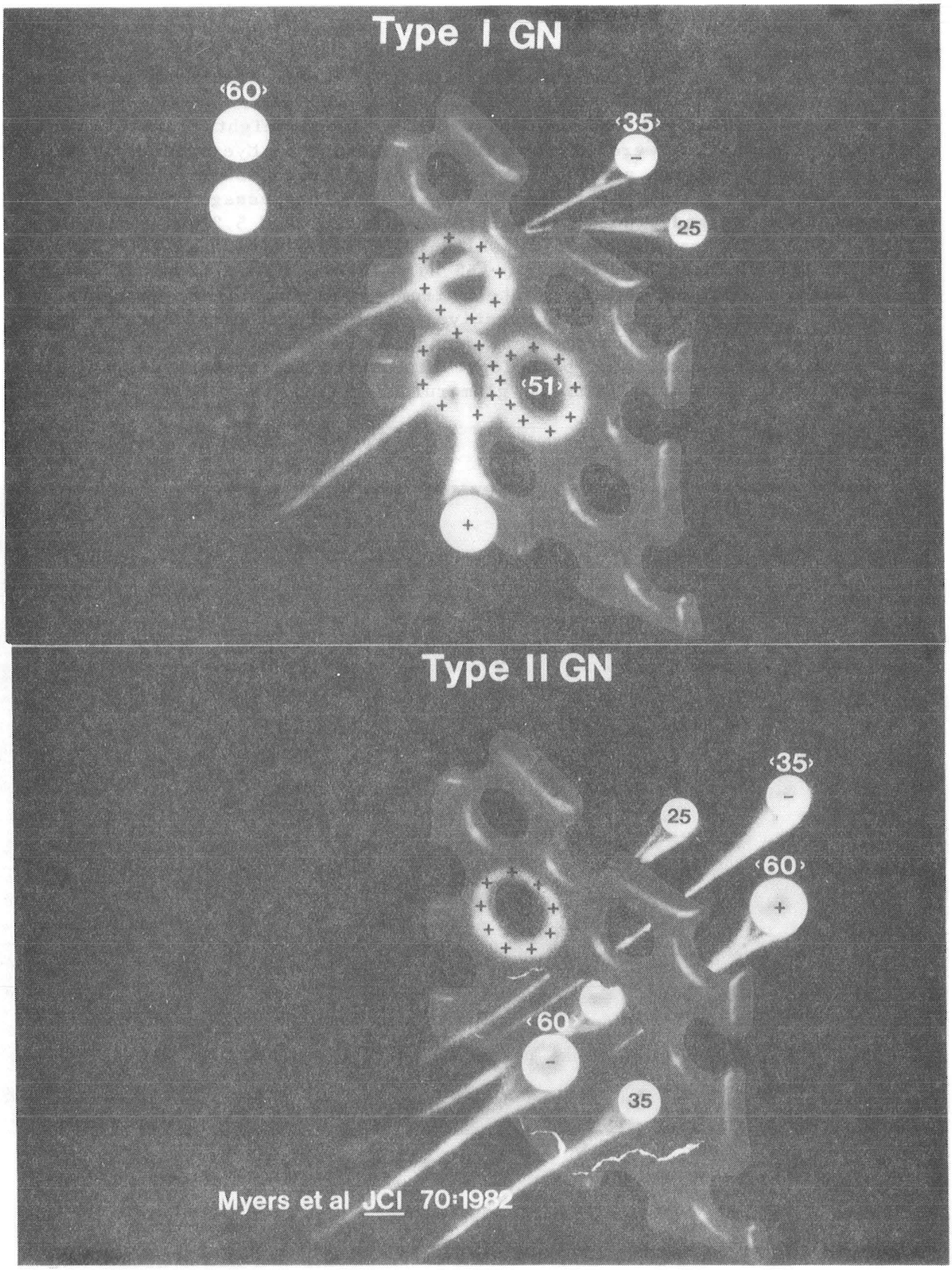


Figure 15B & 15C Model of the derangements in the glomerular basement membrane in patients with glomerulonephritis from the observations of Myers et al. In 15B the defect in patients with selective proteinuria is identified as loss of net negative charge. In 15C the defect in patients with non-selective proteinuria is identified as destruction in the failure of the GBM leading to enlarged pores.

In those forms of glomerulonephritis characterized by selective proteinuria pore size remained undisturbed but the net negative charge conferred on the pore by the several structural proteins had been lost. Negatively charged proteins which can have passed through the pore by virtue of its size but which had been excluded from Bowman's space by the like-charge propulsion are no longer excluded and appear in the filtrate. Proteins, regardless of charge, of large size greater than the pore itself remained excluded. In other forms of glomerulonephritis, of which idiopathic membranous GN is an example, pore size is greatly increased, up to 97 Å or almost two-fold more than the small pore of the basement membrane, almost as if the pathologic process had "ripped" holes in the membrane thus permitting indiscriminate wastage of plasma protein into the urinary filtrate. It is possible that the immune complex which presumptively is the pathogenetic force in membranous glomerulopathy is the culprit in producing these abnormally large pores leading to excess protein excretion of a non-selective type, hypoalbuminemia, edema, the nephrotic syndrome.

The Natural History Of Idiopathic Membranous Glomerulopathy

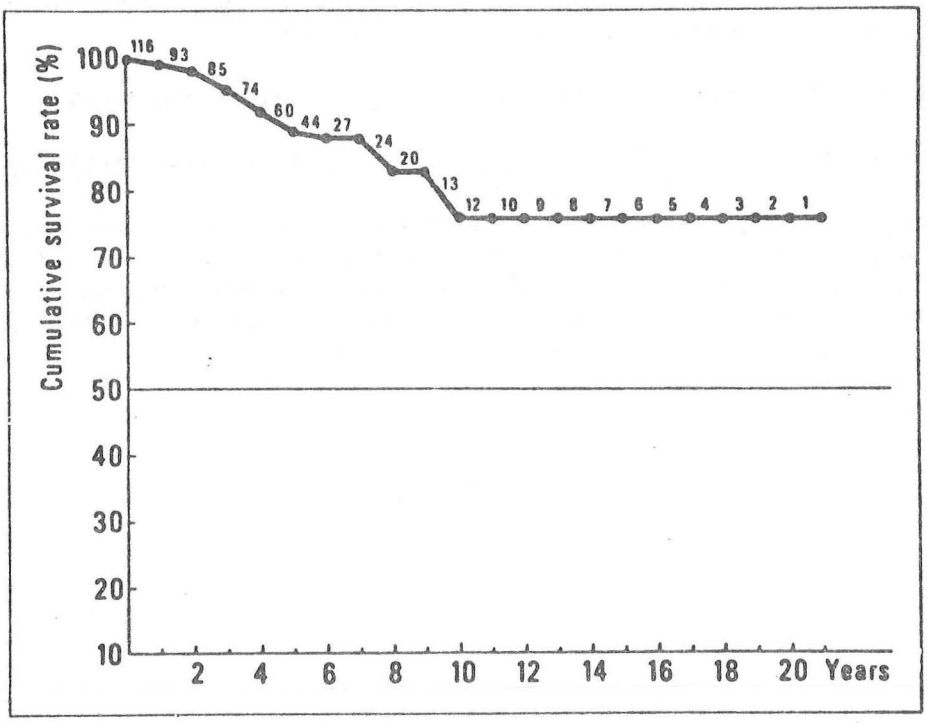
More than 10 separate studies have examined the natural history of patients with idiopathic MGN in the last 20 years. Examining these studies one is struck by the uniformity of the experience in this patient population supporting the concept that MGN is a distinct clinical disorder. This uniformity of clinical experience has been observed in spite of marked variance in therapeusis. The series are reviewed in Table IV from which several points are clear.

TABLE IV

NATURAL HISTORY OF PATIENTS WITH MGN

		n	Nephrotic Sx %	Remission %	Ultimate Renal Failure %
Erwin	1973	48	75	25	25
Laver & Kincaid-Smith	1979	38	80	29	--
Noel	1979	116	62	23	19
Forland & Spargo	1969	19	74	26	47
Baldwin	1973	38	62	29	16
Franklin	1973	32	72	22	44
Pierides	1977	37 Children	77	25	18
Habib	1979	50	48	52	10
Olbing	1973	9	33	33	33
Row	1975	72	56	40	4

Firstly, MGN is generally a benign disease. In children benignity is even more striking with 5 to 6% of the 131 children in three series developing renal failure. About 45% of children experience a complete remission as defined by normal renal excretory function and the absence of proteinuria. The disease is somewhat less benign in adults with an average of 28% of 328 reported cases from the series in the table developing renal failure while 25% of the patients entered a spontaneous remission. The study of Noel and associates is most instructive in that 116 untreated patients were followed for a mean of 4.5 years in Paris. 76% of the patients presented with fully developed nephrotic syndrome while 24% had lesser degrees of proteinuria, suggesting a slightly less severely affected group of patients at the beginning of the follow period. Clinical remission in this untreated group occurred in approximately 23% of the cases contrasted to 19% who developed renal insufficiency. The actuarial survival curve for this group demonstrated 76% of the patients alive at ten years.



Noel, et al. Am. J. Med., 1979

Figure 16 Actuarial survival curve of 116 patients with MGN left untreated.

The recent study of Davison and associates following 66 untreated patients for two to ten years further advanced the concept of a reasonably benign course for patients with membranous glomerulopathy untreated. Pollak's review of several series revealed the generally optimistic course of a natural history of MGN as shown in Figures 17 and 18.

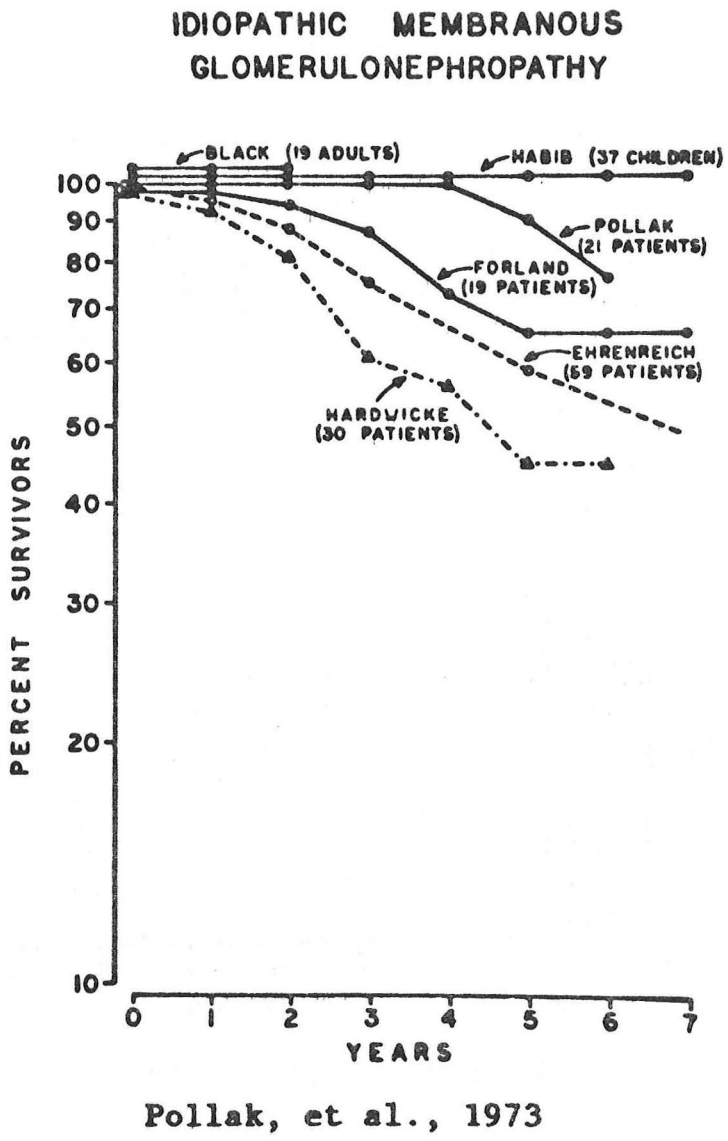


Figure 17 Patient survival in several groups of patients with MGN as reviewed by Pollak. The series of Hardwicke and Ehrenreich contain patients with MGN related to systemic lesions.

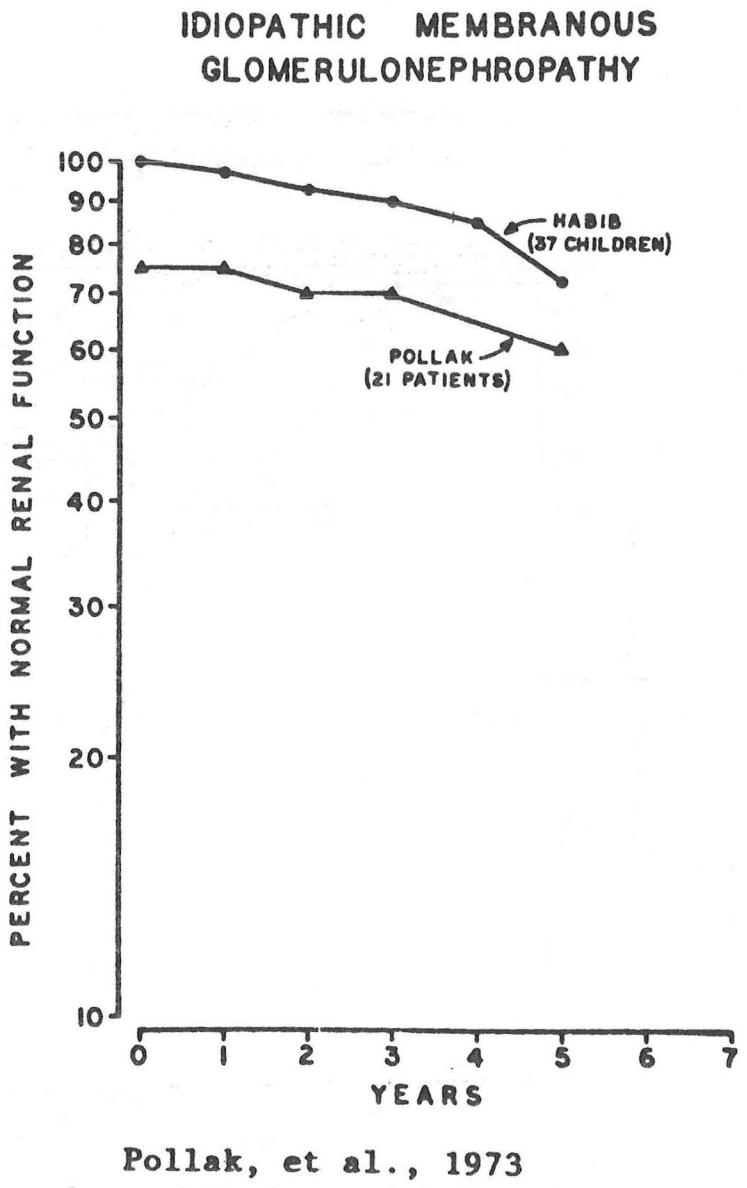


Figure 18 Natural history of renal excretory function in idiopathic MGN. Children have a more benign disease.

The data also raise several caveat. These data clearly demonstrate again how benign the disorder is in children as revealed by the analysis of the Habib Paris study. The less than sparkling results in earlier studies described in these data can be laid to the inclusion of patients with systemic diseases (the Erhenreich and Hardwicke series) and of patients with biopsies that contain glomerular proliferation leading to the supposition of misdiagnosis.

The pictorial synthesis adapted and redrawn from Cameron's data (Figure 19) reflects a general overview of the natural history of idiopathic MGN in the adult.

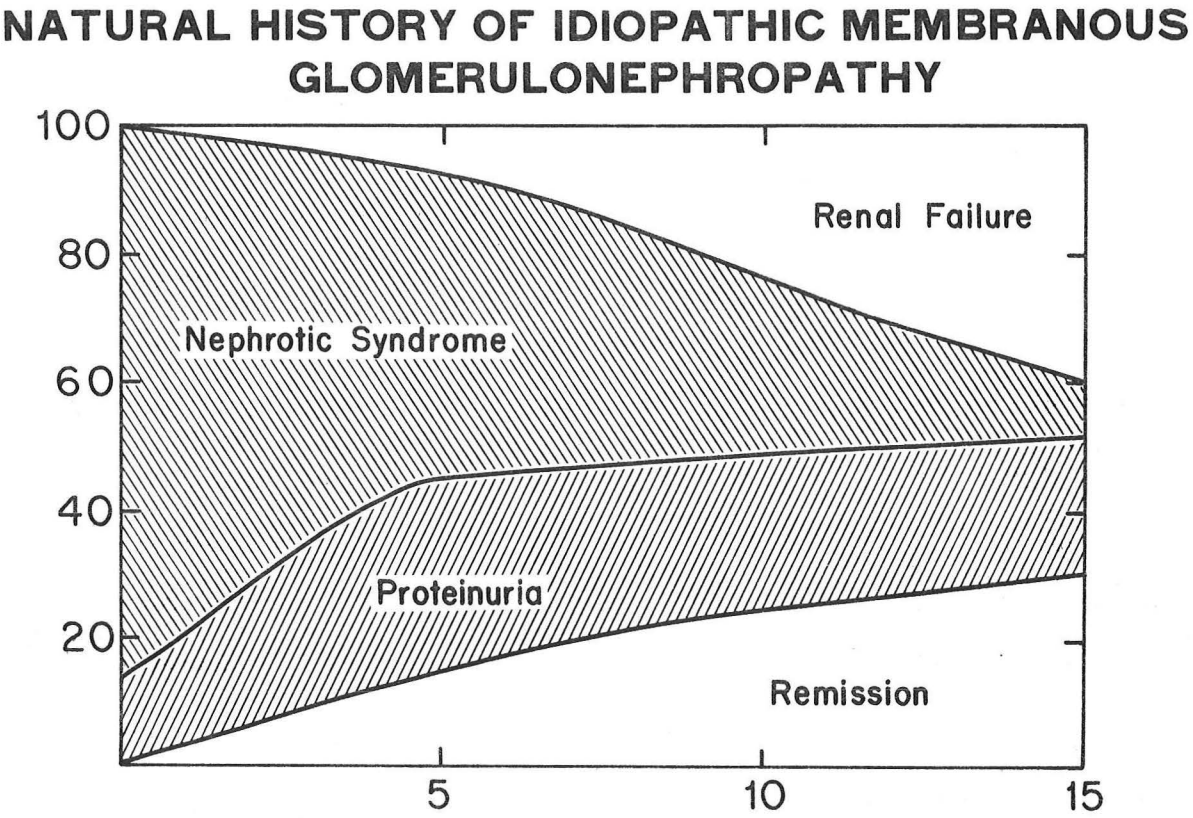


Figure 19: Natural history of MGN

I have taken the liberty of adjusting the figure based on my own review of the subject which includes all the series reviewed by Cameron and several newer ones available to me. Analysis of the figure provides an excellent summary of the disease with which we have been dealing. All patients presented with proteinuria. About 85% of the patients presented with the nephrotic syndrome. There is a finite rate of complete and spontaneous remission. Rates approach 15% in the first five years, become 25% by the later years and may be as high as 30% in those series with 15 years of follow up. Renal failure rate is slowly progressive for the first five years of the disease but may be expected in 40% of the cases after 15 to 20 years of the disorder. As renal failure ensues or patients enter remission, the amount of daily protein excretion in this group of patients is reduced while the renal function of patients with abnormal protein excretion below 3 gm is about the same.

The recent study of Davison and his colleagues of 66 untreated patients in England followed from two to ten years further increases our understanding of the natural history of MGN with respect to excretory function.

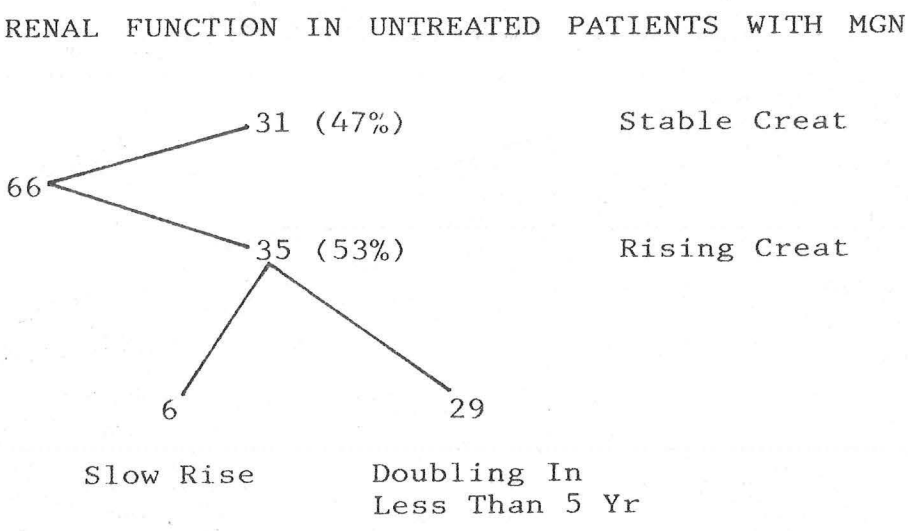


Figure 20

Thirty-one patients, 47%, maintained stable glomerular filtration rates throughout the observation period, while 35% demonstrated steadily declining function. In the latter group, plasma creatinine doubled within five years in 29 or 44% and 12 patients (18%) exhibited a rapid rise in serum creatinine in less than two years despite entering the study with a normal creatinine. There appeared in this group to be a clear separation between groups with "stable" and "progressive" disease.

From the studies reviewed an understanding of a set of prognostic indicators in MGN may be generated as listed in Table V.

TABLE V

PROGNOSTIC INDICATIONS IN MGN

Better Prognosis	Worse Prognosis
Minimal Thickening of the Basement Membrane	Severely Thickened Basement Membrane
Children and Women	Men
Younger Patients	Older Patients
Non Nephrotic	Nephrotic Syndrome
No Superimposed Complications	Superimposed Complications
	---Renal Vein Thrombosis
	---Interstitial Nephritis

First, the pathologic examination of the tissue permits an accurate prediction of that group of patients likely to ultimately wend their way to renal failure. As previously discussed, there is a good correlation between the degree of thickening of the capillary loop, the bulk of deposit material found in the capillary loop, and the clinical outcome. Secondly, the disease is decidedly more benign in children and somewhat less rapid in women than men. If patient mortality is the end point of analysis, then older patients do less well, perhaps related to the volume stresses that nephrotic syndrome imposes on a compromised cardiovascular system present in many older individuals. The degree of pathologic protein excretion predicts outcome based not only on the distinction between nephrotic and non-nephrotic range of proteinuria, but also quantitatively within these two groups. Lastly, the superimposition of complications related to MGN hastens renal failure. Patients with MGN are susceptible to the development of intravascular coagulation especially of the renal veins, a subject that deserves extensive review in its own right elsewhere. Renal vein thrombosis alters the clinical picture in general but in particular enlivens the tempo of the movement to renal failure. Most patients with extensive proteinuria and edema will be treated symptomatically with diuretics so that a small but real incidence of diuretic-related allergic interstitial nephritis has been encountered in patients with MGN. Such a second disease also alters the generally benign tempo of the disorder reflected by a more rapid movement into renal failure.

In summary, idiopathic membranous glomerulopathy is a disease with an identifiable pathologic picture which remains relatively stable. It has a definitive clinical presentation and natural history. A clear understanding of the morphologic derangemnt and clinical presentation of any given patient permits a fairly accurate prognosis to be discerned. One can easily support the view that MGN is a unique disorder in man with patients readily recognizable using clinical acumen and appropriate

morphologic classification. If care is taken to delete the patients with the systemic disorders associated with the pathologic pattern of membranous GN, one can evaluate a relatively homogeneous group of patients for whom a logical and effective therapeutic approach may be constructed.

Therapy

It is in the area of a definitive therapeutic approach to patients with idiopathic MGN that important new information has developed in the last four years which has generated a brisk controversy in the medical literature. This controversy has grown out of the findings of an inter-hospital collaborative study of the controlled use of prednisone in patients with MGN in the United States. Prior to this controlled collaborative study, it has been generally acknowledged that immuno-suppressive therapy of any variety offered little advantage in the treatment of adult patients with membranous GN. The initial feeling with respect to therapy in MGN obtains from an examination of the natural history of the disease as discussed above. In the group of studies reviewed in the previous section (review Table IV), therapy in patients ranged from nothing to various combinations of immunosuppressives. What impressed most reviewers in this field was the uniformity of natural history despite a widely variant therapeutic approach. Indeed, using mortality as the end point, one could see that total patient survival was significantly greater in a group of untreated patients as compared to two previous series in which all patients reported were treated with steroids. The general applicability of the patient experience in this untreated group as reported by Noel and Habib from the Hôpital Necker in Paris will be discussed later. The findings in occasional uncontrolled series that purported to show that prednisone offered some benefit to patients with membranous glomerulopathy has been discussed by most clinicians based on the data that clearly demonstrated in large groups of patients followed for many years that improvement on drug was no greater than that offered by the spontaneous remission in untreated patients. Most important for the creation of the view that membranous glomerulopathy had no definitive response to immunosuppres-sive therapy was the result of a controlled, multi-center prospective study of steroid treatment of the nephrotic syndrome conducted by the Medical Research Council of Britain. There appeared to be no statistic-ally significant differences between a control, untreated group of patients or the steroid treated group with respect to protein excretion rate, patient survival, or maintenance of renal excretory function. Although it is possible to re-analyze this study and demonstrate that the steroid treated groups tended to have lower serum creatinines and protein excretions, most readers of the study concluded that steroids were not effective in treating membranous GN nor was the risk of therapy warranted for whatever small benefit might accrue. Thus, a nihilistic approach to the therapy of membranous glomerulopathy has been the rule. The MRC study also explored the utility of steroid treatment in other forms of nephrotic syndrome pointing out how very responsive patients with nil change disease were to such treatment. This stark difference in steroid responsiveness between patients separable almost uniquely by renal morphology was taken as strong support for the importance of the

renal biopsy in patients with the nephrotic syndrome. Such a biopsy could discern the steroid sensitive group with nil disease from the non-responsive group with membranous GN permitting precise therapy for the one and preventing useless therapy in the other.

This approach of benign therapeutic neglect for patients with MGN no longer can be cavalierly sustained in the face of the new data obtained by the collaborative study in the United States. These new data have opened an important controversy which is presently raging in nephrologic circles and therefore warrants careful review. Originally conceived as an inter-hospital study group comprised of 18 separate institutions to examine the nature of and treatment for idiopathic nephrotic syndrome, the collaborative study concentrated, for internal reasons, on the treatment of adult patients with idiopathic membranous nephropathy. Seventy-two adult patients between the ages of 16 and 65 met the stringent entrance criteria (summarized in Table VI) for evaluation by the study group.

TABLE VI

U.S. COLLABORATIVE STUDY

ENTRANCE CRITERIA

1. Referral from participating center
2. Age 16 to 65
3. > 3.5 g protein/day per 1.73 mm^2 (2 measurements)
4. Absence of associated systemic disease - SLE, PSGN, amyloid, HgSS, hepatitis, drug usage
5. Serum creatinine < 2.0 mg/dl, GFR 42 ml/min
6. Bx of membranous GN

Several critical features of the entrance criteria should be highlighted. The group made major efforts to eliminate patients with systemic disorders which might be associated with membranous changes in renal tissue. For example, special efforts were made, using serologic criteria, to exclude disseminated lupus erythematosus. All patients were administered a series of glucose tolerance tests with an abnormal performance by standard criteria causing the exclusion from analysis of a given patient. Amyloidosis was eliminated by absence of amyloid staining material in renal tissue available on all subjects. In contrast to this careful search for associated illnesses, the evaluation of the potential complication of renal vein thrombosis was poorly done. Patients with renal vein thrombosis were excluded if there were sug-

gestive signs, symptoms, or if special histologic features of that disease were present. Renal vein angiography was not required for admission to the study. Only a few patients were actually studied in this manner at their respective centers. The problem of renal vein thrombosis in this group will be discussed below. Another important feature of the entrance criteria was the manner in which the pathologic diagnosis of membranous was made. All pathologic specimens were examined by a central pathology board in a coded fashion along with a series of biopsies from a wide range of disorders culminating in nephrotic syndrome. Light microscopy, electron microscopy, and immunofluorescence was available on 62 of the 72 patients included in the study. In ten of the subjects adequate tissue for analysis was available for only two of the three modalities. All biopsies had to have electron dense deposits which stained for immunoglobulin when immunofluorescence was available for inclusion in this study. Although, by definition, all patients had to have the nephrotic syndrome as defined by excretion of 3.5 gm of albumin in the urine or more per day, renal excretory function was excellent. Patients had to have a GFR of at least 42 ml/min or a serum creatinine below 2.0 mg/dl for entrance into the study at the time of randomization into a treatment or control group.

Characteristics of the 72 patients who met the entrance criteria are summarized in Table VII.

TABLE VII

CHARACTERISTICS OF PATIENTS IN COLLABORATIVE NEPHROTIC SYNDROME STUDY

1.	Sex Ratio	42 males 30 females	
2.	Age	16-30 yr 31-45 yr 46-65 yr	24 pt 24 pt 24 pt
3.	Mean Protein Excretion Rate		8.8 g/d
4.	Mean Serum Creatinine		1.0 mg/dl
5.	Entrance BP		130/83

The sex ratio was virtually identical to that expected in such a large series with males predominating 3:2. Similarly the bulk of the patients were found in the third to fifth decade of life. Not only were these patients all nephrotic, but protein excretion rates were relatively severe with a mean of 8.8 gm of albumin passed in a 24 hr urine specimen. By definition, patients had excellent renal excretory function; the mean of the group had a serum creatinine of 1.0 mg/dl at the beginning of randomization. Interestingly, blood pressure was normal in virtually every patient. The histologic stratification of the patients was also interesting. The bulk of the patients had Ehrenreich and Churg class II pathology, which means to say moderately severe subepithelial electron dense deposits imbedded in the basement membrane with "spike" of membrane material surrounding the deposit. Only 14 of the 72 subjects entered with quite mild deposit formation or class I. Thus, the bulk of the patients had moderately severe disease by pathologic criteria. Moreover, the requirment that all patients had to have the nephrotic syndrome potentially biased this study for poor outcomes on clinical grounds as reviewed in an earlier section.

TABLE VIII

OUTCOME VARIABLES IN

COLLABORATIVE NEPHROTIC SYNDROME STUDY

1. **Treatment failure - maintenance of nephrotic range proteinuria**
2. **Complete response - < 200 mg protein/24 hr**
3. **Partial response - 20/mg to 2 g protein/24 hr**
4. **Δ GFR during study**

The overall goal of the collaborative study was to ascertain whether short-term, every-other-day prednisone therapy could alter importantly the outcome of the patients with idiopathic MGN with two outcome variables analyzed: 1) the degree of proteinuria; 2) maintenance of renal excretory function over time (Table IX).

TABLE IX

TREATMENT PROTOCOL OF

COLLABORATIVE NEPHROTIC SYNDROME STUDY

1. Random assignment to "steroid" or "placebo" groups

2. Steroids - 100 to 150 mg/qod

3. Taper

   a. Non responder - 4 wk to no drug
   b. Responder - 8 wk to no drug

4. Relapse of responders could permit additional 4 wk course of the high dose therapy followed by taper

The study design did not permit a blind crossover approach to therapy although patients were randomized by computer by stratification of histologic diagnosis into the prednisone treatment group, 100-150 mg q.o.d. for eight weeks, or an untreated control group. The untreated control group received placebo tablets in an identical number to maintain an attempt at a blinded sample. After eight weeks of such therapy a taper schedule was employed. If a patient demonstrated complete or partial response, the taper period was somewhat more prolonged than for the non-responder. Responders were evaluated particularly with respect to the degree of proteinuria present during and at the completion of therapy with a complete response defined as a reduction in albumin excretion rates to 200 mg/day or less and a partial response being defined as a reduction in protein excretion to between 200 mg and 2 gm/day. If a responder had a relapse, a second eight week course of therapy was permitted.

Because steroid therapy had potential risks and toxicities, the study group built into the design ten "stop points" as listed in Table 10 which were recorded and utilized to delete patients from analysis. The important findings in the study deal with the renal excretory and proteinuric aspects of the stop points and will be discussed below. One of the interesting findings in this study was the lack of clinical toxicity observed in those patients receiving steroid therapy in this protocol. Only one patient of the 34 patients who received every-other-day steroids experienced any of the stop points listed in Table X, that being a mild GI bleed.

TABLE X

STOP POINTS IN COLLABORATIVE

NEPHROTIC SYNDROME STUDY

1. Persistent increase in serum creat to two-fold entrance value
2. Hypoproteinemia with hypotension
3. Severe anasarca reducing life to bed-chair existence
4. G.I. bleeding
5. Serious infections
6. Psychosis
7. Hypertension
8. Embolic or thrombotic phenomena
9. Severe steroid side effects
10. Proximal myopathy

One control patient was removed from the study after eight months of follow because of psychiatric disturbance. The physicians caring for the 34 prednisone treated patients were able to identify the correct therapy of their patient in eight instances after one month of therapy and 12 instances after 12 months of therapy and were equally incorrect in assigning the appropriate therapy to their control patients. One can conclude from this aspect of the study that the every-other-day steroid regimen was at least safe.

The other issue at hand, however, was whether such therapy was actually beneficial. Although steroid treated patients were more likely to experience at least one episode of partial or complete remission (22 of 34 patients treated), the final number of patients who were completely free of significant proteinuria at the completion of the two year observation period was absolutely not different than the patients who received no treatment at all. Indeed, that only four patients of the steroid group were in remission after two years of follow (12%) represents a different natural history for this group of patients than has been observed over time in treated or untreated groups in many different studies. I will return to this point later in this discussion. In contrast to this somewhat tenuous benefit of steroid treatment for the proteinuria of idiopathic MGN was the finding with respect to changes in GFR. Eleven of the 38 placebo patients achieved a creatinine stop point with nine either dead secondary to renal disease or in renal

failure. The renal failure rate in the two years of follow-up in this group was a substantial 26%. In contrast, only two patients in the steroid treated group reached a creatinine stop point with only one of 34 patients in renal failure. The differences between the groups regarding GFR was statistically significant at two years. The conclusions of the study group as described by Cecil H. Coggins as the spokesman are detailed in Table XI.

TABLE XI

CONCLUSION IN COLLABORATIVE

NEPHROTIC SYNDROME STUDY

1. Although steroids induce more frequent remissions, they have no lasting effect on reduction in proteinuria.

2. Steroids at 125 mg/qod for 8 weeks are very safe.

3. Significant reduction in renal function can be avoided or delayed by steroid therapy.

The starkly variant conclusion of the study group from the bulk of previous studies has engendered the significant controversy in nephrology eluded to earlier. Several features of this study have been severely criticized. Cameron points out that the natural history described by most series is that of a slowly progressive disorder such that short term trials of several years duration may show no effect or have an effect only on a unique subgroup of patients who would have experienced a more rapid course into renal failure than is typical of the larger group. Indeed, his group has generated information that such a subgroup of more severely affected patients with membranous exists. This subgroup may be the unique subgroup of patients amenable to steroid therapy.

Many have criticized the collaborative study by pointing out that, by chance, a group of patients more severely affected were studied with an increased likelihood of degenerating into of renal failure. All patients had to have nephrotic syndrome, most with morphologically more severe renal lesions. Although this argument is true, it cannot explain the differences between the treatment and non-treatment group in the collaborative study alone. There were no differences in morphology or clinical prognostic criteria between the treatment or placebo group. Moreover protein excretion rates were also equal between the two study groups.

A problem does arise, however, in interpretation of this study based on the possible skew of the data afforded by the possibility of a secondary complication. The care taken to control for the onset of renal vein thrombosis was inadequate. In fact steroids may actually reduce the propensity for clotting and RVT in the treated group perhaps accounting for its mechanism of action. Nor were patients with a rapid decline in renal function re-evaluated pathologically to assure the absence of allergic interstitital nephritis which may occur in nephrotic patients treated with diuretics. Although these points have been raised by many critics of the study, it seems unlikely to me to account for the difference in outcome for treated patients with brief courses of medicine and examined several years later. Statisticians, on the other hand, point out that a difference in outcome of only two patients from the treated or placebo groups moving into or out of the renal failure group would have made the observations from this study not statistically significant. Thus it is possible that missing two cases of renal vein thrombosis in the placebo group could have altered importantly the basic conclusion of the study, that prednisone is beneficial therapy for MGN.

My own reading of the literature and of the results of the collaborative study has generated a more optimistic recommendation of the therapy of idiopathic MGN in the United States than the previous nihilistic approach. With all the problems of a multi-center trial in terms of patient selection, entrance of patients, and enthusiasm of various investigator teams, the collaborative study just reviewed offers the only prospective and well planned set of observations concerning the treatment of this disease in the United States. Although problems may be detected in study design, no major methodologic difficulties would a priori obviate the study findings. One must always assign a risk to benefit ratio in interpreting such studies. The data supplied by the collaborative study suggests that there is little risk from using this dose of steroids in the every-other-day pattern. Because of the important potential for at least retarding the development of renal insufficiency in even a subgroup of patients with membranous GN with little risk, I recommend that patients be treated with a similar protocol as defined by the collaborative study group. Continued entrance of patients into the study with continued follow-up past five and ten years will allow a clearer view of the response of this disorder to therapy. At this point in time, the few complications of therapy do not permit, in my estimation, one to deny the potential benefit of the therapy for this category of patients.

Pathogenesis Of MGN

The immunopathology of idiopathic membranous glomerulopathy with the finding of electron dense deposits containing serum immunoglobulin and the complement has led to the assignment of the pathogenesis of this disorder to an immunologically mediated mechanism. The classic hypothesis that explains this disorder suggests that membranous glomerulopathy is mediated by the passive infiltration of the basement membrane of the glomerulus by immune complexes formed in the circulation. A new understanding of the biology of the basement membrane and the immunopathogenic mechanisms which underlie animal models of MGN has challenged not

only this hypothesis in particular but also the entire categorization of immunologic mechanisms in renal disease. In order to characterize this novel view of the description of immune mechaninisms in renal disease, I will review the classical understanding that grew out of experiments performed more than 30 years ago and which were etched in stone in an important review by Dixon in 1968.

Dixon developed a two-part model of immunologically mediated renal disease. In the first formulation, the kidney serves as the antigen itself. A single event, perhaps an acute infection, initiates synthesis of an antibody which crossreacts with structural elements of the glomerular basement membrane.

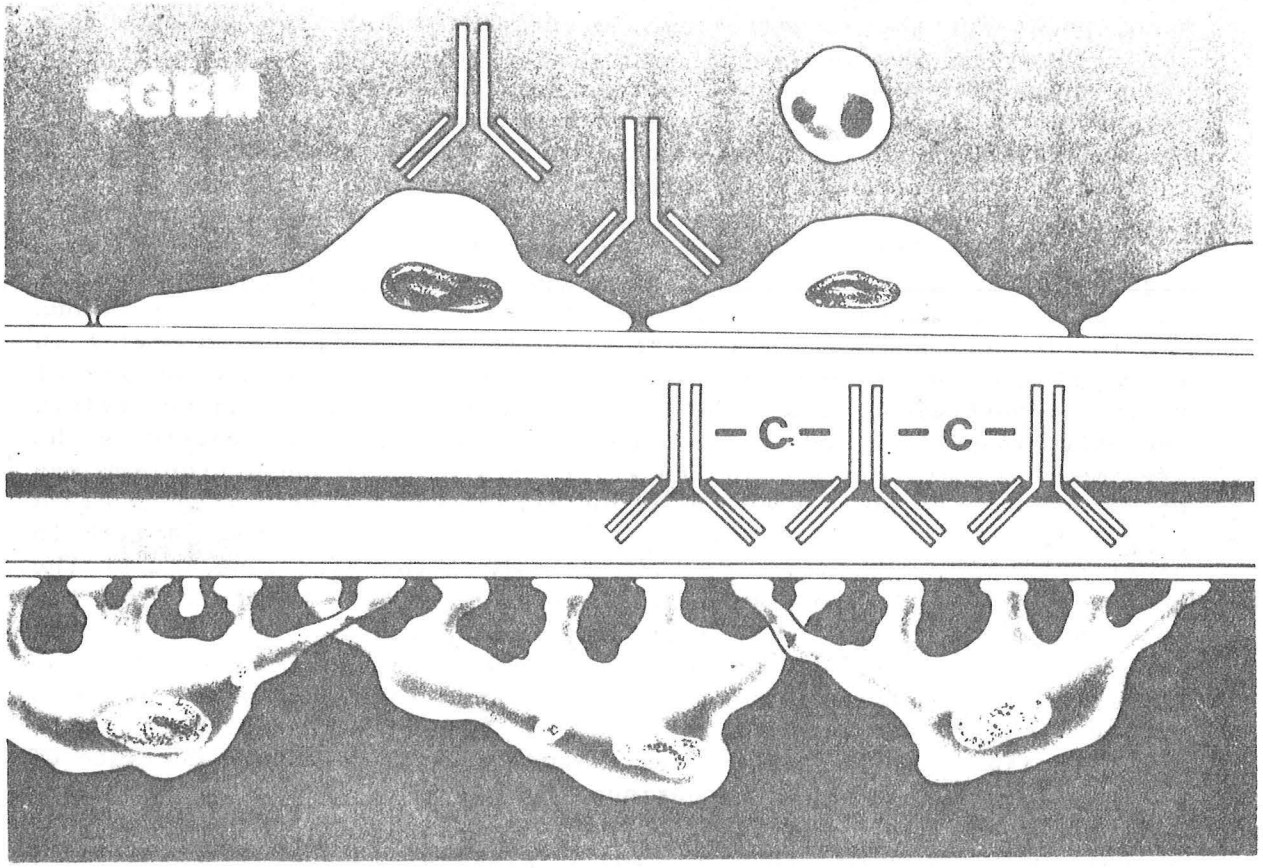


Figure 21 Artist's view of the immune mechanism responsible for anti-GBM nephritis.

As shown in Figure 21, this non-collagen structural antigen is assaulted directly by antibody to the protein. Because the antigen is distributed in an almost continuous fashion along the capillary wall, one can identify the bound antibody with immunofluorescent techniques in a "linear" pattern. In patients and most animal models, the binding of the antibody to the structural protein initiates a cascade of events, many of which are inflammatory in nature, culminating in immediate and delayed alterations in renal function. In man this form of immune injury occurs in a disorder called Goodpasture's Disease, which is relatively rare.

The second Dixon formulation is depicted in cartoon fashion in Figure 22.

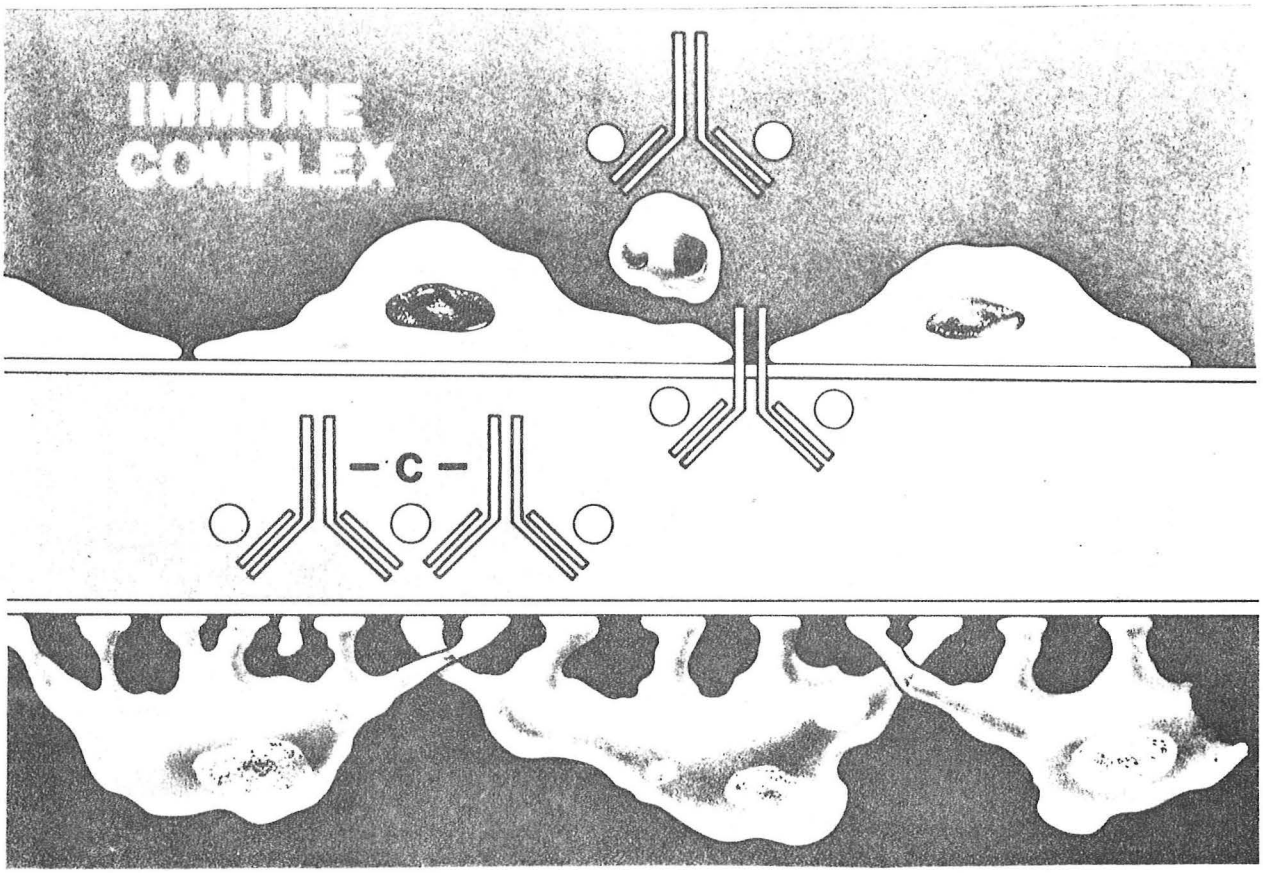


Figure 22 The Dixon hypothesis of immune complex nephritis.

In this view the kidney serves as an innocent bystander which is injured incidental to its capacity as a hemodialyzer. Patients respond to an antigenic stimulation by antibody formation. In the plasma space the antigen is coupled in a certain size and charge configuration to the antibody producing an immune complex. These immune complexes then filter through the basement membrane, lodging in that structure, initiating a series of events including activation of phlogistic factors leading to injury and clinical dysfunction. Employing immunofluorescent techniques, one will detect discontinuous, discrete aggregations of immunoglobulin and complement in a "lumpy-bumpy" pattern. This very immunofluorescent pattern characterizes idiopathic membranous glomerulopathy, thus this disease has been assigned an immune complex pathogenesis.

The immune complex hypothesis of the Dixon model grew directly out of the observations made by Germuth and Dixon concerning the acute, one-shot serum sickness animal model in which labeled bovine serum albumin was injected into a rabbit and its fate followed as shown in Figure 23.

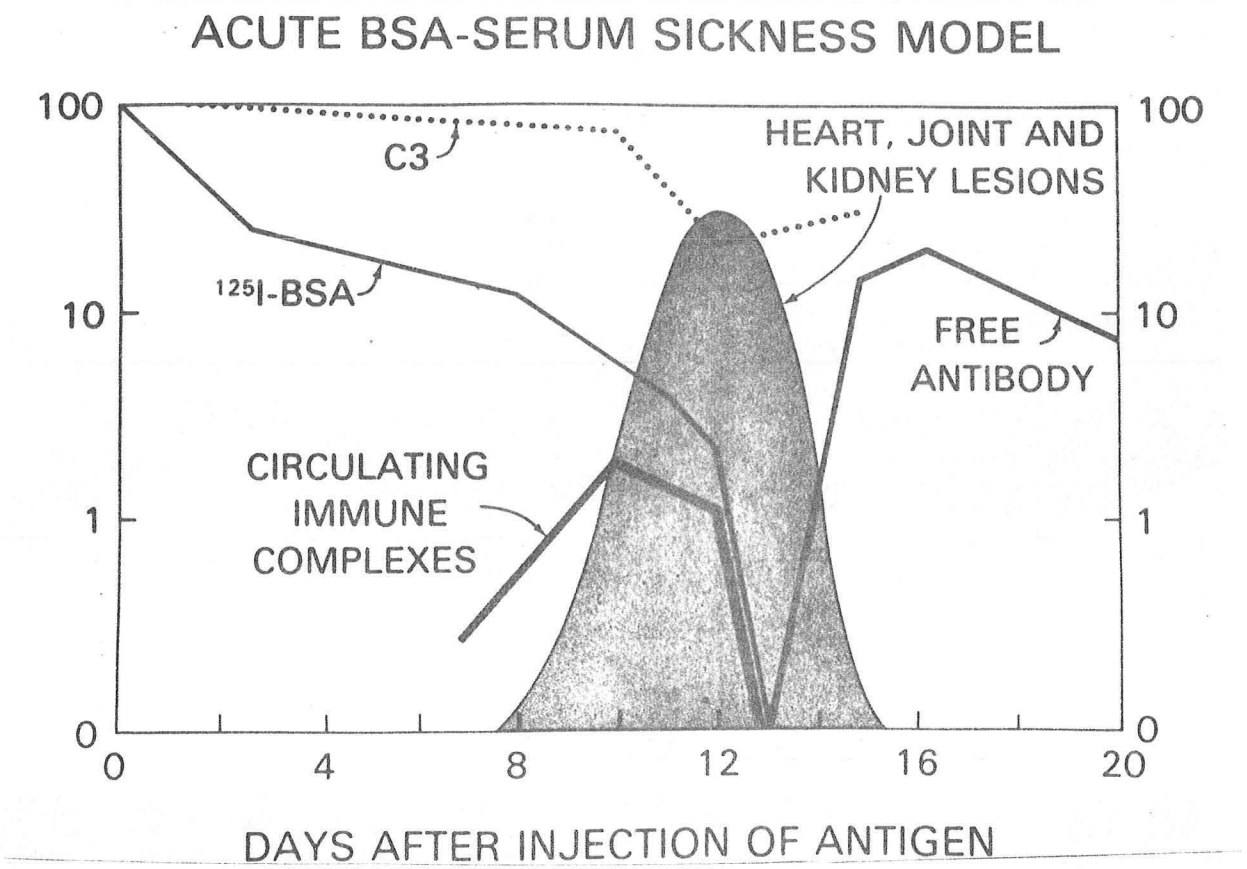
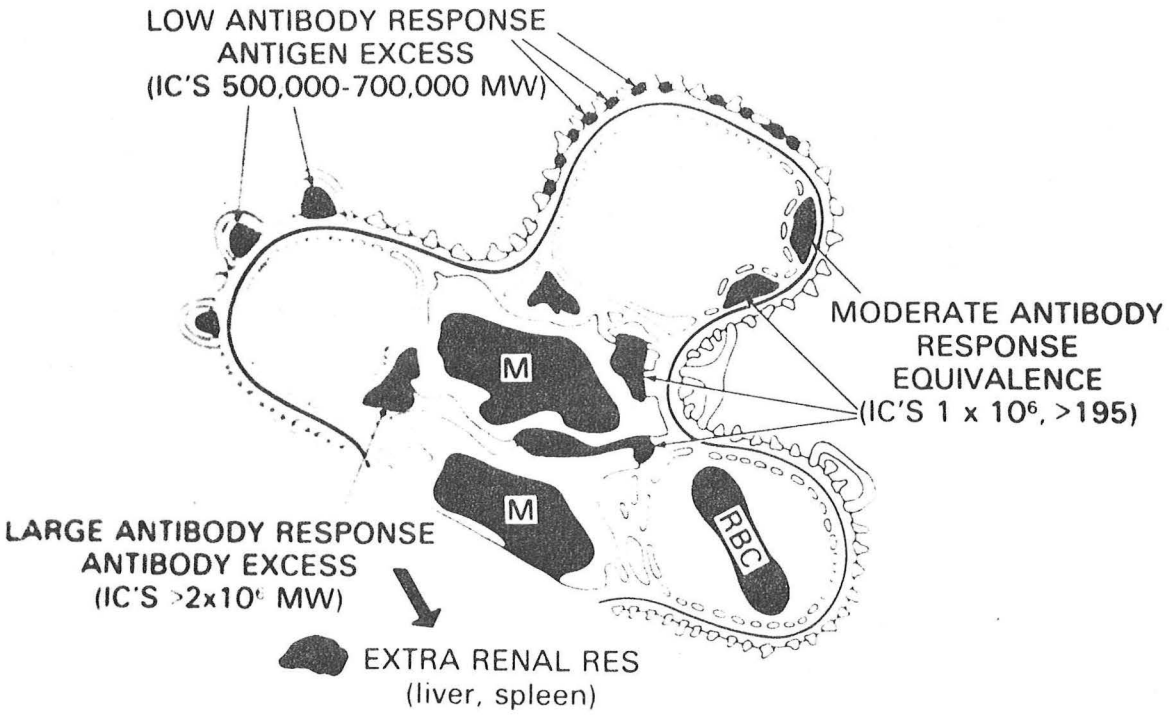


Figure 23 The fate of injected albumin in the single shot, acute serum sickness nephritis in the rabbit.

After an initial distribution phase the elimination of the BSA antigen attains a relatively fixed rate. By day 3, antibodies to the BSA are beginning to appear in the circulation and by day 6 circulating immune complexes may be measured. The presence of increasing concentrations of antibody altered the elimination rate of the antigen hastening its removal from the circulation and ultimate disappearance. Serum sickness lesions appeared only after the formation of circulating complexes and began to disappear when complexes were absent. The temporal relationship between the glomerular lesion and the appearance of complex in the circulation led to the formulation of the hypothesis that complexes were pathogenic and reached the kidney from the circulation itself.

Examination of the pathologic tissue from rabbits actively and chronically presented with antigen led to the second concept that stated that the site of glomerular immune deposit formation in the glomerular capillary wall, thus the type of histologic glomerular lesion which would be produced, depended on the antibody response which determines the antigen:antibody ratio and importantly the circulating immune complex size. This notion is pictorially demonstrated in Figure 24.



GLOMERULAR DEPOSITS AND ANTIBODY RESPONSE
BSA-SERUM SICKNESS MODELS

Figure 24 The relationship between the vigor of the antibody response to chronically administered antigen and the place in the glomerulus the immune comple xwill lodge. Courtesy of Dr. William Couser.

If an animal makes a great deal of antibody in response to a given antigen, large soluble circulating complexes may be formed which easily can be cleared by the mononuclear phagocytic system. Little or no renal deposits will accrue in this setting and those that are found are confined to the mesangial region, an area of the kidney which participates in the functions of the RES system. If a given animal synthesizes a concentration of antibody equivalent to the concentration of antigen present in the circulation, medium size, often soluble, circulating immune complexes are formed which lodge in the mesangium and the subendothelial region of the capillary wall leading to a severe proliferative glomerulonephritis with activation and proliferation of the endothelial cells. Lastly, if an animal makes a meager antibody response, antigen excess will ensue and small circulating immune complexes will be formed which are thought to lodge in the subepithelial region of the glomerular capillary wall. In this location, the deposits are less likely to induce a proliferative response of the endothelium or an inflammatory cellular response from the blood side producing membranous glomerulopathy. Dixon supported this view with results obtained in the

"active", chronic serum sickness rabbit model in which he could change the lesion by controlling the amount of antigen injected based on the antibody response of a given rabbit.

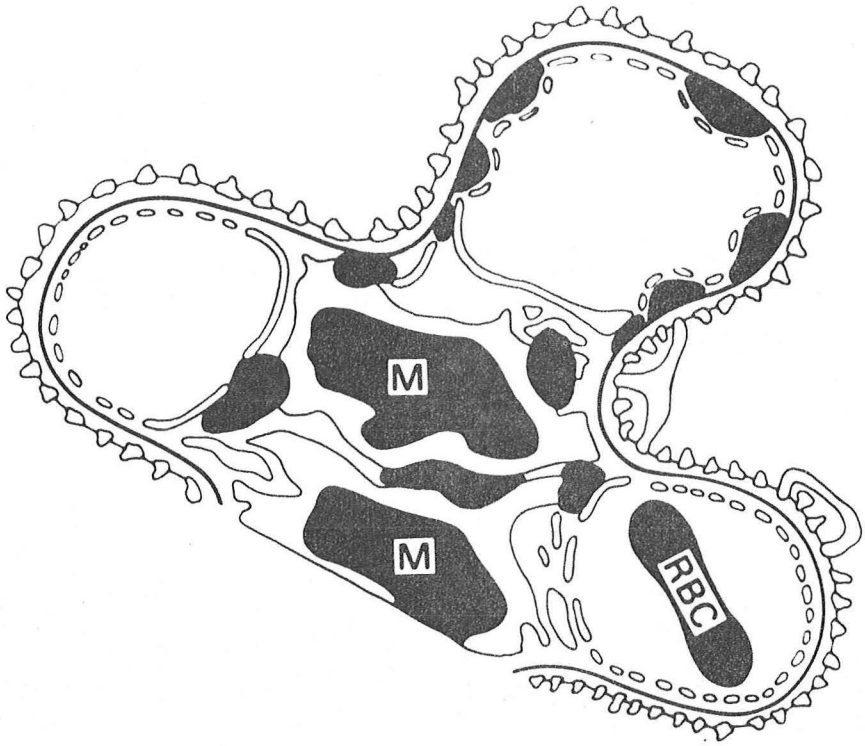
The first assault on this impregnable concept of circulating immune complex mediated nephropathy was launched by the failure to reproduce deposit lesions in the kidney upon the passive introduction of preformed immune complexes into the circulation. Table XII details the large group of studies all of which failed to reproduce subepithelial glomerular deposits with the introduction of preformed complexes into the circulation regardless of the antigen-antibody system employed. Whether the protein system is an anti-BSA system, a haptenated protein-anti-hapten system, or one composed of protein aggregates, which usually can replace complexes in most assay systems, the glomerular capillary tuft was devoid of deposit material. Indeed the majority of deposits that were found in renal biopsy material were confined to the mesangial region or rarely the subendothelial space.

TABLE XII

The Failure of Passively Administered Immune Complexes to Induce Subepithelial Deposit Formation

Report	Material injected	Methods of localization	Results
	Immune complexes		
Mellors, 1962 [54]	Rabbit anti-BSA or OA-FITC IC's into mice	IF	Mesangial deposits, BSA-FITC IC's and BSA-FITC alone on capillary wall
McCluskey, 1962 [50]	Rabbit anti-DNP-DNP-BGG IC's to mice	IF	Mesangial deposits
Weiser, 1962 [55]	Rabbit anti-BSA IgG-FITC-BSA IC's to mice	IF	"Diffuse" glomerular deposits, no illustrations
Okumura, 1971 [59]	Rabbit anti-BSA IC's to mice	IF, EM	Mesangial and subendothelial deposits
Wright, 1973 [61]	Dog anti-canine adenovirus IC's to mice	IF, EM	Mesangial and subendothelial deposits
Haakenstad, 1975 [13]	Rabbit anti-HSA IC's to mice	IF	Mesangial deposits
Ford, 1975 [12, 56]	Rabbit anti-BSA IC's to mice	IF	Mesangial deposits, mesangial and capillary wall deposits with RES blockade
Haakenstad, 1976 [10]	Rabbit anti-HSA IC's to mice	IF, EM	Mesangial and subendothelial deposits
Van Damme, 1978 [22]	Rabbit anti-rat Fx1A IC's to rats	IF	Mesangial deposits
Koyama, 1978 [60]	Rabbit anti-DNP-BSA or BGG to mice Rabbit anti-TNP BSA	IF, EM	Mesangial and subendothelial deposits
	Protein aggregates		
Michael, 1967 [62]	Aggregated HSA, IgG to mice	IF, EM	Mesangial and subendothelial deposits
Mauer, 1972 [63]	Aggregated IgG to rabbits	IF	Mesangial and subendothelial deposits
Ford, 1975 [64]	Aggregated BSA to mice	IF	Mesangial and "pericapillary" deposits
Kijlstra, 1978 [65]	Aggregated IgM	IF, EM	Mesangial and subendothelial deposits

Couser & Salant. Kidney Int., 1980



GLOMERULAR IMMUNE DEPOSITS IN "PASSIVE" SERUM SICKNESS (PRE-FORMED IC INFUSION)

Figure 25 Passively administered immune complexes are found primarily in the mesangium or in the subendothelial region of the GBM. Courtesy of Dr. William Crouser.

No single example of subepithelial deposit consequent to administration of pre-formed immune complexes have been experimentally described to date without vigorous chemical alteration of the basement membrane.

The active administration of the cationic probe ferritin, which can be followed morphologically due to its electron dense nature, afforded initial hints as to the mechanism of subepithelial deposit formation. This antigen led to an immune response, circulating immune complexes composed of ferritin and anti-ferritin antibodies, and later subepithelial deposit.

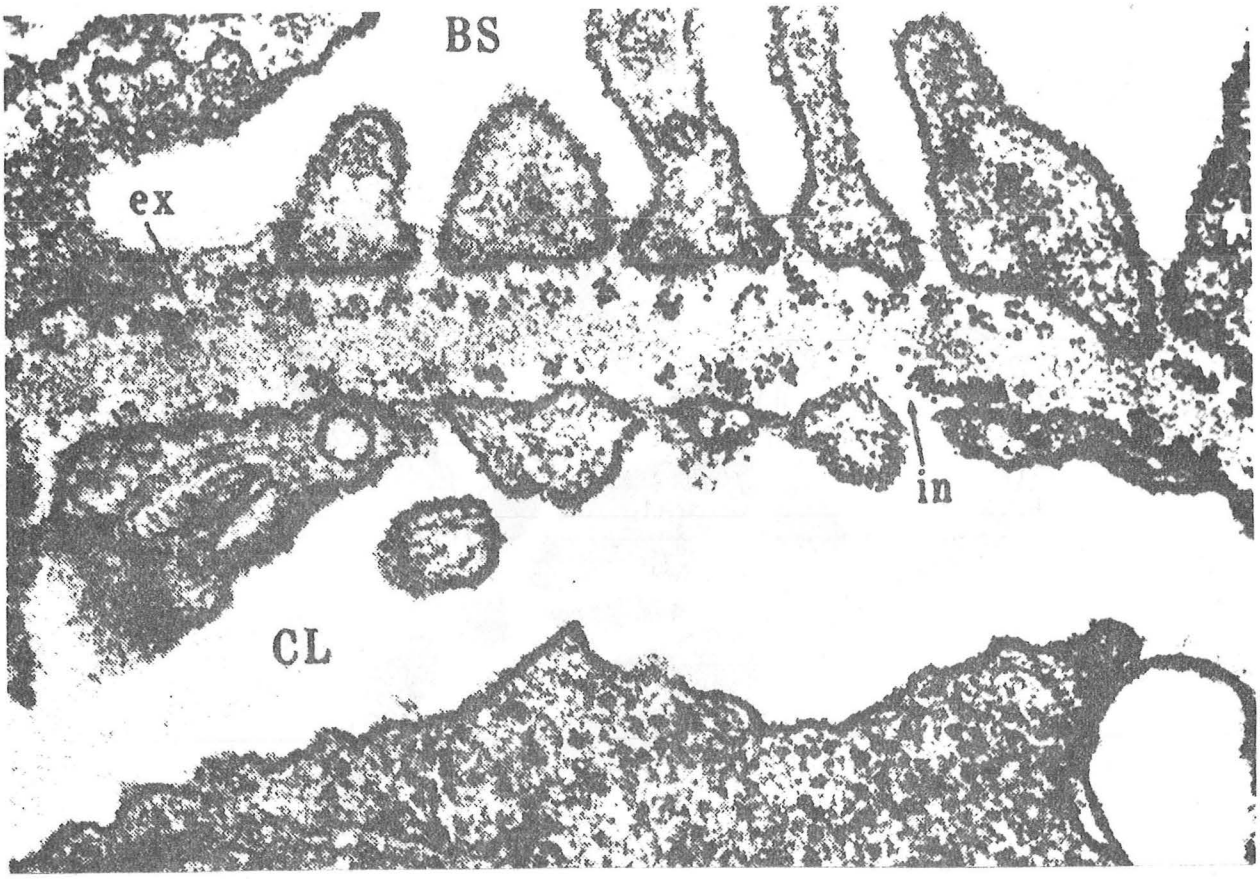


Figure 26 Ferritin granule deposition, the electron dense grains in this photomicrograph, are planted in the regions of the GBM bearing a net negative charge.

In this tightly controlled experimental model, the subepithelial deposit lesion could not be demonstrated to have temporally followed closely the formation of circulating complex. Indeed, one could show the presence of the ferritin in the basement membrane prior to significant antibody formation in the circulation or the formation of complex. The lessons of the ferritin experiments were lost until the animal model of MGN was studied in depth.

The failure to produce subepithelial deposits by passive administration of complex was initially felt to be related to the absence of inflammation and elaboration of vasoactive substances consequent to active presentation of antigen. The argument was advanced that complexes found in the circulation would lodge in the basement membrane only after elaboration of vasoactive compounds during inflammatory reaction to the antigen. Experimental exploration of this view by vasoactive administration of a range of materials in combination with preformed immune complexes failed to produce subepithelial deposit formation. Indeed maneuvers which sought to increase glomerular permeability tended to reduce rather than increase subepithelial deposits in the active, chronic serum sickness model. The failure to produce

glomerular deposits after injection of preformed immune complexes with or without modulation of permeability by vasoactive substances strongly shook the Dixon model.

The second major assault on the classic Dixon immune complex hypothesis has come from the experimental evaluation of the rat model of membranous glomerulopathy, a model called autologous immune complex nephropathy (AICN) or the nephritis of Heymann. In this model, animals immunized with antigen derived from the protein present in the luminal brush border of proximal tubular epithelial cells and obtained by fractionation of crude homogenants, thus its abbreviated name Fx1A, develop a renal disease indistinguishable from human membranous glomerulopathy. Based on the observation that a small number of animals so immunized will have circulating anti-tubular antibodies and immune complexes in the circulation, the pathogenesis of Heymann's nephritis has been felt to be an example of immunologically mediated renal disease in a setting of large antigen excess with subepithelial deposit formation. A similar pathologic lesion can be rapidly created by the passive injection of preformed Fx1A-anti Fx1A antibody. A membranous pattern of renal morphology with subepithelial basement membrane deposits results from such a maneuver. Building on the observation of Van Damme and associates that anti Fx1A directly binds to renal slices, Couser demonstrated that anti Fx1A presented to the kidney during ex vivo isolated renal perfusion not only bound to the glomerulus but was uniquely found in the subepithelial space.

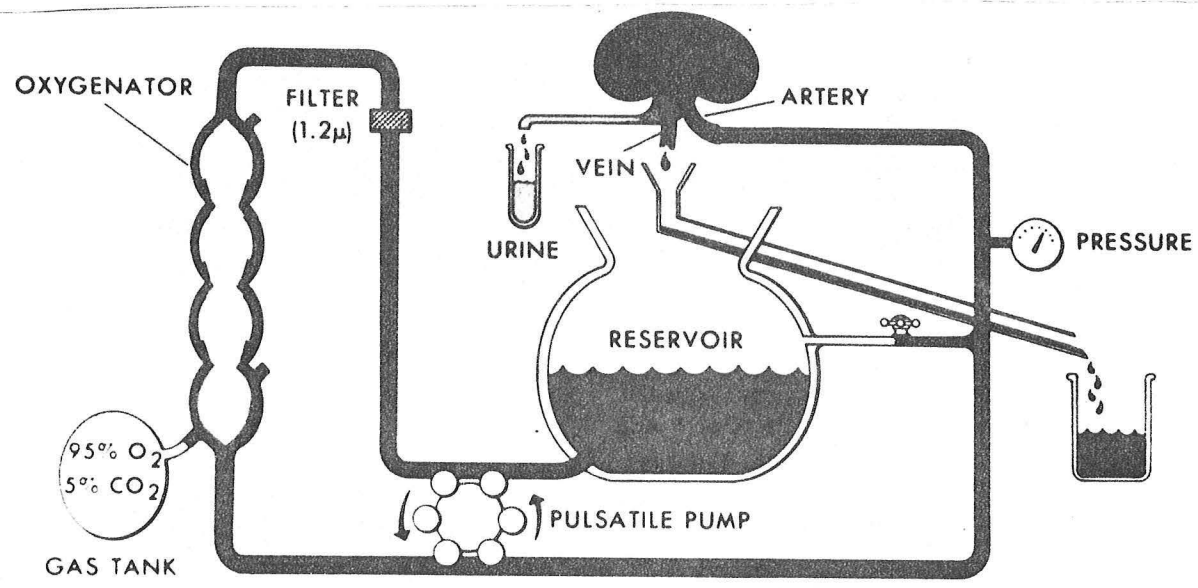


Figure 27 Ex vivo isolated perfusion protocol employed by Couser and associates to demonstrate that anti-Fx1A antibody binds directly to a structural element of the glomerulus.

In the experimental setting of Couser (shown in Figure 27) no circulating antigen was present, anti Fx1A was introduced into the perfusate and was found to develop subepithelial deposits in situ as a reaction of the free antibody with a fixed, endogenous antigen which is a constituent element of the glomerulus found in or near the outer portion of the glomerular capillary wall. The normal rat glomerulus Couser argues, possesses antigenic determinants which engage the antibody in situ as shown in Figure 28.

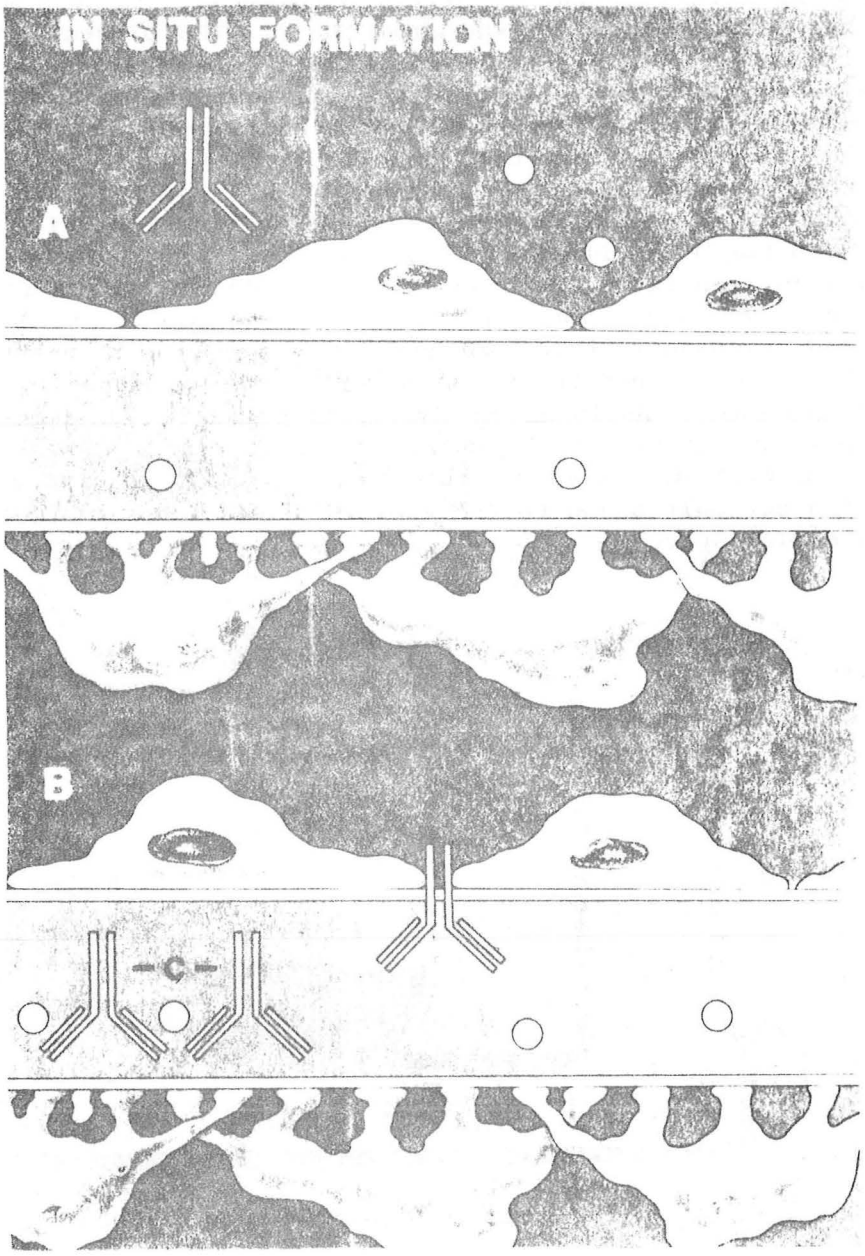


Figure 28 In situ formation of immune complex in the glomerulus of the Heymann's nephritis model.

Recently Farquhar has characterized the antigenic material recognized by the anti-brush border antibody as a glycoprotein synthesized by the glomerular epithelial cell which is localized in unique sections of the cell membrane of the podocyte foot processes called coated pits. This GP330 antigen then is found in what morphologically is a subepithelial location where an antibody may engage it thus altering the glomerular capillary filtration pore. Because the GP330 antigen is discontinuously distributed through the basement membrane, immunofluorescence in the Heymann's model will perforce be of the "lumpy-bumpy" variety. This observation raised the question of whether "lumpy-bumpy" immunofluorescence, characteristic of human membranous glomerulopathy, necessarily indicates a circulating immune complex mediated mechanism. The amendment of the in situ formation model is depicted in Figure 29.

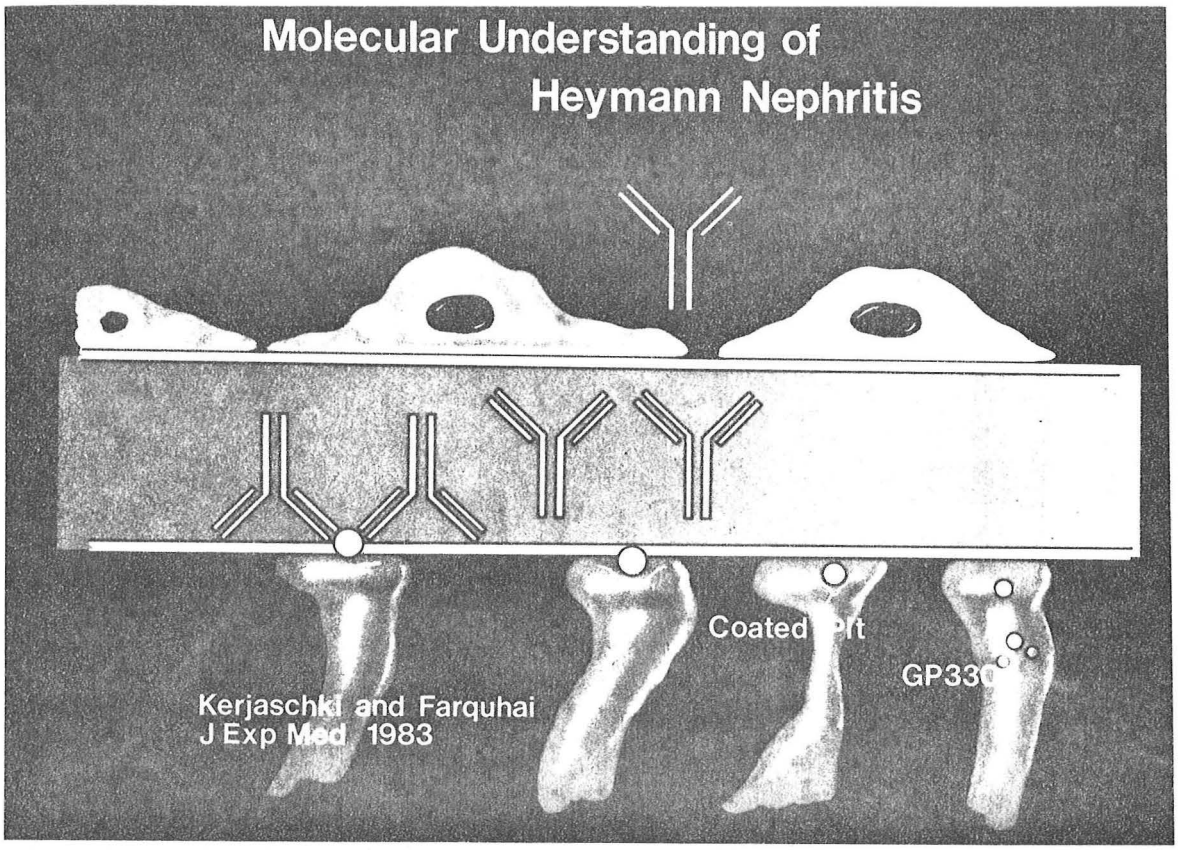


Figure 29 In situ formation of immune complex with a glycoprotein elaborated by the glomuerular epithelia cell, GP330, serving as the antigen.

Couser reinterpreted the ferritin experiment previously discussed as supporting an in situ model of complex formation with the cationic ferritin engaging the negatively charged sialoproteins at the subepithelial portion of the capillary wall where antibody finds the ferritin and a deposit is formed.

The third piece of evidence that argues against a circulating immune complex mediated mechanism to explain the pathogenesis of membranous glomerulopathy stems from an extensive review of the literature that speaks to the presence of such complexes in patients with this disorder. Table XIII reproduces the series that have explored this issue. Using a wide variety of laboratory techniques to discover and quantitate circulating immune complexes, no more than 20% of the patients in most series in which well performed assays were employed will have circulating complexes of any sort documented during their nephrotic period.

TABLE XIII

DETECTION OF CIRCULATING IMMUNE COMPLEXES IN PATIENTS WITH IDIOPATHIC MEMBRANOUS GN--ANY ASSAY

		No. Positive	No. Examined	% Positive
Cairns et al	1982	34	36	94
Sobel et al	1976	18	26	69
Gluckman et al	1978	26	47	54
Szabo et al	1979	7	14	50
Abrass et al	1980	22	55	40
Ooi et al	1980	5*	13	38
Russell et al	1978	6	16	38
Border	1979	54	235	23
Didgeon et al	1977	2	10	20
Meroni et al	1979	2	11	18
Stachura et al	1981	1	6	17
Woodroffe et al	1979	2	44	14
Rossen et al	1979	1	9	11
Robinson et al	1979	1	10	10
Zager et al	1979	1	15	7
Simpson et al	1978	0	5	0
Tung et al	1978	0	8	0
Solling and Olsen	1981	0	7	0
Ooi et al	1977	0	13	0

*all had renal vein thrombosis

Moreover, in those patients that do have circulating complexes, one cannot demonstrate that the complexes in the plasma space correlate to the electron dense material or immunoglobulin deposits seen pathologically in the "membranous" kidney. This experience relating circulating complexes to patients with MGN stands in stark contradistinction to that seen in patients with proliferative forms of systemic lupus erythematosus glomerulonephritis in which more than 95% of patients can have circulating immune complexes demonstrated which contain antigens and antibodies similar to those found in the basement membrane of biopsy material. A demonstration that deposits can be formed <u>in situ</u> in the basement membrane in animal models of membranous glomerulopathy and a

failure to detect circulating immune complexes in the majority of patients with this disease, strongly argue against an immune complex mediated mechanism to explain membranous glomerulopathy in man.

On the other hand, one cannot uncritically accept the view that an identical mechanism extant in the Heymann nephritis animal model can explain human membranous GN. Subsequent to the observations of Couser and colleagues, an assiduous search for the presence of antitubular antibodies in patients with MGN has generally been unrewarding. While it is true that several forms of membranous glomerulopathy in man has been associated with antitubular antibodies such as that following renal transplant rejection, obstructive uropathy, or sickle cell anemia, the majority of patients do not have such an antibody. An alternative hypothesis to explain most cases of human MGN that incorporates in situ complex formation and the acquisition into the GBM of foreign material which serves as antigen has been generated from an understanding of the physicochemical nature of the glomerulus itself that might permit foreign antigen material to be "planted" in the basement membrane where it can serve as both an immunogen and a target for the in situ formation of immune deposit leading to glomerular dysfunction. That such an antigen may be planted in the GBM has been demonstrated experimentally by in vivo perfusion of kidneys with conconavalin A which avidly binds directly to glomerular membrane glycoproteins where it initiates an immune response resulting in membranous glomerulopathy and proteinuria. One can also "plant" an antigen in the basement membrane using probes of specific electrical charges. I have already discussed how the cationic material ferritin will bind to the negatively charged portions of the glomerular basement membrane culminating in membranous glomerulopathy. Such a demonstration has now been repeated using other materials such as protamine or positively charged bovine serum albumin. Lastly, immunoglobulin itself may serve as an antigen planted in the capillary wall by virtue of initial binding to C' receptors present in this region of the glomerular tuft eliciting an antibody response and ultimately renal damage. It is reasonable therefore to suggest that foreign antigen because of chemical interactions, charge considerations, or receptor mediated binding events can localize in the basement membrane where it may form an immune complex in situ initiating the disease we call membranous glomerulopathy.

Understanding the means by which antigen can get to the glomerulus provides a view of only half of the pathogenetic equation in MGN. We must also account for the mechanism which leads to a setting of gross antigen excess in response to a given antigen. This circumstance permits the conditions for antigen to plant in the GBM and escape immune elimination by the RES. In some way a given antigen must evoke only a weak antibody response. That this notion is potentially correct can be discerned from that unique category of patients with systemic lupus erythematosus with distinctly membranous rather than proliferative biopsy lesions. These patients either do not have circulating anti DNA or have a very low titer of ANA. Initially, Izui and colleagues demonstrated that small amounts of anti-DNA antibody may cross-react with elements of the basement membrane and cause a membranous picture in animals similar to the Heymann nephritis story. Most intriguing is the observation of Ooi and colleagues that lymphocytes obtained from

patients with idiopathic membranous glomerulopathy have a diminished capacity to make antibody after a polyclonal challenge with a B cell mitogen. Moreover there is an important association between certain HLA genotypes and the occurrence of MGN.

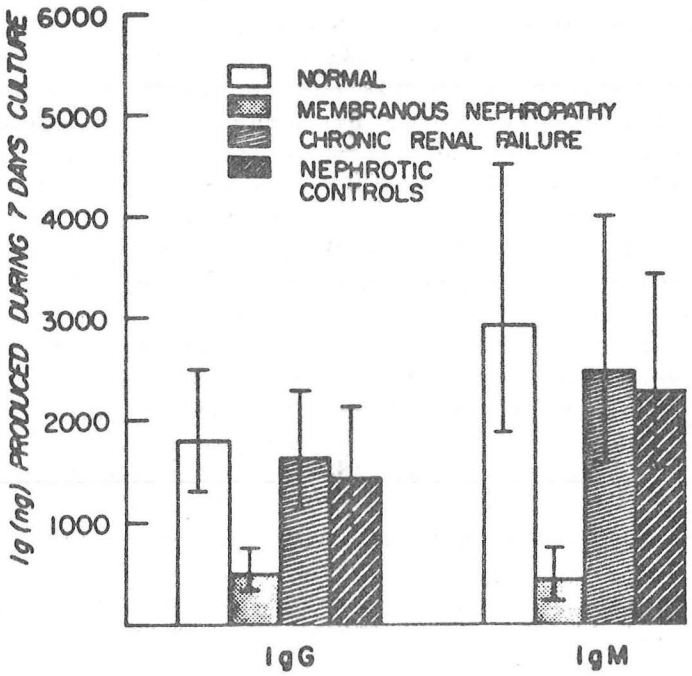


Figure 30 Immunoglobulin response of peripheral blood lymphocytes after polyclonal B cell activation in patients with MGN and relevant controls.

As shown in Figure 30, patients with MGN have defective immunoglobulin synthetic response compared not only to normal subjects but also to non-membranous nephrotic controls. This defect in cellular immunity is relatively nonspecific in that lymphocyte blastic response to various mitogens is also impaired (Figure 31) during active disease.

Subjects	Number of cases	Stimulation index (SI)	
		Con A	PHA
Normal controls	24	85.1 ± 9.3	161.0 ± 31.7
MN			
With NS	5	59.0 ± 12.8	113.0 ± 9.1
In remission	6	74.3 ± 1.8	142.0 ± 47.3
CGN			
With NS	5	82.5 ± 25.2	161.3 ± 13.1
Without NS	5	88.7 ± 42.3	162.0 ± 67.3

Matsumoto, et al. Int. Archs Allergy Appl. Immun., 1981

Figure 31 Mitogen responses of lymphocytes from patients with MGNand relevant controls.

The genetically determined immune hyporesponsiveness found in patients with MGN permits a hypothesis of the pathogenesis of this disorder in man. This hypothesis states that given patients are genetically destined to make meager antibody responses to certain antigens. If those antigens by virtue of their size, charge, chemical interactions, or binding capacities are capable of planting in the subepithelial area of the basement membrane and escape elimination by the meager antibody response, an immune complex will form in situ and initiate renal damage which, because of its location in the capillary wall, leads to proteinuria rather than predominant renal inflammation.

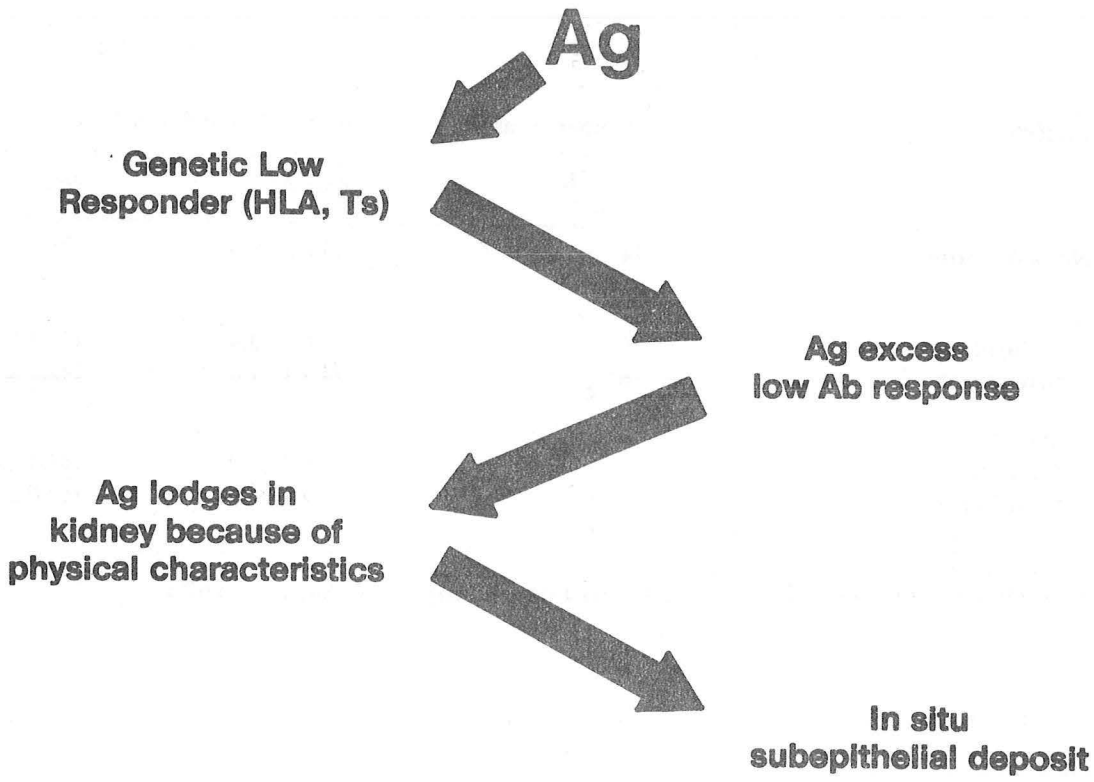


Figure 32 Pathogenesis of idiopathic membranous glomerulopathy.

These new data concerning membranous glomerulopathy allow a general revision of the classic Dixon hypothesis as detailed in Table XIV.

TABLE XIV

DIXON'S FORMULATION OF IMMUNE RENAL DISEASE-REVISED

I. The kidney serves as antigen

A. anti GBM nephritis – Goodpasture's Disease
B. anti GP330 nephropathy – some forms of MGN

II. The kidney is injured incidental to its filtering properties

A. circulating immune complex deposition-SLE, PSGN
B. in situ formation of immune complex by circulating antibody and antigen planted in the GBM by virtue of its chemical properties – most MGN

It is useful in my view, to retain the bipartite Dixon model with injury resulting from the kidney serving as antigen or as an innocent bystander. When the renal antigen involved in the immune injury is the non-collagen portion of the glomerular capillary wall then severe inflammation and nephritis ensues. When the renal antigen is a glycoprotein elaborated by the visceral epithelial cell of the glomerular tuft then discrete complexes are formed in situ and membranous glomerulopathy ensues. On the other hand the kidney may be injured by immune reactants which reach the kidney as small complexes formed in the circulation and lodging under the capillary endothelial cell initiating an inflammatory nephritis or first as antigens which plant in the basement membrane by virtue of charge, chemical reactions, or receptor binding where they are found by small concentrations of antibody in order to form a complex causing disruption of the filtration barrier.

SUMMARY

Idiopathic membranous glomerulopathy is a disease with a distinctive morbid anatomy and natural history. The pathologic diagnosis is constant with little shifting, that is it "breeds true". An understanding of the clinicopathologic features of the disease carries prognostic import. It is now possible that accurate diagnosis of this disorder may lead to potentially effective therapy. An understanding of the pathogenesis of this disorder has greatly altered our understanding of immune mechanisms in renal disease and has engendered a greater degree of collaboration between morphologists, renal physiologists, immunologists, and clinicians.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Dr. William Couser of the University of Washington, Seattle for providing a wealth of materials which detailed his views concerning the pathogenesis of MGN. Beyond these materials he has graciously allowed me to use the schematic representations of the data which he has designed. These have made my task significantly less arduous while improving the clarity of the argument. I owe thank yous as well to Dr. Fred Silva of the UTHSCD Department of Pathology who has given me the best examples of pathologic material available, most better than previously published material. I appreciate the supreme patience of the UTHSCD Medical Illustrations Department who have done a marvelous job of illustration. Most importantly, I want to acknowledge the magnificant job of manuscript preparation by our secretary Ms. Karen Williams.

BIBLIOGRAPHY

Abrass, C.K., C.L. Hall, W.A. Border, et al. Circulating immune complexes in adults with idiopathic nephrotic syndrome. Kidney Int. 17:545, 1980.

Anderson, J.L., and J.A. Quinn. Restricted transport in small pores. A model for steric exclusion and hindered particle motion. Biophys. J. 14:130, 1974.

Andrews, P.M. Glomerular epithelial alterations resulting from sialic acid surface coat removal. Kidney Int. 15:376, 1979.

Barabas, A.Z., and R. Lannigan. Induction of an autologous immune-complex glomerulonephritis in the rat by intravenous injection of heterologous anti-rat kidney tubular antibody: I. Production of chronic progressive immune complex glomerulonephritis. Br. J. Exp. Pathol. 55:47, 1974.

Barbanel, C. and L-H. Noël. Membranous glomerulonephritis in Renal Diseases. Black, D.A.K. and N.F. Jones (ed.) Blackwell, Oxford, p. 489, 1979.

Bariety, J., P. Druet, G. Lagrue, et al. Les (glomerulopathies extra-membraneuses) (gen) etude morphologique en microscopie optique electronic en immunofluorescence. Pathologic Biologie (Paris) 18:5, 1970.

Barnett, E.V., D.W. Knutson, C.K. Abrass, et al. Circulating immune complexes: Their immunochemistry, detection, and importance. Ann. Int. Med. 91:430, 1979.

Bell, E.T. Lipoid nephrosis. Am. J. Pathol. 5:587, 1929.

Benacerraf, B., J.L. Potter, R.T. McCluskey, and F. Miller. The pathologic effects of IV administered soluble antigen-antibody complex: II. Acute glomerulonephritis in rats. J. Exp. Med. 111:195, 1960.

Bennett, C.M., R.J. Glassock, R.L.S. Chang, et al. Permselectivity of the glomerular capillary wall: Studies of experimental glomerulonephritis in the rat using dextran sulphate. J. Clin. Invest. 57:1287, 1976.

Beregi, E., and I. Varga. Analysis of 260 cases of membranous glomerulonephritis in renal biopsy material. Clin. Nephrol. 2:215, 1974.

Bernard, D.B., E.A. Alexander, W.G. Couser, and N.G. Levinsky. Renal sodium retention during volume expansion in experimental nephrotic syndrome. Kidney Int. 14:478, 1978.

Bhan, A.K., E.E. Schneeberger, A.B. Collins, and R.T. McCluskey. Evidence for a pathogenic role of a cell-mediated immune mechanism in experimental glomerulonephritis. J. Exp. Med. 148:246, 1978.

Black, D.A.K., G. Rose, and D.B. Brewer. Controlled trial of prednisone in adult patients with the nephrotic syndrome. Br. Med. J. 2:421, 1970.

Bohrer, M.P., C. Baylis, H.D. Humes, et al. Permselectivity of the glomerular capillary wall. Facilitated filtration of circulating polycations. J. Clin. Invest. 61:72, 1978.

Bohrer, M.P., W.M. Deen, C.R. Robertson, et al. Influence of molecular configuration on the passage of macromolecules across the glomerular capillary wall. J. Gen. Physiol. 74:583, 1979.

Bolton, W.K., N.O. Atuk, B.C. Sturgil, et al. Therapy of the idiopathic nephrotic syndrome (INS) with alterante day steroids. Am. J. Med. 62: 60, 1977.

Border, W.A. Immune complex detection in glomerular diseases. Nephron 24:105, 1979.

Bourdeau, J.E., and F.A. Carone. Protein handling by the renal tubule. Nephron 13:22, 1974.

Brenner, B.M., C. Baylis, and W.M. Deen. Transport of molecules across renal glomerular capillaries. Physiol. Rev. 56:502, 1976.

Cairns, S., P. Klouda, P. Dyer, et al. Role of the major histocompatibility complex system in idiopathic membranous nephropathy: Prediction of outcome and response to treatment. Kidney Int. 18:802, 1980 (Abstract).

Cairns, S.A., R.A. London, and N.P. Mallick. Circulating immune complexes in idiopathic glomerular disease. Kidney Int. 21:507, 1982.

Cameron, J.S. Clinicopathological correlations in glomerular disease in Kidney Disease--Present Status. Churg, J. and B.F. Spargo (ed.) IAP Monographs No. 20, 1978.

Cameron, J.S. Nephrology Forum: Pathogenesis and treatment of membranous nephropathy. Kidney Int. 15:88, 1979.

Cameron, J.S. The natural history of glomerulonephritis in Renal Disease. Black, D.A.K. and N.F. Jones (ed.) Blackwell, Oxford, p. 329, 1979.

Cameron, J.S. Glomerulonephritis: Current problems and understanding. J. Lab. Clin. Med. 99:755, 1982.

Cameron, J.S., and W.F. Clark. A role for insoluble antibody--antigen complexes in glomerulonephritis? Clin. Neph. 18:55, 1982.

Cameron, J.S., C.S. Ogg, D.R. Turner, et al. Membranous nephropathy in Progress In Glomerulonephritis. P. Kincaid-Smith (ed.) p. 473, 1979.

Cattran, D., C. Cardella, R. Charron, et al. Preliminary results of controlled trial of alternate day prednisone (ADS) in idiopathic membranous glomerulonephritis (IMGN). VIIIth Int. Cong. Nephrol., Athens, 1981 CN146.

Caulfield, J.P., and M.G. Farquhar. Distribution of anionic sites in glomerular basement membrane: Their possible role in filtration and attachment. Proc. Natl. Acad. Sci. USA 73:1646, 1976.

Caulfield, J.P., and M.G. Farquhar. Distribution of anionic sites in normal and nephrotic glomerular basement membranes. J. Cell Biol. 70:92a, 1976 (abstract).

Caulfield, J.P., and M.G. Farquhar. Loss of anionic sites from the glomerular basement membrane in aminonucleoside nephrosis. Lab. Invest. 39:505, 1978.

Chang, R.L.S., C.R. Robertson, W.M. Deen, and B.M. Brenner. Permselectivity of the glomerular capillary wall to macromolecules: I. Theoretical considerations. Biophys. J. 15:861, 1975.

Chang, R.L.S., I.F. Ueki, J.L. Troy, et al. Permselectivity of the glomerular capillary wall to macromolecules: II. Experimental studies in rats using neutral dextran. Biophys. J. 15:887, 1975.

Chatenoud, L., and M.A. Bach. Abnormalities of T cell subsets in glomerulonephritis and systemic lupus erythematosus. Kidney Int. 20:267, 1981.

Chiu, J., and K.N. Drummond. Chemical and histochemical studies of glomerular sialoprotein in nephrotoxic nephritis in rats. Am. J. Pathol. 68:391, 1972.

Chonko, A.M., W.H. Bay, J.H. Stein, and T.F. Ferris. The role of renin and aldosterone in the salt retention of edema. Am. J. Med. 63:881, 1977.

Churg, J., and T. Ehrenreich. Membranous nephropathy in Glomerulonephritis. P. Kincaid-Smith, et al (ed.). John Wiley, New York, p. 443, 1973.

Cochrane, C.G. Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels: I. Structural studies. J. Exp. Med. 118:489, 1963.

Cochrane, C.G. Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels: II. Pathogenetic and pharmacodynamic studies. J. Exp. Med. 118:503, 1963.

Cochrane, C.G. Mechanisms involved in the deposition of immune complexes in tissues. J. Exp. Med. 134:79s, 1971.

Cochrane, C.G., and D.J. Hawkins. Studies on circulating immune complexes: III. Factors governing the ability of circulating complexes to localize in blood vessels. J. Exp. Med. 127:137, 1968.

Cochrane, C.G., and D. Koffler. Immune complex disease in experimental animals and man. Adv. Immunol. 16:185, 1973.

Cochrane, C.G., E. Unanue, and F.J. Dixon. A role of polymorphonuclear leukocytes and complement in nephrotoxic nephritis. J. Exp. Med. 122: 99, 1965.

Cohen, S.L., C. Fisher, J.F. Mowbray, et al. Circulating and deposited immune complexes in renal disease and their clinical correlation. J. Clin. Pathol. 32:1135, 1979.

Collins, A.B., G.A. Andres, and R.T. McCluskey. Lack of evidence for a role of renal tubular antigen in human membranous glomerulonephritis. Nephron 27:297, 1981.

Cortney, M.A., L.L. Swain, and D.D. Weiss. Renal tubular protein absorption in the rat. J. Clin. Invest. 49:114, 1970.

Couser, W.G. What are circulating immune complexes doing in glomerulonephritis? New Eng. J. Med. 304:1230, 1981.

Couser, W.G., J.R. Hoyer, M.M. Stilmant, et al. The effect of aminonucleoside nephrosis on immune complex localization in autologous immune complex nephropathy in rats. J. Clin. Invest. 61:561, 1978.

Couser, W.G., and D.J. Salant. In situ immune complex formation and glomerular injury. Kidney Int. 17:1, 1980.

Couser, W.G., D.J. Salant, M.M. Stilmant, et al. The effects of aminonucleoside of puromycin and nephrotoxic serum on subepithelial immune deposit formation in passive Heymann nephritis. J. Lab. Clin. Med. 1979.

Couser, W.G., D.J. Salant, and M.M. Stilmant. Mechanisms of proteinuria induced by anti-kidney antibodies in non-inflammatory experimental glomerulonephropathy in Proceedings of the Conference on Immune Mechanisms in Renal Disease. A.F. Michael and N.B. Cummings (ed.), Plenum Press, New York, 1980, in press.

Couser, W.G., M.M. Stilmant, and C. Darby. Autologous immune complex nephropathy: I. Sequential study of immune complex deposition, ultrastructural changes, proteinuria and alterations in glomerular sialoprotein. Lab. Invest. 34:23, 1976.

Couser, W.G., D.R. Steinmuller, M.M. Stilmant, et al. Experimental glomerulonephritis in the isolated perfused rat kidney. J. Clin. Invest. 62:1275, 1978.

Couser, W.G., J.B. Wagonfeld, B.H. Spargo, and E.J. Lewis. Glomerular deposition of tumor antigen in membranous nephropathy associated with colonic carcinoma. Am. J. Med. 57:962, 1974.

D'Achiardi-Rey, R. and V.E. Pollak. Membranous glomerulonephropathy: There is no significant effect of treatment with corticosteroids. Am. J. Kidney Diseases 1:386, 1982.

Davison, A.M. J.S. Cameron, and D.N.S. Kerr. Natural history of untreated idiopathic membranous glomerulonephritis in adults. Abst. VIII Int. Cong. Nephrol., Athens, 1981, CN196.

Digeon, M., M. Laver, J. Riza, and J.F. Bach. Detection of circulating immune complexes in human sera by simplified assays with polyethylene glycol. J. Immunol. Methods 16:165, 1977.

Dixon, F.J. The pathogenesis of glomerulonephritis. Am. J. Med. 94: 493, 1969.

Dixon, F.J., J.D. Feldman, and J.J. Vazquez. Experimental glomerulonephritis: The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. J. Exp. Med. 113:899, 1961.

Dixon, F.J., J.J. Vazquez, W.O. Weigel, and C.G. Cochrane. Pathogenesis of serum sickness. Arch. Pathol. 68:18, 1958.

Donadio, J.V., K.E. Holley, C.F. Anderson, and W.F. Taylor. Controlled trial of cyclophosphamide in idiopathic membranous nephropathy. Kidney Int. 6:431, 1974.

Dorhout Mees, E.J., J.C. Ross, P. Boer, et al. Observations on edema formation in the nephrotic syndrome in adults with minimal lesions. Am. J. Med. 67:378, 1979.

Edgington, T.S., R.J. Glassock, and F.J. Dixon. Autologous immune complex nephritis induced with renal tubular antigen: I. Identification and isolation of the pathogenetic antigen. J. Exp. Med. 127:555, 1968.

Edgington, T.S., R.J. Glassock, and F.J. Dixon. Autologous immune complex pathogenesis of experimental allergic glomerulonephritis. Science 155:1432, 1967.

Ehrenreich, T., and J. Churg. Pathology of membranous nephropathy. Pathol. Ann. 3:145, 1968.

Ehrenreich, T., J.G. Porush, J. Churg, et al. Treatment of idiopathic membranous nephropathy. New. Eng. J. Med. 295:741, 1976.

Erwin, D.T., J.V. Donadio, and K.E. Holley. The clincial course of idiopathic membranous nephropathy. Mayo Clin. Proc. 48:197, 1973.

Feenstra, K., R.V.D. Lee, H.A. Greben, et al. Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens: I. The natural history: A histologic and immunohistologic study at the light microscopic and ultrastructural level. Lab. Invest. 32:235, 1975.

Fillit, H.M., and J.B. Zabriskie. Cellular immunity in glomerulonephritis. Am. J. Pathol. 109:227, 1982.

Fish, A.J., A.F. Michael, R.L. Vernier, and R.A. Good. Acute serum sickness nephritis in the rabbit: An immune deposit disease. Am. J. Pathol. 49:997, 1966.

Fleuren, G.J., and Ph.J. Hoedemaeker. The role of fixed antigens in the pathogenesis of autologous immune complex glomerulonephritis. Abst. VII Int. Congr. Nephrol, Montreal, 1978, p. K3.

Foidart, J.B., J.P. Salmon, F.J. Berthoux, and P. Mahieu. Binding of soluble immune complexes to human glomerular complement receptors. Kidney Int. 15:303, 1979.

Ford, P.M. The effect of manipulation of reticuloendothelial system activity on glomerular deposition of aggregated protein and immune complexes in two different strains of mice. J. Exp. Pathol. 56:523, 1975.

Ford, P.M. Glomerular localization of aggregated protein in mice: Effect of strain difference and relationship to systemic macrophage function. Br. J. Exp. Pathol. 56:307, 1975.

Ford, P.M. Passive serum sickness in the mouse: effect of interstrain differences on glomerular deposition of immune complexes. Br. J. Exp. Pathol. 56:199, 1975.

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

Franklin, W.A., R.B. Jennings, and D.P. Earle. Membranous glomerulonephritis: Long-term serial observations on clinical course and morphology. Kidney Int. 4:36, 1973.

Friend, P.S., and A.F. Michael. Hypothesis: Immunologic rationale for the therapy of membranous lupus nephropathy. Clin. Immunol. Immunopathol. 10:35, 1978.

Garovoy, M.R., L.R. Kupor, and J.D. Mullins. Treatment of autologous immune complex glomerulonephritis with vasoactive amine antagonists. J. Lab. Clin. Med. 90:638, 1977.

Gärtner, H.V., H. Fischbach, H. Wehner, et al. Comparison of clinical and morphological features of peri-(epi-extra) membranous glomerulonephritis. Nephron 13:288, 1974.

Gelfand, M.C., M.M. Frank, and I. Green. A receptor for the third component of complement in the human renal glomerulus. J. Exp. Med. 142:1029, 1975.

Germuth, F.G., Jr. A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. J. Exp. Med. 97:257, 1953.

Germuth, F.G., Jr., C. Flanagan, and M.R. Montenegro. The relationships between the chemical nature of the antigen, antigen dosage, rate of antibody synthesis and the occurrence of arteritis and glomerulonephritis in experimental hypersensitivity. Johns Hopkins Med. J. 101:149, 1957.

Germuth, F.G., Jr., and G.E. McKinnon. Studies on the biological properties of antigen-antibody complexes: I. The anaphylactic shock induced by soluble antigen-antibody complexes in unsensitized normal guinea pigs. Johns Hopkins Med. J. 101:13, 1953.

Germuth, F.G., Jr., M.G. Pace, and J.C. Tippett. Comparative histologic and immunologic studies in rabbits of induced hypersensitivity of the serum sickness type: II. The effect of sensitization to homologous and cross-reactive antigens on the rate of antigen elimination and the development of allergic lesions. J. Exp. Med. 101:135, 1955.

Germuth, F.G., Jr., and E. Rodriguez. Immunopathology of the Renal Glomerulus: Immune Complex Deposit and Anti-basement Membrane Disease. Little Brown and Company, Boston, 1973.

Germuth, F.G., A.J. Valdes, L.B. Senterfit, and A.D. Pollack. A unique influence of cortisone on the transit of specific macromolecules across vascular walls in immune complex disease. Johns Hopkins Med. J. 122: 137, 1968.

Gitlin, D., C.A. Janeway, and L.E. Farr. Albumin metabolism in children with nephrosis. J. Clin. Invest. 35:44, 1956.

Glassock, R.J. Corticosteroid therapy is beneficial in adults with idiopathic membranous glomerulopathy. Am. J. Kidney Dis. 1:376, 1982.

Glassock, R.J., T.S. Edgington, J.I. Watson, F.J. Dixon. Autologous immune complex nephritis induced with renal tubular antigen: II. The pathogenetic mechanism. J. Exp. Med. 127:573, 1968.

Gluck, M.C., G. Gallo, J. Lowenstein, et al. Membranous glomerulonephritis. Evolution of clinical and pathologic features. Ann. Int. Med. 78:1, 1973.

Gluckman, J.C., N. Jacob, H. Beaufils, et al. Clinical significance of circulating immune complexes detection in chronic glomerulonephritis. Nephron 22:138, 1978.

Golbus, S., and C.B. Wilson. Experimental glomerulonephritis induced by in situ formation of immune complexes in glomerular capillary wall. Kidney Int. 16:148, 1979.

Grausz, H., R. Lieberman, and L.E. Earley. Effect of plasma albumin on sodium reabsorption in patients with nephrotic syndrome. Kidney Int. 1:47, 1972.

Grupe, W.E., and M.H. Kaplan. Demonstration of an antibody to proximal tubular antigen in the pathogenesis of experimental autoimmune nephrosis in the rat. J. Lab. Clin. Med. 74:400, 1969.

Gur, A., P.Y. Adefuin, N.J. Siegel, and J.P. Hayslett. A study of the renal handling of water in lipoid nephrosis. Pediat. Res. 20:1976, 1976.

Haakenstad, A.O., J.B. Case, M. Mannik. Effect of cortisone on the disappearance kinetics and tissue localization of soluble immune complexes. J. Immunol. 114:1153, 1975.

Haakenstad, A.O. and M. Mannik. The disappearance kinetics of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. Lab. Invest. 35:283, 1976.

Haakenstad, A.O., and M. Mannik. Saturation of the reticuloendothelial system with soluble immune complexes. J. Immunol. 112:1939, 1974.

Haakenstad, A.O., G.E. Striker, and M. Mannik. The glomerular deposition of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. Lab. Invest. 35:293, 1976.

Habib, R., and C. Kleinknecht. Idiopathic extramembranous glomerulonephritis in children. Morphology and natural history in Glomerulonephritis. P. Kincaid-Smith, et al (ed.) John Wiley, New York, p. 461, 1973.

Habib, R., and C. Kleinknecht. The primary nephrotic syndrome of childhood. Classification and clinicopathologic study of 406 cases. Pathol. Ann. p. 417, 1971.

Habib, R., C. Kleinknecht, and M.C. Gubler. Extramembranous glomerulonephritis in children. Report of 50 cases. J. Pediatr. 82:754, 1973.

Habib, R., P. Michielsen, E. de Montera, et al. Clinical microscopic and electron microscopic data in the nephrotic syndrome of unknown origin in Ciba Foundation Symposium on Renal Biopsy: Clinical and Pathologic Significance, 1961.

Hardwicke, J., J.D. Blainey, D.B. Brewer, et al. The nephrotic syndrome. Proc. 3rd Int. Congr. Nephrol. 3:69, 1967.

Hawn, C.V.Z., and C.A. Janeway. Histologic and serological sequences in experimental hypersensitivity. J. Exp. Med. 85:571, 1947.

Hayslett, J.P., M. Kashgarian, K.G. Bensch, et al. Clinicopathologic correlations in the nephrotic syndrome due to primary renal disease. Medicine (Baltimore) 52:93, 1973.

Hebert, L.A., C.L. Allheiser, and S.M. Koethe. Some hemodynamic determinants of immune complex trapping by the kidney. Kidney Int. 14:452, 1978.

Hebert, L.A., K.A. Stuart, C.L. Allheiser, and G.E. Rodney. Measurement of the fractional uptake of macromolecules by the renal vascular bed compared to other vascular beds. J. Lab. Clin. Med. 88:716, 1976.

Heymann, W., D.B. Hacket., J. Harwood, et al. Production of nephrotic syndrome in rats by Freunds adjuvants and rat kidney suspension. Proc. Soc. Exp. Biol. Med. 100:660, 1959.

Hoffsten, P.E., A. Swerdlin, M. Bartell, et al. Reticuloendothelial and mesangial function in murine immune complex glomerulonephritis. Kidney Int. 15:144, 1979.

Hopper, J., Jr., L.G. Biava, and W.H. Tu. Membranous nephropathy: High-dose alternate-day therapy with prednisone. West. J. Med. 35:1, 1981.

Hopper, J., P.A. Trew, and C.G. Biava. Membranous nephropathy: Its relative benignity in women. Nephron 29:18, 1981.

Hoyer, J.R., J.C. Elema, and R.L. Vernier. Unilateral renal disease in the rat: II. Glomerular mesangial uptake of colloidal carbon in unilateral aminonucleoside nephrosis and nephrotoxic serum nephritis. Lab. Invest. 34:250, 1976.

Hoyer, J.R., and M.W. Seiler. Pathophysiology of Tamm-Horsfall protein. Kidney Int. 16:279, 1979.

Ichikawa, I., and B.M. Brenner. Mechanism of inhibition of proximal tubule fluid reabsorption after exposure of the rat kidney to the physical effects of expansion of the extracellular fluid volume. J. Clin. Invest. 64:1466, 1979.

Idelson, S.A., N. Smithlin, G.W. Smith, and J.T. Harrington. Prognosis in steroid-treated idiopathic nephrotic syndrome in adults. Analysis of major predictive factors after ten-year follow-up. Arch. Int. Med. 137:891, 1977.

Imbasciati, E., L. Cagnoli, N. Case, et al. Controlled study of monthly alternated courses of steroids and chlorambucil in membranous nephropathy (MN). VIIIth Int. Congr. Nephrol., Athens, 1981 CN-066.

Izui, S., P.H. Lambert, and P.A. Miescher. In vitro demonstration of a particular affinity of glomerular basement membrane and collagen for DNA: A possible basis for local formation of DNA-anti-DNA complexes in systemic lupus erythematosus. J. Exp. Med. 144:428, 1976.

Jensen, H., N. Rossing, S.B. Andersen, and S. Jarnum. Albumin metabolism in the nephrotic syndrome in adults. Clin. Sci. 33:445, 1967.

Johnson, K.J., and P.A. Ward. Biology of disease. New concepts in the pathogensis of immune complex-induced tissue injury. Lab. Invest. 47: 218, 1982.

Jones, D.B. Nephrotic glomerulonephrits. Am. J. Pathol. 33:313, 1957.

Kaitz, A.L. Albumin metabolism in nephrotic adults. J. Lab. Clin. Med. 53:186, 1959.

Kelley, V.E., and R.S. Cotran. Mesangial and subepithelial localization of ferritin immune complexes in mouse glomeruli. Lab. Invest. 27:144, 1972.

Kerjaschki, D., and M.G. Farquhar. Immunocytochemical localization of the Heymann nephritis antigen (GP330) in glomerular epithelial cells of normal Lewis rats. J. Exp. Med. 157:667, 1983.

Kida, H., T. Asamoto, and N. Hattori. Long term prognosis of membranous nephropathy. Abst. VIIIth Int. Cong. Nephrol., Athens, 1981, CN073.

Kijlstra, A., V.D.L. Allegonda, D.W. Knutson, et al. The influence of phagocyte function on glomerular localization of aggregated IgM in rats. Clin. Exp. Immunol. 32:207, 1978.

Klassen, J., R.T. McClusky, and F. Milgrom. Non-glomerular renal disease produced in rabbits by immunization with homologous kidney. Am. J. Pathol. 63:333, 1971.

Kleinknecht, C., M. Broyer, and R. Habib. Steroids in idiopathic membranous nephritis. New Eng. J. Med. 296:49, 1977.

Kleinknecht, C., M. Levy, M-F. Gagnadoux, and R. Habib. Membranous glomerulonephritis with extra-renal disorders in children. Medicine 58:219, 1979.

Klouda, P.T., J. Manos, E.J. Acheson, et al. Strong association between idiopathic membranous nephropathy and HLA-DRW3. Lancet Oct. 13, 1979, p. 770.

Kniker, W.T. Amelioration of progressive complex-induced chronic glomerulonephritis by antagonists of vasoactive amines. Fed. Proc. 29:287, 1970 (abstract).

Kniker, W.T., and C.G. Cohcrane. The loaclization of circulating immune complexes in experimental serum sickness: the role of vasoactive amines and hydrodynamic forces. J. Exp. Med. 127:119, 1958.

Kobayashi, Y., S. Tateno, Y. Hiki, et al. A prospective study of prednisone treatment in non-nephrotic patients with idiopathic membranous nephropathy (IMN). VIIIth Int. Cong. Nephrol., Athens, 1981 CN-076.

Kobayashi, Y., S. Tateno, H. Shigematsu, and Y. Hiki. Prednisone treatment in non-nephrotic patients with idiopathic membranous nephropathy. Nephron 30:210, 1982.

Koyama, A., Y. Niwa, H. Shigematsu, et al. Studies on passive serum sickness: II. Factors determining the localization of antigen-antibody complexes in the murine renal glomerulus. Lab. Invest. 38:253, 1978.

Kuriyama, T. Chronic glomerulonephritis induced by prolonged immunization in the rabbit. Lab. Invest. 28:224, 1973.

Lambert, P.H., F.J. Dixon, R.H. Zubler, et al. A collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum. J. Lab. Clin. Immunol. 3:1, 1980.

Laver, M.C., and P. Kincaid-Smith. The natural history and treatment of membranous glomerulonephritis in Glomerulonephritis. P. Kincaid-Smith, T.H. Mathew, E.L. Becker (ed.). John Wiley, New York, p 461, 1973.

Levinsky, R.J. Role of circulating immune complexes in renal diseases. J. Clin. Pathol. 34:1214, 1981.

Levinksy, R.J., Malleson, P.N., T.M. Barratt, and J.F. Soothill. Circulating immune complexes in steroid-responsive nephrotic syndrome. New Eng. J. Med. 298:128, 1978.

Levy, M., M-F Gagnadoux, A. Beziau, and R. Habib. Membranous glomerulonephritis associated with anti-tubular and anti-alveolar basement membrane antibodies. Clin. Nephrol. 10:158, 1978.

Levy, M., and J. Seely. Pathophysiology of edema formation in The Kidney. Brenner, B.M., and F.C. Rector (ed.), W.B. Saunders, Philadelphia, p. 723, 1981.

Libit, S.A., B. Burker, A.F. Michael, and R.L. Vernier. Extramembranous glomerulonephritis in childhood: Relationship to systemic lupus erythematosus. J. Pediat. 88:394, 1976.

Littman, E. Membranous glomerulonephritis: Long-term survival with persistent nephrotic syndrome. J. Urol. 108:680, 1972.

Llach, F., S. Papper, and S.G. Massry. The clinical spectrum of renal vein thrombosis: Acute and chronic. Am. J. Med. 69:819, 1980.

Lyons, S.K., V.W. Pinn, S. Cortell, et al. Allergic interstitial nephritis causing reversible renal failure in four patients with idiopathic nephrotic syndrome. New Eng. J. Med. 288:124, 1973.

MacKenzie, G.M., and W.H. Leake. Relation of antibody and antigen to serum disease susceptibility. J. Exp. Med. 33:601. 1921.

McCluskey, R.T., and B. Benacerraf. Localization of colloidal substances in vascular endothelium. A mechanism of tissue damage: II. Experimental serum sickness with acute glomerulonephritis induced passively in mice by antigen-antibody complexes in antigen excess. Am. J. Pathol. 35: 275, 1959.

McCluskey, R.T., B. Benacerraf, and F. Miller. Passive acute glomerulonephritis induced by antigen-antibody complexes solubilized in hapten excess. Proc. Soc. Exp. Biol. Med. 111:764, 1962.

McCluskey, R.T., B. Benacerraf, J.L. Potter, and F. Miller. The pathologic effects of intravenously administered soluble antigen-antibody complexes: I. Passive serum sickness in mice. J. Exp. Med. 111:181, 1960.

McCluskey, R.T., C.L. Hall, and R.B. Colvin. Immune complex mediated diseases. Human Pathol. 9:71, 1978.

Mannik, M. Physiocochemical and functional relationships of immune complexes. J. Invest. Derm. 74:333, 1980.

Mannik, M., and Arend, W.P. Fate of preformed immune complexes in rabbits and rhesus monkeys. J. Exp. Med. 134:19s, 1971.

Mantel, N. Evaluation of survival data and the new rank order statistics arising in its consideration. Cancer Chemotherapy Reports 50:163, 1977.

Matsumoto, K., K. Osakabe, H. Katayama, et al. Cell-mediated immunity in idiopathic membranous nephropathy. Int. Archs Allergy Appl. Immun. 66:310, 1981.

Matsumoto, K., K. Osakabe, H. Katayama, et al. Concanavalin-A-induced suppressor cell activity in idiopathic membranous nephropathy. Int. Archs Allergy Appl. Immun. 69:26, 1982.

Mauer, S.M., A.J. Fish, E.B. Blau, and A.F. Michael. The glomerular mesangium: I. Kinetic studies of macromolecular uptake in normal and nephrotic rats. J. Clin. Invest. 51:1092. 1972.

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

Mellors, R.C., J. Arias-Stella, M. Seigel, and D. Pressman. Analytical pathology: II. Histopathologic demonstration of glomerular localizing antibodies in experimental glomerulonephritis. Am. J. Pathol. 31:687, 1955.

Mellors, R.C., and W.J. Brzosko. Studies in molecular pathology: I. Localization and pathogenic role of heterologous immune complexes. J. Exp. Med. 115:891, 1962.

Mellors, R.C., M. Siegel, and D. Pressman. Analytical pathology: I. Histochemical demonstration of antibody localization in tissues, with special reference to the antigenic components of kidney and lung. Lab. Invest. 4:69, 1955.

Meroni, P.L., P. Dall'Aglio, G. Balestrieri, et al. Lymphocyte markers and circulating immune complexes in primary glomerulonephritis. Proc. EDTA 16:714, 1979.

Michael, A.F., A.J. Fish, and R.A. Good. Glomerular localization and transport of aggregated proteins in mice. Lab. Invest. 17:14, 1967.

Miller, F., B. Benacerraf, R.T. McCluskey, and J.L. Potter. Production of acute glomerulonephritis in mice with soluble antigen-antibody complexes prepared from homologous antibody. Proc. Soc. Exp. Biol. Med. 104: 706, 1960.

Miller, R.B., J.T. Harrington, C.P. Ramos, et al. Long term results of steroid therapy in adults with idiopathic nephrotic syndrome. Am. J. Med. 46:919, 1969.

Movat, H.Z. and D.D. MacGregor. The fine structure of the glomerulus in membranous glomerulonephritis (lipoid nephrosis) in adults. Am. J. Clin. Pathol. 32:109, 1959.

Müller, G.A., C. Müller, G. Liebau, et al. Strong association of idiopathic membranous nephropathy (IMN) with HLA-DR 3 and MT-2 without involvement of HLA-B 18 and no association to BfF1. Tissue Antigens 17:332, 1981.

Myers, B.D., T.B. Okarma, S. Friedman, et al. Mechanisms of proteinuria in human glomerulonephritis. J. Clin. Invest. 70:732, 1982.

Naruse, T., T. Fukasawa, N. Hirokawa, et al. The pathogenesis of experimental membranous glomerulonephritis induced with homologous nephritogenic tubular antigen. J. Exp. Med. 114:1347, 1976.

Naruse, T., T. Fukasawa, S. Umegae, et al. Experimental membranous glomerulonephritis in rats. Correlation of ultrastructural changes with the serum level of autologous antibody against tubular antigen. Lab. Invest. 39:120, 1978.

Naruse, T., D. Kitamura, Y. Miykawa, and S. Shibata. Deposition of renal tubular epithelial antigen along the glomerular capillary walls of patients with membranous glomerulonephritis. J. Immunol. 110:1163, 1973.

Naruse, T., Y. Miyakawa, K. Kitamura, and S. Shibata. Membranous glomerulonephritis mediated by renal tubular epithelial antigen-antibody complexes. J. Allerg. Clin. Immunol. 54:311, 1974.

Nöel, L.H., M. Zanetti, D. Droz, et al. Long term prognosis of idiopathic membranous glomerulonephritis. Study of 116 untreated patients. Am. J. Med. 66:82, 1979.

Nyberg, M., E. Pettersson, G. Tallqvist, and A. Pasternack. Survival in idiopathic glomerulonephritis. Acta Path. Microbiol. Scand. 88:319, 1980.

Nydegger, U.E., P.H. Lambert, H. Gerber, and P.A. Miecher. Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen. J. Clin. Invest. 54:297, 1974.

Okumura, K., Y. Kondo, and T. Tada. Studies on passive serum sickness: I. The glomerular fine structure of serum sickness nephritis induced by pre-formed antigen-antibody complexes in the mouse. Lab. Invest. 24:383, 1971.

Olbing, H.J., I. Greifer, B.P. Bennett, et al. Idiopathic membranous nephropathy in children. Kidney Int. 3:381, 1973.

Ooi, B.S., Ooi, Y.M., Hsu, A., et al. Diminished synthesis of immunoglobulin by peripheral lymphocytes of patients with idiopathic membranous glomerulopathy. J. Clin. Invest. 65:789, 1980.

Ooi, B.S., Y.M. Ooi, and V.E. Pollak. Identification of circulating immune complexes in a subpopulation of patients with membranous glomerulonephropathy. Clin. Immunol. Immunopathol. 16:447, 1980.

Ooi, Y.M., B.S. Ooi, and V.E. Pollak. Relationship of levels of circulating immune complexes to histologic patterns of nephritis: A comparative study of membranous glomerulonephropathy and diffuse proliferative glomerulonephritis. J. Lab. Clin. Med. 90:891, 1977.

Ooi, Y.M., B.S. Ooi, E.H. Vallota, et al. Circulating immune complexes after renal transplantation. J. Clin. Invest. 60:611, 1977.

Ooi, Y.M., E.H. Vallota, and C.D. West. Serum immune complexes in membranoproliferative and other glomerulonephridities. Kidney Int. 11:275, 1977.

O'Regan, S., M. Smith, and K.N. Drummond. Immune complex infusion in the rat: Renal functional and morphological changes. Clin. Exp. Immunol. 24:110, 1976.

Ozawa, T., M.F. Mass, S. Guggenheim, et al. Autologous immune complex nephritis associated with sickle cell trait: Diagnosis of the hemoglobinopathy after renal structural and immunological studies. Br. Med. J. 1:369, 1976.

Ozawa, T., R. Pluss, J. Lacher, et al. Endogenous immune complex nephropathy associated with malignancy: I. Studies on the nature and immunopathogenic significance of glomerular bound antigen and antibody, isolation and characterization of tumor specific antigen and antibody and circulating immune complexes. Q. J. Med. 44:523, 1975.

Pappenheimer, J.R. Passage of molecules through capillary walls. Physiol. Rev. 33:387, 1953.

Pascal, R.R., C.S. Sian, J.M. Brensilver, et al. Demonstration of an antibody to tubular epithelium in glomerulonephritis associated with obstructive uropathy. Am. J. Med. 69:944, 1980.

Pierides, A.M. and D.N.S. Kerr. Idiopathic membranous nephropathy. Nephron 20:301, 1978.

Pierides, A.M., P. Malasit, A.R. Morley, et al. Idiopathic membranous nephropathy. Q. J. Med. 46:163, 1977.

Pincus, T., R. Haberkern, and D.L. Christian. Experimtnal chronic glomerulitis. J. Exp. Med. 127:891, 1968.

Pollak, V.E., C.L. Pirani, and D.H. Clyne. The natural history of membranous glomerulonephropathy in Progress In Glomerulonephritis. P. Kincaid-Smith (ed.) p. 429, 1979.

Pollak, V.E., S. Rosen, C.L. Pirani, et al. Natural history of lipid nephrosis and of membranous glomerulonephritis. Ann. Int. Med. 67: 1171, 1968.

Pussell, B.A., C.M. Lockwood, D.M. Scott, et al. Value of immune-complex assays in diagnosis and management. Lancet Aug. 12, 1978, p. 359.

Ramzy, M.H., J.S. Cameron, D.R. Turner, et al. The long-term outcome of idiopathic membranous nephropathy. Clin. Nephrol. 16:13, 1981.

Rastogi, S.P., J. Hart-Mercer, and D.N.S. Kerr. Idiopathic membranous glomerulonephritis in adults: Remission following steroid therapy. Quart. J. Med. 38:335, 1969.

Rennke, H.B., R.S. Cotran, and M.A. Venkatachalam. Role of molecular charge in glomerular permeability. Tracer studies with cationized ferritins. J. Cell. Biol. 67:638, 1975.

Rennke, H.G., Y. Patel, and M.A. Venkatachalam. Glomerular filtration of proteins: Clearance of anionic, neutral, and cationic horseradish peroxidase in the rat. Kidney Int. 13:278, 1978.

Rennke, H.G., and M.A. Venkatachalam. Glomerular permeability: In vivo tracer studies with polyanionic and polycationic ferritins. Kidney Int. 11:44, 1977.

Rennke, H.G., and M.A. Venkatachalam. Glomerular permeability of macromolecules. Effect of molecular configuration on the fractional clearance of uncharged dextran and neutral horseradish peroxidase in the rat. J. Clin. Invest. 63:713, 1979.

Robinson, M.F., J.L. Roberts, J.V. Jones, and E.J. Lewis. Circulating immune complex assays in patients with lupus and membranous glomerulonephritis. Clin. Immunol. Immunopathol. 14:348, 1979.

Rosen, S. Membranous glomerulonephritis: current status. Human Pathology 2:209, 1971.

Rossen, R.D., M.A. Reisberg, D. Singer, et al. The effect of age on the character of immune complex disease: A comparison of the incidence and relative size of materials reactive with C1q in sera of patients with glomerulonephritis and cancer. Medicine (Baltimore) 58:65, 1979.

Rossen, R.D., M.A. Reisberg, D.B. Singer, et al. Soluble immune complexes in sera of patients with nephritis. Kidney Int. 10:256, 1976.

Rothschild, M.A., M. Oratz, and S.S. Schreiber. Albumin synthesis. New Eng. J. Med. 286:816, 1972.

Row, R.G., J.S. Cameron, D.R. Turner, et al. Membranous nephropathy. Long term follow-up and association with neoplasia. Q. J. Med. 44: 207, 1975.

Ryan, G.B., S.J. Hein, and M.J. Karnovsky. Glomerular permeability to proteins: Effects of hemodynamic factors on the distribtuion of endogenous immunoglobulin G and exogenous catalase in the rat glomerulus. Lab. Invest. 34:415, 1976.

Salant, D.J., S. Belok, M.M. Stilmant, et al. The role of complement (C) in proteinuria induced by in situ glomerular immune deposit (ID) formation. Kidney Int. 14:717, 1978 (Abstract).

Salant, D.J., S. Belok, M.M. Stilmant, et al. Determinants of glomerular localization of subepithelial immune deposits: Effects of altered antigen: antibody ratio, steroids, vasoactive amine antagonists and aminonucleocide of puromycin on passive Heymann nephritis in rats. Lab. Invest. 48: 89, 1979.

Salant, D.J., C. Darby, and W.G. Couser. A quantitative analysis of subepithelial immune deposit (SID) formation in "in situ" immune complex glomerulonephropathy (ICGN). Kidney Int. 14:717, 1978 (Abstract).

Schoenfeld, L.S., and R.J. Glassock. Renal tubular antigen excretion in normal urine: I. Immunochemical identification. Kidney Int. 3:309, 1973.

Schur, P.H. Immune-complex assays: The state of the art. New Eng. J. Med. 298:161, 1978.

Seiler, M.W., and J.R. Hoyer. Altered in situ formation of complexes in experimental nephrotic syndrome. Kidney Int. 14:718, 1978 (Abstract).

Seiler, M.W., H.G. Rennke, M.A. Venkatachalam, and R.A. Cotran. Pathogenesis of polycation-induced alteration ("fusion") of glomerular epithelium. Lab. Invest. 36:48, 1975.

Seiler, M.W., M.A. Venkatachalam, and R.S. Cotran. Glomerular epithelium: Structural alterations induced by polycations. Science 189:390, 1975.

Sellers, A.L., J. Katz, and S. Rosenfeld. Plasma albumin catabolism in experimental nephrosis. Nature 192:562, 1961.

Shearn, M.A., J. Hopper, and C.G. Biava. Membranous lupus nephropathy initially seen as idiopathic membranous nephropathy. Arch. Int. Med. 140:1521, 1980.

Shin, M.L., M.C. Gelfand, R.B. Nagel, et al. Localization of receptors for activated complement on visceral epitheial cells on the human renal glomerulus. J. Immunol. 118:869, 1977.

Simpson, I.J., P.B. Doak, I.C. Williams, et al. Circulating immune complexes in primary glomerulonephritis and systemic lupus erythematosus. Aust. NZ J. Med. 8:241, 1978 (Abstract).

Solling, J., and S. Olsen. Circulating immune complexes in glomerulonephritis. Clin. Neph. 16:63, 1981.

Solling, J. Molecular weight of circulating immune complexes in patients with glomerulonephritis. Nephron 30:137, 1982.

Stachura, I., Whiteside, and R.H. Kelly. Circulating and deposited immune complexes in patients with glomerular disease. Immunopathologic correlations. Am. J. Pathol. 103:21, 1981.

Stack, J.I., and P.M. Zabetakis. Erythrocytosis associated with idiopathic membranous glomerulopathy. Clin. Nephrol. 12:87, 1979.

Starling, E.H. On the absorption of fluid from the connective tissue space. J. Physiol. (London) 19:312, 1896.

Stilmant, M.D., W.G. Couser, and R.S. Cotran. Experimental glomerulonephritis in the mouse associated with mesangial deposition of autologous ferritin immune complexes. Lab. Invest. 32:746, 1975.

Strauss, J., V. Pardo, M. Koss, et al. Nephropathy associated with sickle cell anemia: an autologous immune complex nephritis: I. Studies of the nature of the glomerular bound antibody and antigen identification in a patient with sickle cell disease and immune deposit glomerulonephritis. Am. J. Med. 58:382, 1975.

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

Suki, W.N., and A. Chavez. Membranous nephropathy: Response to steroids and immunosuppression. Am. J. Nephrol. 1:11, 1981.

Szabó, T., M. Szabó, Á. Lenkey, and J. Szabó. Circulating immune complexes and phogocytic activity of polymorphonuclear cells in patients with glomerulopathies. Clin. Neph. 12:243, 1979.

Takekoshi, Y., M. Tanaka, N. Shida, et al. Strong association between membranous nephropathy and hepatitis-B surface antigenaemia in Japanese children. Lancet Nov. 18, 1978, p. 1065.

Takekoshi, Y., Tanaka, M., Y. Miyakawa, et al. Free "small" and IgG-associated "large" hepatitis Be antigen in the serum and glomerular capillary walls of two patients with membranous glomerulonephritis. New. Eng. J. Med. 300:814, 1979.

Theofilopoulos, A.N., B.S. Andrews, M.M. Urist, et al. The nature of immune complexes in human cancer serum. J. Immunol. 199:657, 1977.

Theofilopoulos, A.N. and F.J. Dixon. Immune complexes in human diseases. A review. Am. J. Pathol. 100:531, 1980.

Tiller, D.J., A.R. Clarkson, T. Mathew, et al. A prospective randomized trial in the use of cyclophosphamide, dipyridamole and warfarin in membranous and mesagniocapillary glomerulonephritis. Proc. VIIIth Int. Cong. Nephrol., Athens, p. 345, 1981.

Trevillian, P., and J.S. Cameron. Interaction of anti-GBM antibody and immune complexes in rabbits. Kidney Int. 14:295, 1978 (Abstract).

Trew, P.A., C.G. Biava, R.D. Jacobs, et al. Renal vein thrombosis in membranous glomerulonephropathy: Incidence and association. Medicine 57:69, 1978.

Tung, S.K., A.J. Woodroffe, T.D. Ahlin, et al. Application of the solid phase Clq and Raji cell radioimmune assays for the detection of circulating immune complexes in glomerulonephritis. J. Clin. Invest. 62:61, 1978.

Unanue, E.R., and F.J. Dixon. Experimental glomerulonephritis: V. Studies on the interaction of nephrotoxic antibodies with tissues of the rat. J. Exp. Med. 121:697, 1965.

Unanue, E.R., and F.J. Dixon. Experimental glomerulonephritis: Immunological events and pathogenetic mechanisms. Advances in Immunology 6:1, 1967.

Van Damme, B.J.C., G.J. Fleuren, W.W. Bakker, et al. Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens: IV. Fixed glomerular antigens in the pathogenesis of heterologous immune complex glomerulonephritis. Lab. Invest. 38:502, 1978.

Van Es, L.A., A.P.R. Blok, L. Schoenfeld, and R.J. Glassock. Chronic nephritis induced by antibodies reacting with glomerular-bound immune complexes. Kidney Int. 11:106, 1977.

Venkatachalm, M.A., and H.G. Rennke. The structural and molecular basis of glomerular filtration. Circ. Res. 43:337, 1978.

Weiser, R.S., and C. Laxon. The fate of fluorescein-labelled soluble antigen-antibody complex in the mouse. J. Infect. Dis. 111:55, 1962.

Whitworth, J.A., S. Leibowitz, M.C. Kennedy, et al. Absence of glomerular renal tubular epithelial antigen in membranous glomerulonephritis. Clin. Nephrol. 5:159, 1976.

Wilson, C.B. Recent advances in the immunological aspects of renal disease. Fed. Proc. 36:2171, 1977.

Wilson, C.B. Nephritogenic antibody mechanisms involving antigens within the glomerulus. Immunol. Rev. 55:257, 1981.

Wilson, C.B., and F.J. Dixon. Quantitation of acute and chronic serum sickness in the rabbit. J. Exp. Med. 134:75, 1971.

Wilson, C.B., and F.J. Dixon. Diagnosis of immunopathologic renal disease. Kidney Int. 5:389, 1974.

Wilson, C.B., and F.J. Dixon. The renal response to immunological injury in The Kidney. B. Brenner and F. Rector (ed.) W.B. Saunders, Philadelphia, 1976.

Woodroffe, A.J., W.A. Border, A.N. Theofilopoulos, et al. Detection of circulating immune complexes in patients with glomerulonephritis. Kidney Int. 12:268, 1977.

Woodroffe, A.J., M. Foldes, P.E. McKenzie, et al. Serum immune complexes and disease. Aust. N.Z.J. Med. 9:129, 1979.

Wright, N.G., W.I. Morrison, H. Thompson, and H.G.C. Cornwall. Experimental adenovirus immune complex glomerulonephritis. Br. J. Exp. Pathol. 54:528, 1973.

Zager, R.A., W.G. Couser, B.S. Andrews, et al. Membranous nephropathy: A radioimmunologic search for anti-renal tubular epithelial antibodies and circulating immune complexes. Nephron 24:10, 1979.

Collaborative study of the adult nephrotic syndrome. A controlled study of short-term prednisone treatment in adults with membranous nephropathy. New Eng. J. Med. 301:1301, 1970.

Controlled trial of azathioprine in the nephrotic syndrome secondary to idiopathic membranous glomerulonephritis. Western Canadian Glomerulonephritis Study Group. CMA Journal 115:1209, 1976.

Medical Research Council Working Party. Controlled trial of azathioprine and prednisone in chronic renal disease. Br. Med. J. 2:239, 1971.