Kidney International, Vol. 15 (1979), pp. 88–103

Pathogenesis and treatment of membranous nephropathy

Principal Discussant: J. STEWART CAMERON

Department of Renal Medicine, Guy's Hospital, London, England

The *Nephrology Forum* is designed to relate the principles of basic science to clinical problems in nephrology.

Editors
JORDAN J. COHEN
JOHN T. HARRINGTON
JEROME P. KASSIRER

New England Medical Center Hospital
Boston, Massachusetts

Case presentation

A 19-year-old woman was admitted to New England Medical Center Hospital (NEMCH) for evaluation of the nephrotic syndrome. The patient was in excellent health until proteinuria was first detected 10 months earlier during the third month of her first pregnancy. Twenty-four-hour urine protein excretion remained in the 5 to 10 g range, and progressive pedal, hand, and periorbital edema developed. During the final weeks of the pregnancy, hypertension was present (systolic blood pressure, 140 to 150 mm Hg; diastolic blood pressure, 90 to 96 mm Hg). A healthy infant girl was delivered approximately 3 months prior to admission. Following delivery, the edema persisted. The patient's serum albumin concentration varied between 3.1 and 2.0 g/100 ml, serum creatinine concentration remained normal, and 24-hr urine protein excretion varied between 4 and 15 g.

There was no history of dysuria, gross hematuria, urinary frequency, flank pain, or fever. There was no history of previous streptococcal infections or prior renal disease. She had no known allergies and was using no medications.

On admission to NEMCH, the patient was in no distress. The physical examination revealed the following: blood pressure, 132/80 mm Hg; pulse, 60/min without postural change; respirations, 12/min; and temperature, 37° C; the head, eyes (including optic fundi), ears, nose, and throat were unremarkable; the chest was clear to auscultation and percussion. The cardiac examination and peripheral pulses were normal. The abdominal, pelvic, and rectal examinations were unremarkable. Presacral edema and pitting edema (2+) from the knees to the ankles were present. The neurologic examination was within normal limits. There were no skin rashes, petechiae, or eruptions present. Laboratory findings disclosed the following data: hemoglobin, 14.5 g/100 ml; white blood cell count, 10,400 mm^3 with a normal differential; platelet count, 265,000 mm^3; serum creatinine, 0.9 mg; blood urea nitrogen, 11 mg/100 ml; serum sodium, 141 mEq; serum potassium, 4.3 mEq; serum chloride, 107 mEq; serum bicarbonate, 26 mEq/liter; total serum protein, 5.5 g; serum albumin, 3 g; serum cholesterol, 315 mg; serum calcium, 9.1 mg; serum phosphorus, 4.4 mg; and serum uric acid, 5.3 mg/100 ml. Liver enzymes were normal. Fasting and 2-hr postprandial blood sugar concentrations were normal. Results of chest x-ray and electrocardiogram were unremarkable. Results of urinalysis revealed the following: specific gravity, 1.023; pH, 6; protein 4+; no glucose; white blood cells, 10 to 15/high power field (HPF); red blood cells, 4 to 5/HPF; no casts were present. Two urine cultures revealed less than 30,000 colonies of staph species and diptheroids. The 24-hr urine protein excretion was 4.4 g. An i.v. urogram revealed kidneys of normal size with prompt bilateral function and no evidence of obstruction. An antinuclear antibody test was negative, and serologic tests for rheumatoid factor, syphilis, and streptozyme level were negative. The total hemolytic complement activity was 195 U (normal, 150 to 250 U) and the complement 3 (C3) concentration was 0.73 mg/ml (normal, 0.87 to 2.2 mg/ml).

Results of an open renal biopsy revealed membranous nephropathy (Fig. 1).

The patient was entered into the interhospital Study of Adult Idiopathic Nephrotic Syndrome (120 mg/day of prednisone versus placebo). The patient began her assigned "medication" (which later was disclosed to be placebo) 2 months after admission and continued for 8 weeks. She developed mild-to-moderate acne and gained weight. At the end of the treatment period, the serum creatinine concentration was unchanged at 0.8 mg/100 ml, and the serum albumin concentration remained at 2.8 g/100 ml; the 24-hr urine protein excretion was 15 g. Since there was no evidence of any response to therapy, the medication was discontinued in accordance with the experimental protocol.

Presently, approximately 3.5 years after biopsy, the patient is in the second trimester of her second pregnancy and doing well clinically although she continues to have heavy proteinuria and significant hypoalbuminemia. Her renal function, however, as measured by her serum creatinine concentration of 0.6 mg/100 ml, remains normal and she is normotensive.

Discussion

DR. J. S. CAMERON: This woman presented at age 19 approximately 4 years ago. She had proteinuria in the nephrotic range then and since, but edema only during her pregnancy. One of the questions that arises immediately is, How far can one work up

Presentation of the Forum is made possible by grants from Hoechst-Roussel Pharmaceuticals Inc., Smith Kline & French Laboratories, Burroughs Wellcome Company, Ciba Pharmaceutical Company, and Boehringer Ingelheim Ltd.

0085-2538/79/0015-0088 $03.20

such a patient in the middle of pregnancy? Clearly, there was no great problem for this patient because she went through to a full-term normal delivery. One can, of course, do a needle renal biopsy during pregnancy in the sitting position if necessary. The problem of localizing the kidneys is increased, however, because of the radiation dose to the fetus. Like the physicians treating this patient, we tend not to biopsy during pregnancy, but to wait for delivery and then do the full investigation. One of the other questions that arises at this point is, How long had she had proteinuria. As with so many patients with glomerular disease, the answer is that we don't know. All we can say is that the condition was found at this point, and we can suspect rather strongly that it preceded her pregnancy and was not precipitated by it. First pregnancy is one of the occasions, like induction into the Armed Forces or insurance examinations, when we discover proteinuria. When we talk about follow-up and we show long-term survival statistics, we have to remember that these are data all related to the *apparent clinical onset* of the disease. The biological onset of the disease is something we never know.

A renal biopsy was done early in the patient's workup and showed according to the report typical optical and immunofluorescent findings of membranous nephropathy. I thought perhaps we might pause and review this biopsy first.

DR. S. ROSEN (*Beth Israel Hospital, Boston*): The renal biopsy, obtained through an open surgical procedure, was an excellent specimen containing much renal cortex (Fig. 1). The glomerular tufts had apparent diffuse thickening of peripheral capillary walls and mild mesangial hypercellularity accompanied by matrical increase. In scattered glomeruli the process was more severe, and segmental capillary lumenal obliteration, adhesions, and fibrocellular crescents were observed. A typical spike pattern of the glomerular capillary wall was noted in silver methenamine preparations. Focal interstitial fibrosis, tubular atrophy, and chronic inflammation were seen; the vessels were unremarkable. Immunofluorescent findings were that of a glomerular 3+ IgG coarse peripheral granular pattern with a slightly less intense B_1C and a trace to 1+ IgM deposition in a similar distrubution. The 1μ epon sections stained with methylene blue-azure II-basic fuschin clearly delineated glomerular capillary subepithelial deposits. Electron microscopic studies disclosed diffuse subepithelial electron-dense deposits and extensive foot process fusion; no subendothelial or mesangial deposits were recognized. Mesangial hypercellularity and matrical increase, as seen by light microscopy, were also noted. In conclusion, this pattern is that of membranous nephropathy with mild proliferation and focal sclerosis.

DR. J. S. CAMERON: This biopsy showed mesangial hypercellularity and segmental sclerosing lesions. I think most people now would accept both these features [1] as complications or associated features of membranous nephropathy, the latter especially in advanced cases. On the other hand, the combination of these observations makes one wonder, based on the findings of optical microscopy alone, about systemic lupus erythematosus (SLE). I think perhaps we might usefully discuss at this point what is for or against SLE in this particular biopsy and how to tell the difference between an SLE membranous biopsy and an idiopathic one.

Does this patient have SLE? The obvious answer is to do the appropriate tests: look for antibodies to double-stranded native DNA by either the Farr Test or the more recently introduced *Crithidia Luciliae* kinetoplast test, which is cheap, quick, and easy as opposed to the Farr Test. Finally, our decision as to whether or not a patient with no obvious clinical stigmata of SLE can be called SLE depends on this information. I assume that the tests in this patient were and remain negative, and that she does *not* have SLE. Usually, even in a patient with SLE membranous nephropathy, there are some subendothelial or mesangial deposits. Pure membranous nephropathy, however, *can* occur in SLE without any mesangial or subendothelial deposits. Can immunofluorescence help us to distinguish SLE membranous from idiopathic membranous nephropathy? The answer is yes, but only to a limited extent. Most of us expect to find a "full house" of immunoglobulins—IgG, IgM, and IgA—together with early components of complement, such as Clq and C4 [2], which were not looked for in this particular biopsy. Their absence makes one suspicious that the patient does not have SLE. This isn't an invariable rule, and certainly we have some patients who seem to have idiopathic membranous nephropathy with a "full house" of immunoglobulins—about 20–30% of our patients. Of 42 of our patients with idiopathic membranous nephropathy, all biopsies showed IgG, and 75% showed C3, as everyone reports. About 30% of patients had IgA, or IgM, or both; about 30% had fibrin deposition; and 20% had early complement components. Thus, if the kidney biopsy in a patient who appears to have idiopathic membranous nephropathy shows all complement components and immunoglobulins, it raises the

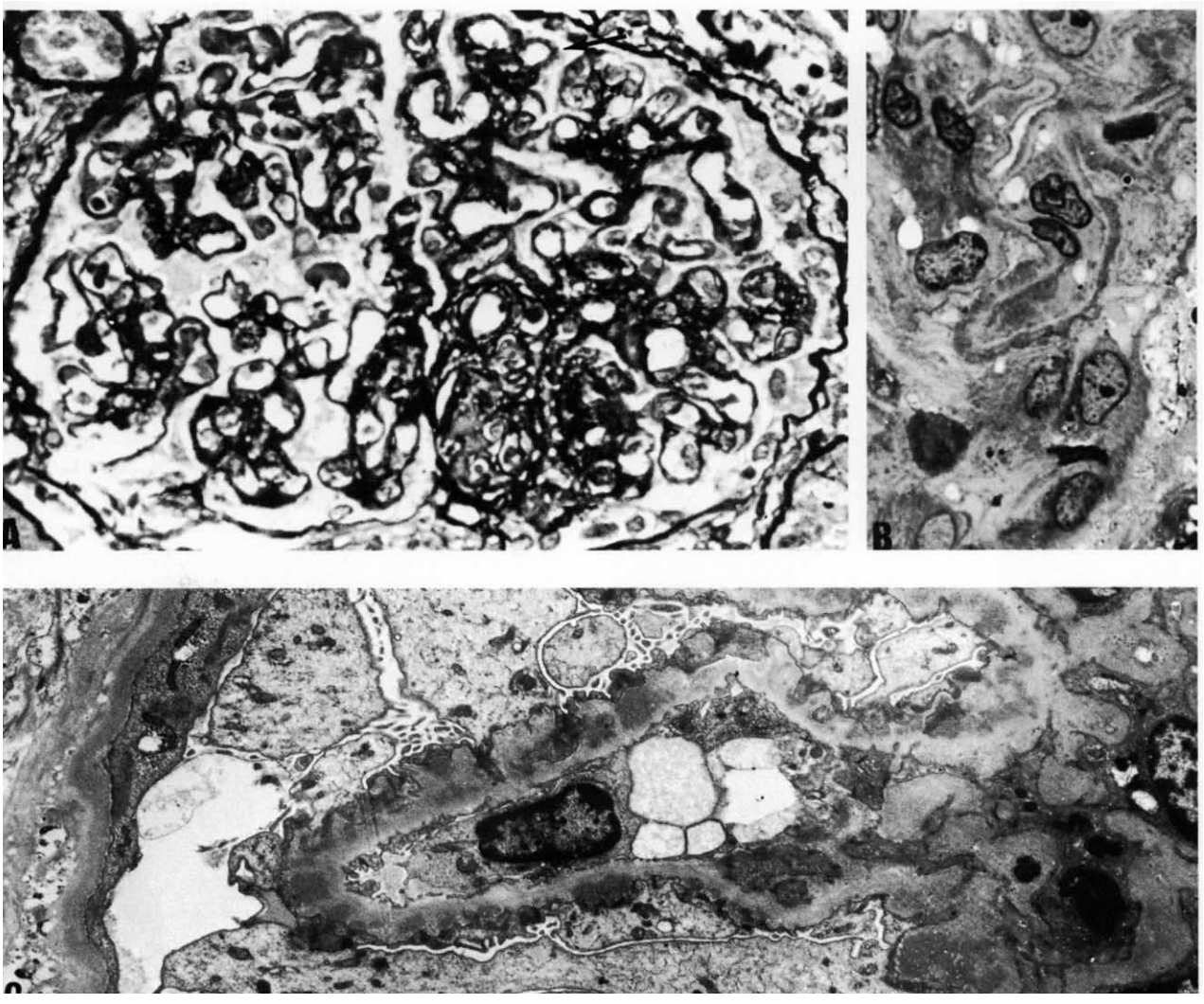

Fig. 1. *Renal biopsy. (A)* In this silver methenamine preparation, segmental glomerular sclerosis, mild mesangial hypercellularity, and a focal basement membrane spike pattern *(arrow)* can be observed (×570). *(B)* The subepithelial deposits are distinct in the stained 1 μ epon section (×1068). *(C)* Subepithelial electron dense deposits, basement membrane projections, and extensive foot fusion are noted in this electron micrograph (×3204).

question of whether the patient has SLE. IgA was absent in the patient presented today.

I assume that this patient was screened for Australian antigenemia [3], and that she is not C2 deficient [4], which are two clinical associations one can detect. I am just mentioning these because later on we will be talking about the circumstances associated with membranous nephropathy. Our own series of membranous nephropathy patients is now just around one hundred patients: two-thirds male and one-third female. A woman 19 years old is a prime subject for SLE. We should not forget that idiopathic membranous nephropathy with no tests positive for SLE may evolve into florid clinical and immunological SLE [5, 6]. We had two such cases, and I think this possibility justifies doing tests for SLE and complement concentrations not just once, but from time to time.

We should also ask whether this biopsy falls into the group described by Burkholder, Hyman, and Krueger [7] as mixed membranous and proliferative glomerulonephritis, *type* III. The study described a small group of patients with mesangial proliferation, sometimes some segmental accentuation of the proliferation, and not only epimembranous deposits but also subendothelial and mesangial deposits. Since we have seen the electron microscopy, we can say that the present patient doesn't fit into this

category. Had we not had that available to us, I think we might have just wondered whether she was one of this group of patients. Because they have been little discussed, I thought I would briefly mention our experience with ten such patients. All their tests for SLE were negative, all their complement concentrations were normal, and they did not have the complement abnormalities we associate with mesangiocapillary glomerulonephritis (MCGN). Rather than consider this disorder an extra variety of MCGN or membranoproliferative glomerulonephritis (MPGN), we regard it as a separate variety: "glomerulonephritis with deposits at multiple sites." Both of the well-known forms of MCGN, of course, may show some subepithelial deposits, but these are rather infrequent. Nobody talks about these patients, and they are excluded from published papers. Some regard them as an atypical form of membranous nephropathy.

Could we have diagnosed membranous nephropathy on clinical grounds? I don't think there are any specific characteristics in the differential diagnosis of nephrotic patients that will allow you to pick out membranous patients from the others. Patients with membranous nephropathy are more often male, usually have microscopic hematuria as this patient did, and even in mild cases differential protein clearances are usually nonselective. Renal function and blood pressure may both be normal or abnormal. Recently, two attempts to predict histologic data on clinical grounds have been published. One was in adults using a sequential Bayesian analysis [8], and the other was in children using discriminant function analysis [9]. Both suggested that you could progress a long way clinically toward discriminating among different patients using these analyses on all the data available. I think that while this may be possible in children, our current attitude is that all adult nephrotics should have renal biopsy as part of their evaluation, if only so that we can give the patient or relatives a more accurate prognosis apart from the question of treatment. I would be interested in learning your views about renal biopsy in nephrotic patients.

The patient under discussion was entered in the collaborative Study of Adult Idiopathic Nephrotic Syndrome [10], and we will hear more about this later. I gather that despite developing acne and gaining weight, she was receiving a placebo! Four years since she was first documented as having proteinuria, her renal function remains within normal limits, and profuse proteinuria without edema persists. Now she is pregnant again with delivery expected in a few months, and I think we might pause to consider the management of the nephrotic patient who becomes pregnant. During the second trimester, the patient was still normotensive without edema, but had urinary protein excretion of 3.2 g/24 hr. Her serum creatinine concentration was only 0.6 mg/100 ml, which I take as an indication that she had achieved the supranormal glomerular filtration rate (GFR) and creatinine clearance that one would expect as part of the normal second trimester of pregnancy. Her urine is being cultured every month as a routine appropriately. There are few data on this. In one study, however, of 31 pregnancies in 19 patients with the nephrotic syndrome [11], 11 were complicated by bacteriuria, or overt clinical infections, or both, which is of course a much higher figure than the 7% or so that you expect in normal pregnant women. I am frankly not very worried about a patient like this in pregnancy [11, 12], so long as the patient has normal renal function and above all normal blood pressure. I think previous reports in the literature on pregnancy and renal disease have not clearly distinguished the different effects of different renal diseases and the effects of hypertension and diminished renal function on pregnancy. In fact, renal function isn't necessary to become pregnant and carry to term. We (and others) have had a patient on dialysis who delivered a normal child [13]. The dangerous thing is *hypertension*, especially if it is present before the pregnancy begins and above all if it is accompanied, as it was fortunately not in the case of this patient, by reduced renal function. Patients with GFR of around 40 ml/min and fairly vicious hypertension are, to my mind, about the limit at which one can expect to get a pregnancy through to an early conclusion with a live baby. In patients with membranous nephropathy, our own experience and that in the literature [11, 13] is very good. Very few patients have aborted or developed severe hypertension and lost the baby, and I would hope that this patient's second pregnancy will be as successful as the first one was. There is an association between hypoalbuminemia and small babies [11], and since her serum albumin concentration is low (3 g/100 ml), we can expect her baby to be a little smaller than normal. I don't think this indicates dysmaturity or that all of these women are having their babies early; I suspect that it simply reflects the fact that the infants are slightly protein-malnourished, although there is no good evidence for this.

I think this patient will go to term and have a normal baby because she has been "road-tested" once

already and both her renal function and blood pressure are normal. We will discuss her long-term outlook when we discuss membranous nephropathy in general.

Are there any questions so far?

DR. J. T. HARRINGTON: Of interest is your comment about the long time-lag between the onset of apparently idiopathic membranous nephropathy and the subsequent development of SLE in some patients. Could you be more specific?

DR. J. S. CAMERON: Well, one of our patients took between 2 and 3 years, and Libit et al [5] document cases of 1, 3, and 5 years. Simenhoff and Merril's [6] patients took 5 months and 7 years. One of these was a C2 deficient patient, whose course they had earlier published as an association between C2 deficiency and membranous nephropathy. Of course, if the possibility of SLE had not been investigated adequately, some of these patients could have had SLE all the time. I have only mentioned patients in whom it was carefully assessed at the beginning of their illness.

DR. J. J. COHEN: Dr. Coggins, does your recent Study of Adult Idiopathic Nephrotic Syndrome [10] indicate whether or not patients with membranous nephropathy can continue longer than 5 years and then develop SLE.

DR. C. H. COGGINS (*Massachusetts General Hospital, Boston*): There were three patients in the study in whom pathologic reports indicated SLE from the outset, but the serologic tests were negative. Within a fairly short period of time in each case the serologic tests became positive. The patients did not, however, have membranous nephropathy; they had "mixed deposit disease."

DR. J. S. CAMERON: We looked very hard at the pathologic reports of a patient with atypical membranous nephropathy who subsequently developed SLE. She had only subepithelial deposits on the plastic-embedded material. We looked carefully at the mesangium and the subendothelial space, and no deposits were seen initially. Our pathologist commented on the great irregularity in size and number of the deposits around the different capillary loops in our patients, compared with the monotonous regularity of the typical idiopathic membranous biopsy. He suspected SLE, and he was later proven to be right.

Does anyone feel that the patient under discussion should have been biopsied in pregnancy?

DR. J. T. HARRINGTON: We were not caring for this patient at the time of her first pregnancy, but we have never biopsied a patient during pregnancy. In answer to your earlier question about biopsying adults with nephrotic syndrome, I believe most nephrologists in the United States prefer to biopsy virtually all adult patients with nephrotic syndrome, except those with diabetes.

DR. J. S. CAMERON: Perhaps I might add what we do in patients with diabetes. We require some clinical clue, such as the absence of retinopathy or the presence of persistent microscopic hematuria, that suggests something else besides the presence of diabetic nephropathy. Undoubtedly, if you do biopsy these suspicious patients with diabetes, then you find a good portion of them do not have diabetic nephropathy. Wass et al [14] think that most units are biopsying too few patients with diabetic nephropathy. There is no real answer to this. Certainly a lot of *clinically* unnecessary biopsies would be performed if every diabetic with proteinuria were biopsied.

Churg has found a very high incidence of diabetes in his series of patients with membranous nephropathy [15]. In contrast, we only have 1 patient with clinical diabetes in 100 patients with membranous nephropathy. Clearly, the New York population differs in this respect from the London population, or the selection of patients differs markedly.

DR. C. H. COGGINS: That incidence would, of course, depend heavily on whether you decide to biopsy patients with diabetes. An abnormal glucose tolerance test eliminates a patient from our study. I might add that in trying to decide whether to biopsy patients with diabetes we also use the absence of retinopathy as a clinical clue. Recently, a patient in our hospital, who had no retinopathy detectable by fluorescein study, had a nephrectomy (before transplantation) and had clear histologic findings of diabetic nephropathy.

DR. J. S. CAMERON: Rather than discussing in detail the histopathology of membranous nephropathy, which is interesting but very well-documented [16], I want to talk more about what we do and do not know about the immunopathogenesis of this disease. Membranous nephropathy can be found in a variety of circumstances, some of which suggest that like other forms of glomerulonephritis it may be a consequence of glomerular deposition of circulating soluble complexes. Until recently this seemed to be the likely explanation for the pathogenesis of this condition. There are two salient features in membranous nephropathy, however, that require an explanation. *First*, most investigations fail to find, by the various techniques for detecting circulating immune complexes, material in many patients

with membranous nephropathy [17, 18, 19]. The highest percentage I have been able to find is in Border's [20] series from the collaborative Study of Adult Idiopathic Nephrotic Syndrome in which, by one method at least out of the three they employed, almost half the patients with membranous nephropathy were shown to have complex-like material. Most people score less than 20%, and some zero [17]. *Second*, despite the glomerular deposition of immunoglobulins and complement components, as in other forms of nephritis, there is a striking absence of obvious glomerular proliferation in the majority of patients.

The relationship of membranous nephropathy to possible immune complex deposition is a fascinating one. First, we must consider Dixon and Germuth's work. It was demonstrated that a pattern remarkably like that of membranous nephropathy could be induced in rabbits given chronic serum sickness by repeated daily injections of a foreign protein using a constant low-dosage schedule (2.5 mg/day) [21, 22] Germuth showed further that the deposition of complexes in the peripheral capillaries giving a membranous-like pattern was associated with the presence in the circulation of smaller immune complexes. In contrast, rabbits given foreign protein according to different schedules showed deposition of larger complexes principally in the mesangium. You will note that I have avoided saying that these complexes were necessarily being deposited in the glomeruli; but they were in the circulation, and Germuth presumed like many other investigators that this was indeed the case. Further study suggests that the membranous pattern was associated with small quantities of complexes in the circulation [22]. Kuriyama [23], working with ovalbumin-induced nephritis in rabbits, and Koyama et al [24], who injected performed complexes of bovine serum albumin into mice, suggested that the animals, which produced principally low avidity antibody, showed peripheral membranous-like deposition of complexes rather than mesangial deposition.

One can postulate, then, that human glomerulonephritis might arise from the presence in the circulation of small amounts of complexes formed from low avidity antibody, and that this explains the relative difficulty of detecting immune complexes in this disorder. This contrasts with mesangiocapillary or postinfectious glomerulonephritis and SLE, in which complexes are easily found in large amounts in the majority of patients.

Could it be that membranous nephropathy is not the result of the deposition of circulating complexes at all, and that this explains why we cannot find them? I would now like to review some work done during the past few years which supports the idea that complexes may form by combination of antibody and antigen in situ within the glomerulus, and that this may be one mechanism—perhaps the principal mechanism—by which membranous nephropathy arises.

For a time after the work of Dixon and Germuth, we became used to thinking that there were fundamentally two contrasting varieties of nephritis (Fig. 2). One variety was that in which deposition of antibody against glomerular antigens induced the inflammation; the other variety was that due to the deposition in the glomerular capillary walls of immune complexes formed in the circulation, and again inflammation was induced. What we are talking about now is the possibility that a new antigen "planted" either in the glomerular capillary wall or the mesangium might combine with circulating antibody to induce inflammation. In retrospect, of course, the autologous phase of antiglomerular basement membrane antibody nephritis is an example of this: The heterologous antibody fixes to the glomerular basement membrane, and after several days autologous antibody to this foreign protein (in this case heterologous IgG) forms, and it is during the fixation of this autologous antibody that the inflammation and renal damage takes place.

Some of the earlier observations in this field were those of Mauer [25]. He localized aggregated immunoglobulin in the mesangium in experimental animals and then injected an antibody directed against this immunoglobulin. The result was a brisk nephritis. More recently, another experiment was reported by Golbus and Wilson [26] in which Concanavalin A was attached to the glomeruli, an antibody against it injected, fixed, and again a nephritis was produced.

It has become clear that in another of the hallowed models of presumed soluble complex disease—acute serum sickness in rabbits—one of the earliest events that can be detected in the glomerulus is the deposition of the foreign protein *without* any antibody or complement. After a couple of days as the antibody against the foreign protein appears in the circulation, it then appears in the glomeruli. Finally after another 2 or 3 days, immune elimination of the circulating foreign antigen by the antibody occurs with extensive deposition of complexes. It is quite possible, therefore, in even an old-fashioned disease such as acute serum sickness

(which has been with us since Von Pirquet in 1908) that the renal injury is partly mediated through in situ immune complex formation.

The first suggestion (so far as I am aware) that human membranous nephropathy might result from in situ formation of complexes in the capillary wall came from Evans in 1974 [27]. He suggested that differential filtration of various antigens and antibodies through the capillary wall, to produce an antibody-antigen ratio in the subepithelial space suitable for complex formation, might account for the formation of deposits at this site. He further pointed out that once deposited such complexes might be in equilibrium with the antigen and antibody in the circulation. Although more recent work on other determinants of molecular penetration into the glomerular capillary wall besides molecular size, in particular charge [28], has rendered his detailed arguments obsolete, the idea remains a fruitful one.

This brings us to a discussion of the model usually thought to resemble human membranous glomerulonephropathy most closely—Heymann nephritis. In brief, the original descriptions of this model by Glassock et al [29] showed that when rats were given injections of isologous kidney, a nephritogenic antigen could be found in the renal tubular brush border, called renal tubular epithelial (RTE) antigen. When this was prepared crudely and injected, antibody was induced in the host rat, and a chronic membranous nephropathy appeared with the glomerular deposits containing RTE antigen. The antigen was detected in a particular fraction of the tubule preparation called FX 1A, and hence it is referred to as FX 1A in many publications. This fraction contains several antigens, and most work has been done with relatively crude preparations.

Initially it was assumed that this disease resulted from the formation of FX 1A-antiFX 1A immune complexes in the circulation and their subsequent deposition within the kidney. Barabas, Nagi, and Lannigan [30], however, showed that a single injection of antiFX 1A antibody could also lead to prolonged proteinuria. They postulated that the FX 1A released from tubules following the injection led to the formation of complexes with the injected antibody; the complexes thus formed then deposited in the glomeruli. This form of the disease is usually referred to as "passive" Heymann nephritis [31]. Van Es et al [32] showed later that the chronic proteinuria that followed the single injection of rabbit antiFX 1A depended upon secondary autologous antibody directed against the heterologous rabbit antiFX 1A antibody present in the glomeruli, the immune complex thus acting as a "planted" antigen.

Even more fascinating, it has been shown that the glomerular basement membrance contains antigens that cross-react with antiFX 1A, and that formation of complexes within the glomerular wall follows within seconds of injection of the antiFX 1A antibody [33, 34]. The crucial experiments involved in vivo perfusion of the isolated kidney with antiFX 1A antibodies both from the circulation and eluted from the kidneys of rabbits with nephritis, both of which fixed, and FX 1A-antiFX 1A complexes, which did not. This work has been confirmed recently by Steinmuller et al [35] using an in vitro isolated perfused kidney system.

All of this may be relevant to human membranous nephropathy, although the extent to which in situ complex formation contributes is unknown. It is quite possible that both mechanisms—deposition of complexes formed in the circulation and in situ complex formation—are important. In other forms of glomerulonephritis, deposition of soluble complexes may predominate, but membranous nephropathy *may* be a disease in which the majority of the immune material is formed within the subepithelial space. This could explain why one cannot find complexes in the circulation in the majority of patients, and might explain the failure of membranous nephropathy to transmit to grafted kidneys, unlike other forms of glomerulonephritis such as MCGN or mesangial IgA disease [36]. In passing, it is also interesting to remember that SLE, rather surprisingly, does not transmit to grafted kidneys. A priori, this is one of the forms of glomerulonephritis that one would think might transmit with regularity. A suggestion from Izui, Lambert, and Miescher [37], however, is relevant here. These workers proposed that DNA might bind to basement membrane collagen and then react in situ with circulating antiDNA antibody; this might then allow other complexes in the circulation access to the capillary wall from which they were otherwise excluded. Whether there are antiDNA-DNA complexes in the circulation at all is the subject of present debate [38, 39].

One can begin to play with these new ideas to speculate on what determines the initiation and progression of nephritis. Certainly, events that might modify the glomerular capillary wall permeability [29] become crucial if antigen and antibody are to gain access to the subepithelial space. Low-affinity antibody could certainly exist in the circulation

Table 1. Antigens and circumstances reported to be associated with membranous nephropathy

Intrinsic antigens	Extrinsic antigens	Debatable associations
DNA	Hepatitis B	Renal venous thrombosis
Renal tubular epithelial	Tuberculosis pallidum	Diabetes
Carcinoembryonic	Filarial	Rheumatoid arthritis
Other tumor	Mercury and mercurials	Sjögren's syndrome
Thyroglobulin	Gold	Sarcoidosis
	Penicillamine	Tuberculosis
	Tridione	

alongside free antigen and form complexes in the capillary wall while there were few or none in the circulation, as suggested by Evans [28]. It may be that only some special sorts of antigen can do this, but the variety of circumstances in which membranous nephropathy may be found suggests that many antigens must be capable of this. This speculation may lead us not to chase complexes in the circulation, but to go to the kidney to study the actual site where the damage is taking place.

Turning back to human membranous glomerulopathy, Table 1 shows some antigens that are implicated in the human disease. The most interesting in view of the discussion today, is the possibility that some patients may have membranous nephropathy arising from immune complexes with RTE as the antigen. Naruse et al [40, 41] first discussed this possibility, but we [42] were unable to confirm this either in idiopathic or in drug-induced membranous nephropathy using antisera to a slightly different nephrogenic antigen extracted from human urine. I believe other studies have also failed to confirm this interesting finding although Ozawa et al [43] suggested that, as one might expect, RTE was involved in the membranous nephropathy secondary to renal venous thrombosis. Costanza et al [44] described a patient in whom carcinoembryonic antigen (CEA) appeared to be the antigen involved. We [45] and others have been unable to confirm this association, which is surprising since it is now 5 years since that paper was published, and a number of patients have been studied using several different antigenic determinants of the CEA to prepare the antisera. Also with reference to Table 1, it should be pointed out that membranous nephropathy may be associated with Hodgkin's disease, as well as the more usual "minimal change" pattern.

The extrinsic antigens are also listed in Table 1. There are a number of infectious agents and drugs that are particularly associated with membranous nephropathy. The patients with this variety of membranous nephropathy are particularly important because they teach us two things: *First*, the continued deposition of *something* in the glomerulus is necessary for the continuing disease, because if you take away the drug these patients almost invariably get better although it can take 1 or 2 years. *Second*, there is some mechansim by which the glomerulus can remove the subepithelial or intramembranous deposits; the nature of this is obscure.

Finally, some more general and debatable associations of membranous nephropathy are shown in Table 1. We have already discussed the possible association with diabetes mellitus. I will end the discussion of the possible associations of membranous nephropathy by simply stating that my own prejudice is that renal venous thrombosis is in all probability secondary to the glomerular disease in every case.

As I mentioned earlier, I do not wish to discuss the histopathologic changes in detail. They have been well described by Ehrenreich and Churg [15] and by Bariéty et al [46]. I will only make three points: *First*, oil immersion on immunofluorescent material may be necessary to demonstrate the finest granular deposits in stage 1 membranous nephropathy, and one may be tempted to diagnose linear immunofluorescent studies erroneously unless immersion is used. *Second*, the classical "spikes" of stage 2 membranous nephropathy may not be present in early stage 1 cases. To differentiate these from "minimal change" lesions, the capillary walls cut obliquely are more helpful, because if silver preparations under the oil immersion are used one can see a "bubbly" appearance of deposits cut through horizontally. *Third*, after clinical resolution of membranous nephropathy and a normal biopsy appearance on optical microscopy, electron microscopy may still reveal lucent areas within the apparently normal basement membrane, which we believe represent the sites of leached-out deposits. Gärtner et al [47], who described this appearance, have called this stage 5 membranous nephropathy. It is already clear from the lack of correlation between renal

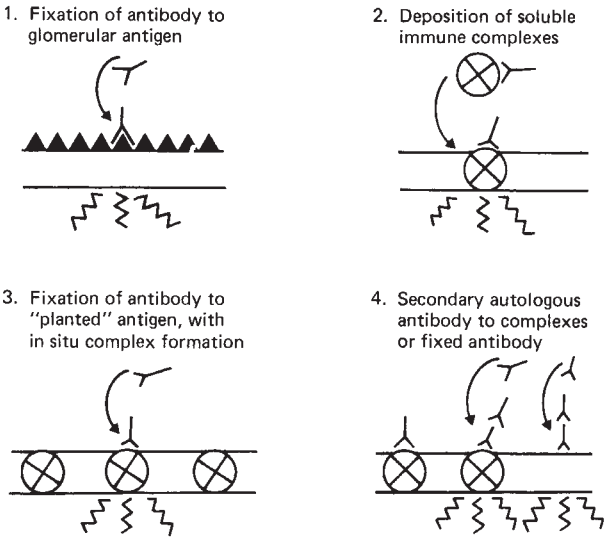

Fig. 2. *Different mechanisms for the generation of injurious immune complex formation in glomerulonephritis. (1)* antiglomerular basement membrane antibody nephritis, *(2)* deposition of circulating immune complexes, *(3)* in situ complex formation, *(4)* secondary autologous antibody deposition. (See text for explanation.)

function and renal biopsy grading [16] (Fig. 2) that stages 1 through 4 do not represent progressively worse manifestations of the disease, although 1 through 3 may represent a histologic evolution. In general, GFR does not correlate well with glomerular changes in glomerular diseases, but correlates much better with interstitial and tubular changes.

Perhaps we can finish by considering the long term outlook for patients with membranous nephropathy. Figure 3 shows the actuarial survival for all the published series of membranous nephropathy up to 1976, including our own data [48]. You can see that by and large this is a slowly progressive disease with renal failure and other causes of death coming in 3 to 5 years from onset. Figure 4 summarizes data from all the patients with membranous nephropathy whom we biopsied more than 10 years ago. All these 33 patients had at least 10 years of potential follow-up. These data are percentages of those analyzed at that particular point, not actuarially calculated figures. The proportion of patients in remission apparently decreases as we lose a few patients from follow-up around the decade mark. I suspect that the proportion in remission would be around 40% at 10 to 15 years. We lost mainly patients who were fit and well, which is what always happens. Another point I particularly want to make is that there are *no* renal deaths in our series until after 3 years, and that any study with an end-point based on terminal renal failure is going to have to run at least 5 or 10 years. Finally, there are quite large numbers of nonrenal deaths, particularly from cardiovascular disease.

Figure 5 is my summary of what probably happens to patients with membranous nephropathy from all the literature and from our own series. By 15 years, there are probably very few patients with active disease and none with a continuing nephrotic syndrome. From our own data, most of the patients who go into renal failure appear to suffer a persisting nephrotic syndrome and transfer from this category to that of renal death.

Recently, Hopper, Trew, and Biava [49] have suggested that male patients do much worse than female patients with membranous nephropathy when renal failure is considered. This has not been the case in our own series of 94 patients. Of 59 men, 8 have died (or required substitution therapy), and only 3 of these deaths (5%) have been from renal failure as against 8 deaths among 35 women, 4 of them (12%) from renal failure. The mean follow-up in our series is similar to Hopper's, and the mean ages almost identical. In his series, one-third of male patients had gone into terminal renal failure before 7 years had passed. Even excluding the few children in our series, the difference persists.

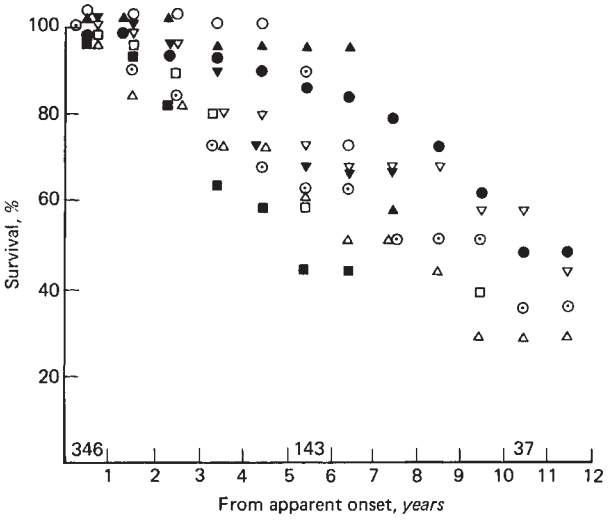

Fig. 3. *Actuarial survival of patients with membranous nephropathy, calculated from series published up to 1976.* Deaths and the requirement for dialysis or transplantation have been included as "deaths". Deaths from "nonrenal" causes (e.g., myocardial infarction) have been included. All series predominantly or entirely of adult patients, except data taken from Ref. 51 represented by *solid triangle* (▲). Data taken from Ref. 48 represented by *solid circle* (●).

An interesting observation is that younger patients do better than older patients. Table 2 shows an analysis of data on adults and children from the literature [48]. This is true even though the proportion of nephrotic patients is about the same in either group. The percentage in renal failure at about 5 years is only 4% in the children, but 20% in the adults. Also the remission rate (that is to say the complete absence of proteinuria or clinical signs of renal disease) is almost 50% in the children, but much lower in the adults. The proportion of remissions might be dependent on how many patients were treated if treatment works, but in crude figures 35% of the children and 8% of the adults went into remission without any treatment whatsoever except diuretics. In our own series (see Table 3), about 25% of the patients went into remission without any treatment. You can compare these data with the more extensive data from Ehrenreich et al [50], which caused such a stir because they suggested an effect of corticosteroids on membranous nephropathy (see Table 3). As you can see in our data, out of 26 patients who received nothing at all 8 went into remission, which is approximately 25% (see Table 3). We, certainly, have not been impressed with the idea that steroids make any difference at all to these patients and we have stopped treating our patients with membranous nephropathy with corticosteroids.

Using current measurement of GFR or creatinine clearance as the end-point for a trial of therapy, many years will be necessary to demonstrate an effect if one is present. Any tool we have is going to be a blunt instrument in under 5 years. This must be remembered when we examine the essentially negative results that have emerged from the trials carried out so far (see Table 4). Most of these trials lasted for only a year or two, and in several the numbers were small; in the Medical Research Council trial, neither the dosage nor the duration of treatment was standardized.

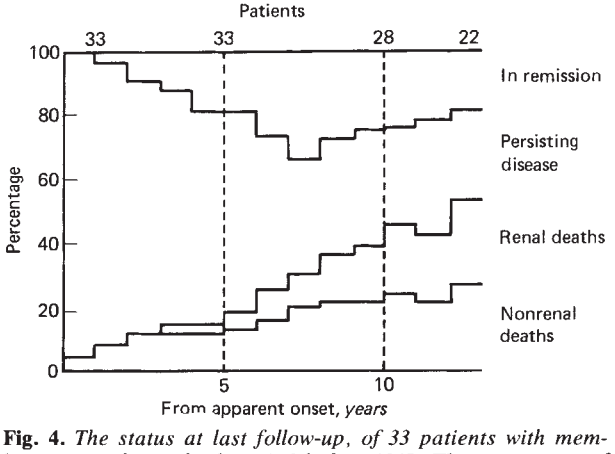

Fig. 4. *The status at last follow-up, of 33 patients with membranous nephropathy biopsied before 1968.* The percentage of patients in each follow-up category is indicated *at left*, and the number of patients available for analysis (including all those then or previously listed as dead or on dialysis) is indicated *at top*.

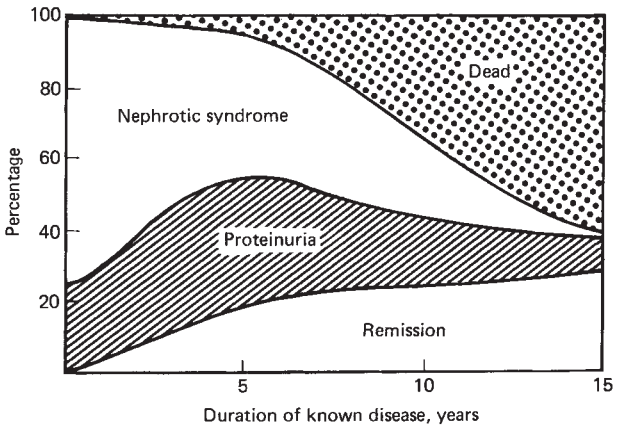

Fig. 5. *A synthesis of the probable long-term outcome of adult patients with membranous nephropathy.* An increase in the proportion of patients with minor proteinuria at apparent onset (i.e., clinical discovery), for example by a change in biopsy policy in isolated proteinuria, would improve the prognosis of the group. A diagram for children would probably be similar, but the proportion with persisting nephrotic syndrome and renal death would be smaller. (Derived from data in Figs. 3 and 4.)

At this point, we must raise the data of the collaborative Study of Adult Idiopathic Nephrotic Syndrome [10]. With Dr. Coggins present, I will only say that at first I viewed these data with great skepticism, but the randomization and analysis seem to have been impeccably performed and appear to show an effect of corticosteroids in delaying renal failure and diminishing proteinuria in adult patients with membranous nephropathy. I am reminded of the epigraph of Toulmin: "A man demonstrates his rationality . . . . by the manner in which, and the occasions on which he *changes* [his] ideas, procedures and concepts" (italics added) [54]. I hope that I will be able to demonstrate my rationality by changing my belief on the absence of specific treatment for membranous nephropathy, but we need more data and must take steps to generate them.

Questions and Answers

DR. J. T. HARRINGTON: Dr. Coggins, will you lead off by briefly summarizing and commenting on the results of the Study of Adult Idiopathic Nephrotic Syndrome?

Table 2. Comparison of outcome in adult and childhood onset patients with membranous nephropathy[a]

	NS[b]	F/U >5 yr	Deaths in CRF	Remissions (Treatment)	Remissions (No treatment)
Adults (N = 435)[c]	85%	207	82 (19%)	34	35
Children (N = 82)[d]	79%	29	4 (4%)	15	25

[a] Data taken from Ref. 48.
[b] Abbreviations used are: N, number of patients studied; NS, nephrotic syndrome; F/U, follow-up; CFR, chronic renal failure.
[c] Adult, >15 years old at apparent onset.
[d] Children, <15 years old at apparent onset.

DR. C. H. COGGINS: For the past several years we have been conducting a prospective cooperative study on the effect of prednisone compared to placebo in adults with the idiopathic nephrotic syndrome [10]. Patients were randomly allocated either to a treatment or control group.

The patients in the group receiving prednisone underwent therapy for 2 months during which they received prednisone every second day. This was followed by tapering of medication for approximately 1 month at the discretion of the individual physician. All patients who remitted and then relapsed again received additional treatment. Of the group of 34 patients, however, only 5 patients received more than one dose. The treated group had significantly more remissions, less proteinuria, and, of the greatest import, better maintenance of creatinine clearance during the follow-up period. We were surprised to find an observable difference and tried to find some way in which the groups were not comparable, or whether the therapeutic group initially contained patients with a better prognosis. We compared a large number of clinical, laboratory, and histologic characteristics of the patients in both groups and found no correlation between the prognosis and these characteristics. Further, we found no selection bias between the treatment and control groups. There seems to be no other explanation for the differences other than the prednisone therapy. The duration of observation of both groups was the same on the average, and each had an equal opportunity to develop problems. It may be a bit surprising that some of the control patients developed renal failure so quickly.

DR. J. S. CAMERON: Yes, I was going to ask you about that. The data (Fig. 2) reveal that it is very unusual for patients to develop renal failure in less than 4 years from apparent onset. The "stop point" in your trial is a doubling of serum creatinine concentration, but I think you have 11 patients who are in renal failure in the control group and only 1 in the treatment group. Is that right?

DR. C. H. COGGINS: Yes, the renal failure for 9 of the 11 patients was rather severe. For 2 patients,

Table 3. The effect of treatment with corticosteroids on long-term follow-up in patients with membranous nephropathy[a]

	Group I[b] (N = 48)		Group II[c] (N = 103)		Group III[d] (N = 67)		Group IV[e] (N = 26)	
	Rx	No Rx	Rx	No Rx	Rx	No Rx	Rx	No Rx
Dead	5	7	6	22	6	1	5	12
Persistent NS	5	2	12	11	15	5	0	0
Persistent proteinuria	8	9	24	11	0	0	0	0
Remission	4	8	17	0	25	15	5	4
	22	26	59	44	46	21	10	16
Follow-up	5.4 yr, mean		5.8 yr, mean		1 to 15 yr		0 to 23 yr	
Duration of Rx	2 to 38 mo (mean 7 mos)		6 wks to 39 mo		NA		NA	

[a] Abbreviations used are: N, number of patients studied; Rx, administration of corticosteroids; NS, nephrotic syndrome; NA, data not available.
[b] 7 patients also received immunosuppressants, and analysis confined to NS since only nephrotic patients treated. Data taken from Ref. 48.
[c] 16 patients also received immunosuppressants. Data taken from Ref. 50.
[d] 24 patients also received immunosuppressants, and 7 of these did not receive corticosteroids. Data taken from Ref. 51.
[e] All NS. Data taken from Ref. 52.

Table 4. Results of controlled studies of immunosuppressant drugs in patients with membranous nephropathy[a]

Agent	Duration	Result
Corticosteroids	2 to 4 yr	none
Azathioprine plus Corticosteroids	2	none
Azathioprine	1 yr	none
Cyclophosphamide	2 yr	none
Chlorambucil	1 yr	P & C_{cr}, same[b]

[a] Data taken from Ref. 53.
[b] P = proteinuria, C_{cr} = creatinine clearance.

however, it shouldn't be called renal failure; their serum creatinine concentrations simply doubled.

DR. J. S. CAMERON: Certainly from our own experience and the published literature, it is unusual for one-third of the 35 control patients with nephrotic syndrome to develop renal failure so soon.

DR. C. H. COGGINS: Within about 2.5 years, one-third of the group doubled their serum creatinine concentrations. During the same time, two-thirds of the patients did not raise their serum creatinine concentrations significantly. It sounds less dramatic when stated that way and doesn't seem surprising. Many patients in both groups are doing well as the years go by with no sign at all of progression of their renal disease. There is another point, which you mentioned earlier, that we can confirm. Any patient who has *any* kind of remission, even a partial remission defined as a reduction in proteinuria to below 2 g/day, has an excellent chance of maintaining GFR in the future, confirming the findings of Idelson et al [55].

DR. J. S. CAMERON: Yes. When the prognosis for patients with proteinuria only is examined and compared with that of patients with a full nephrotic syndrome, a consistent pattern appears with the single exception of patients with SLE, where there is no difference. Patients with mesangiocapillary, membranous, and focal segmental sclerosing lesions, and a nephrotic syndrome show almost identical survival curves with about 30 to 40% having 10-year survival. Those that were never nephrotic showed about 90% survival at the same point. In other words, simple clinical observation at the beginning of the disease, the presence of swelling in the ankles or more than 3 g of proteinuria, gives a useful prognostic index although it is a gloomy one.

DR. J. T. HARRINGTON: Of those patients who developed renal failure within 5 years, how many of those patients unequivocally had normal renal function at 3 years as this patient does? It is my feeling that if a patient survives 3 years and still has unequivocal normal function, the long-term prognosis is very good [55]. Do you have any information on this point?

DR. J. S. CAMERON: We do have data on this [48]. We have seen patients like the patient under discussion who maintain perfectly normal renal function for several years, but then very slowly develop renal failure. I suspect, however, that you are right; if a patient maintains normal renal function for 3 to 5 years, the prognosis could be considered generally good but not invariably so.

DR. C. H. COGGINS: One of the questions that we asked of patients in our study was, When did edema or proteinuria first develop? It is very "soft" data because often the patients don't know but will give an answer. In looking at those answers, we found no difference between the patients with persistence of normal renal function and those in whom renal function deteriorated rather rapidly.

DR. J. S. CAMERON: We have one patient who has had proteinuria for 22 years generally short of the nephrotic range [48]. There are several patients in the literature with similar courses [56]. Certainly, a few patients can persist for long periods with membranous nephropathy without decline in renal function but with persisting urine abnormalities short of a nephrotic syndrome [57]. As we agreed earlier, the presence of a nephrotic syndrome does not necessarily indicate that the patient will do poorly even if it persists for 4 or 5 years, but by and large it marks the individual as a higher risk patient. Looking at the survival curves in Fig. 2, it is an indolent disease. The survival curves are in general flat for the first few years, then bend over, which is rather different from other forms of glomerulonephritis in which a mortality is seen in the first few years.

DR. J. J. COHEN: The hypothesis that glomerular disease develops because of the in situ formation of immune complexes is a very intriguing one. If true, would such a pathogenesis suggest to you a different therapeutic strategy from that presently used?

DR. J. S. CAMERON: It might at the theoretical level, but I don't think at the practical level as yet. If we believe that the generation of immune complexes in the circulation is the crucial event with both the antibody and the antigen in solution, then one can examine a number of things, including the quantity of antibody or the avidity with which the antibody combines with the antigen, and try to modify these with therapeutic agents. If combina-

tion takes place on a surface, however, in this case the outside of the glomerular basement membrane, then the nature of the combination may be very different. We already know, for example, that we can do things in a tube with immune reactants in solution which, probably for reasons of steric hindrance, can't be done if some of the reactants are fixed to the wall of the tube.

Instead of asking how complexes get into the sites they do, in or on the capillary wall, we must now ask how we might influence the access of free antigen and free antibody to the site of reaction. Recently, the influence of charge on the access of immune materials and events that affect the charged molecules within the glomerular capillary wall have been explored. For example, aminonucleoside nephrosis induced in the kidney *denies* access of circulating immune complexes to the capillary wall [58], whereas several other manipulations permit access of the complexes.

I think the real implication for therapy is that anyone who thinks about the therapy of established glomerulonephritis in simplistic terms is in difficulty. Certainly, the old idea of using immunosuppressants because glomerulonephritis was supposed to be a disease of hyperimmunity seems laughable now. The new idea that we might simply "activate the T cells" with something like levamisole and thus improve things is equally naive. Not only at the level of cellular control, but even at the level of local assembly of complexes, it is probably very complicated. The in situ formation of complexes also implies that the material in the circulation may not tell us a thing about what is going on in the kidney; it might even mislead us.

DR. J. DONOHOE (*University Hospital, Boston*): Could I ask you about the comment that you made concerning the pathogenesis of membranous nephropathy? I think you said that it is unusual for membranous nephropathy to recur in a transplant patient.

DR. J. S. CAMERON: Yes. There are several de novo cases in patients who undoubtedly had problems other than nephritis as a cause of renal failure [59, 60], but there are only two patients so far whose courses are reasonably well documented in whom membranous nephropathy recurred following renal transplantation [61, 62].

DR. J. DONOHOE: Don't those cases strengthen the argument in favor of circulating immune complexes rather than in situ formation?

DR. J. S. CAMERON: I suspect that *both* mechanisms—deposition of circulating immune complexes and in situ complex formation—operate in several types of glomerulonephritis, but possibly the balance is in favor of in situ immune complex formation in membranous nephropathy. Unpublished work by Border and Glassock unequivocally demonstrates circulating immune complexes in Heymann nephritis of rats, the same disease in which some others have shown in situ immune complex formation, and I am sure that both are right. There is in this model for the first time good evidence for the operation of both mechanisms. It is almost certainly true, although the evidence is less clear, that both mechanisms operate in acute serum sickness nephritis in rabbits. I suspect that it is a question of predominance of one mechanism or the other in some conditions. One would therefore expect that the minority of patients with membranous nephropathy and large amounts of soluble immune complexes in their circulation might have an aggressive recurrence of the disorder in the graft.

DR. J. J. COHEN: It has recently been suggested that complement receptors for C3b on the glomerular capillary wall play a role in some forms of immune complex deposition. What do you think about this suggestion?

DR. J. S. CAMERON: It *may* be important in human renal disease, but of course we know that rats and many other animals who do not have glomerular C3b receptors can develop membranous nephropathy and other forms of glomerulonephritis that closely resemble the human disease. So, while the possession in humans and a few closely related primates of the C3b receptors (in the interstitium incidentally as well as in the glomerulus) may have an influence on the initiation and progression of disease, I can't believe it is a dominant mechansim. In fact, human beings are a little resistant to the induction of nephritis compared with the incidence of nephritis found in dogs, rats and mice; we are a relatively nephritis-resistant rather than nephritis-prone species. The presence of C3b receptors *could* relate to this, but it should work exactly the other way around. C3b receptors should allow the more avid fixation of complement-fixing immune complexes in the kidney.

With membranous nephropathy, it is interesting that the C3b receptors are believed to be on the epithelial cells of the glomeruli, and of course in membranous nephropathy the immune deposits are subepithelial. We do know also that these C3b receptors may become undetectable and then return in diseases that are exclusively subendothelial, such as mesangiocapillary glomerulonephritis during

complement fixation. I am inclined to think that at the moment C3b receptors are a very nice phenomenon in search of a relation to something clinically important!

DR. D. BERNARD (*University Hospital, Boston*): Dr. Cameron, you mentioned that in your experience the drug-induced varieties of membranous nephropathy will invariably fade away, perhaps over a period of years. Recently, we had a patient who was treated with gold therapy and subsequently developed severe nephrotic syndrome. After some 9 months, the disease persists despite some steroid therapy. Would you give this patient a good prognosis?

DR. J. S. CAMERON: I think I would. Clearly, there is the possibility of a secondary mechanism, such as autologous antibody to immune complexes formed in situ or deposited in the glomerulus. This may happen in malarial nephropathy, for example. There is also evidence in the literature suggesting that not all cases of penicillamine-induced nephropathy remit [63]. The problem is that the patients may not have been followed long enough. I think the best report is by Gärtner et al [64]. They followed-up 31 patients with penicillamine-induced nephropathy for more than 2 years and they found that all patients got better after 2 years although many of them had been fully nephrotic even after 1 year (Nield, personal communication). What might still be causing the nephrotic syndrome at 1 year is fascinating. When you imply that this patient hasn't improved, you have not yet waited for 24 months when many patients finally remit.

Reprint requests to Dr. J. Stewart Cameron, Guy's Hospital London Bridge SE1 9RT England.

References

1. GÄRTNER HV, FISCHBACH H, WEHNER H, BOHLE A, EDEL HH, KLUTHE R, SCHELER F, SCHMULLING RM: Comparison of clinical and morphological features of peri-(epi-, extra-) membranous glomerulonephritis. *Nephron* 13:288–301, 1974
2. SINNIAH R, FENG PH: Lupus nephritis: Correlation between light, electron microscopic and immunofluorescent findings and renal function. *Clin Nephrol* 6:340–351, 1976
3. COMBES B, STASTNY P, SHOREY J, ELGENBRODT EH, BARRERA A, HULL AR, CARTER NW: Glomerulonephritis with deposition of Australia antigen-antibody complexes in glomerular basement membrane. *Lancet* 2:234–237, 1971
4. PICKERING RJ, MICHAEL AF, HERDMAN RC, GOOD RA, GEWURZ H: The complement system in chronic glomerulonephritis—three newly associated aberrations. *J Pediatr* 78:30–43, 1971
5. LIBIT SA, BURKE B, MICHAEL AF, VERNIER R.L: Extramembranous glomerulonephritis in childhood: Relationship to systemic lupus erythematosus. *J Pediatr* 88:394–402, 1976
6. SIMENHOFF ML, MERRILL JP: The spectrum of lupus nephritis. *Nephron* 1:348–375, 1964
7. BURKHOLDER PM, HYMAN LR, KRUEGER RP: Characterization of mixed membranous and proliferative glomerulonephritis. Recognition of three varieties, in *Glomerulonephritis* I, edited by KINCAID-SMITH P, MATHEW TH, BECKER EL, New York, John Wiley & Sons, Publishers, 1973, pp. 557–559
8. WORLD MJ: Variables discriminating between cryptogenic glomerular lesions in adults with the nephrotic syndrome. *Q J Med* 45:451–468, 1976
9. INTERNATIONAL STUDY OF KIDNEY DISEASE IN CHILDREN. Nephrotic syndrome in childhood: Prediction of histopathology from clinical and laboratory characteristics at time of diagnosis. *Kidney Int* 13:159–165, 1978
10. COGGINS CH: Study of adult idiopathic nephrotic syndrome. *Abstr Int Congr Nephrol*, Montreal, 1978, p. D36
11. STUDD JWW, BLAINEY JD: Pregnancy and the nephrotic syndrome. *Br Med J* i:276–280, 1960
12. STRAUCH BS, HAYSLETT JP: Kidney disease and pregnancy. *Br Med J* 4:578–582, 1974
13. EDITORIAL: Pregnancy after treatment for renal failure. *Lancet* 1:861, 1978
14. WASS JAH, WATKINS PJ, DISCHE FE, PARSONS V: Atypical glomerular disease in diabetes mellitus. *Proc EDTA* 13:546–552, 1976
15. CHURG J, EHRENREICH T: Membranous nephropathy, in: *Glomerulonephritis* I, edited by KINCAID-SMITH P, MATHEW TH, BECKER EL, New York, John Wiley and Sons, Publishers, 1973, pp. 443–448
16. EHRENREICH T, CHURG J: Pathology of membranous nephropathy. *Path Ann* 3:145–168, 1968
17. OOI YM, OOI BS, POLLAK VE: Relationship of levels of circulating immune complexes to histologic patterns of nephritis: A comparative study of membranous glomerulopathy and diffuse proliferative glomerulonephritis. *J Lab Clin Med* 90:891–898, 1977
18. STUHLINGER WD, VERROUST PJ, MOREL-MAROGER L: Detection of circulating immune complexes in patients with various renal diseases. *Immunology* 30:43–47, 1976
19. WOODROFFE AJ, BORDER WA, THEOFILOPOULOS AN, GORE O, GLASSOCK RJ, DIXON FJ, WILSON CB: Detection of circulating immune complexes in patients with glomerulonephritis. *Kidney Int* 12:268–278, 1977
20. BORDER W, ABRASS C, HALL C, BROWN C, GLASSOCK R, COGGINS C: Detection of circulating immune complexes (iCK) in adult idiopathic nephrotic syndrome (AINS). *Kidney Int* (abstract) 13:58A, 1978
21. GERMUTH FG, RODRIGUEZ E: The immunopathology of the renal glomerulus. Boston, Little Brown, 1973
22. GERMUTH FG, TAYLOR TJ, SIDDIQUI SY, RODRIGUEZ E: Immune complex disease VI: Some determinants of the varieties of glomerular lesions in the chronic bovine serum albumin-rabbit system. *Lab Invest* 37:162–169, 1977
23. KURIYAMA T: Chronic glomerulonephritis induced by prolonged immunization in the rabbit. *Lab Invest*, 28:224–235, 1973
24. KOYAMA A, NIWA Y, SHIGEMATSU H, TANIGUCHI M, TADA T: Studies on passive serum sickness. II. Factors determining the localization of antigen-antibody complexes in the murine renal glomerulus. *Lab Invest* 38:253–262, 1978
25. MAUER SM, SUTHERLAND DER, HOWARD RJ, FISH AJ, NAJARIAN JS, MICHAEL AF: The glomerular mesangium.

III. Acute immune mesangial injury. A new model of glomerulonephritis. *J Exp Med* 137:553-570, 1973
26. GOLBUS S, WILSON CB: Glomerulonephritis (GN) produced by the in situ formation of immune complexes on the glomerular basement membrane. *Kidney Int* (abstract) 13:61A, 1978
27. EVANS DJ: Pathogenesis of membranous nephropathy. *Lancet* 1:1143-1144, 1974
28. BRENNER BM, BOHRER MP, BAYLIS L, DEEN WM: Determinants of glomerular permselectivity: Insights derived from observations in vivo. *Kidney Int* 12:229-237, 1978
29. GLASSOCK RJ, EDGINGTON TS, WATSON JI, DIXON FE: Autologous immune complex nephritis induced with renal tubular antigen. II. The pathogenic mechanism. *J Exp Med* 127:573-587, 1968
30. BARABAS AZ, NAGI AH, LANNIGAN R: Induction of autologous immune complex nephritis in rats by heterologous antikidney mitochondrial serum. *Int Urol Nephrol* 2:303-308, 1970
31. FEENSTRA K, VAN DER LEE R, GREBEN HA, ARENOS A, HOEDEMAEKER PJ: Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. I. The natural history of a histologic and immunohistologic study of light microscopic and ultrastructural level. *Lab Invest* 32:235-242, 1975
32. VAN ES CA, BLOK APR, SCHOENFELD L, GLASSOCK RJ: Chronic nephritis induced by antibodies reacting with glomerular bound immune complexes. *Kidney Int* 11:106-115, 1977
33. FLEUREN GJ, LEE RVD, GREBEN HA, VAN DAMME BJC, HOEDEMAEKER PHJ: Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. IV. Investigations into the pathogenesis of the model. *Lab Invest* 38:496-501, 1978
34. VAN DAMME BJC, FLEUREN GJ, BAKKER WW, VERNIER RL, HOEDEMAEKER PHJ: Experimental glomerulonephritis in the rat induced by antibodies directed against tubular antigens. *Lab Invest* 38:502-509, 1978
35. STEINMULLER DR, BELOK S, SALANT DJ, STILMANT MM, LOWENSTEIN LM, COUSER WG: Subepithelial (SE) immune complex (IC) deposits in experimental membranous nephropathy (MN) are formed *in situ*, *Abstr Int Congr Nephrol*, Montreal, 1978, p. G10
36. CAMERON JS, TURNER DR: Recurrent glomerulonephritis in allografted kidneys. *Clin Nephrol* 7:47-54, 1977
37. IZUI S, LAMBERT P-H, MIESCHER PA: *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* 144:428-443, 1976
38. IZUI S, LAMBERT P-H, MIESCHER PA: Failure to detect circulating DNA-anti DNA complexes by four radioimmunological methods in patients with systemic lupus erythematosus. *Clin Exp Immunol* 30:384-392, 1978
39. DAVIS JS, GODFREY SM, WINFIELD JB: Direct evidence for circulating DNA/anti DNA complexes in systemic lupus erythematosus. *Arthr Rheum* 21:17-22, 1978
40. NARUSE T, KITAMARA K, MIYAKAWA Y, SHIBATA S: Deposition of renal tubular epithelial antigen along the glomerular capillary walls of patients with membranous glomerulonephritis. *J Immunol* 110:1163-1166, 1973
41. NARUSE T, MIYAKAWA Y, KITAMURA K: Membranous glomerulonephritis mediated by renal tubular epithelial antigen-antibody complex. *J Allerg Clin Immunol* 54:313-318, 1974
42. WHITWORTH JA, LEIBOWITZ S, KENNEDY MC, CAMERON JS, EVANS DJ, GLASSOCK RJ, SCHOENFELD LS: Absence of glomerular renal tubular epithelial antigen in membranous glomerulonephritis. *Clin Nephrol* 5:159-162, 1976
43. OZAWA T, BOEDECKER EA, SCHORR W, GUGGENHEIM S, MCINTOSH RM: Immune-complex disease with unilateral renal vein thrombosis. *Arch Pathol Lab Med* 100:279-282, 1976
44. COSTANZA ME, PINN V, SCHWARTZ RS, NATHANSON L: Carcinoembryonic antigen-antibody complexes in a patient with colonic carcinoma and nephrotic syndrome. *N Engl J Med* 289:520-522, 1973
45. WHITWORTH JA, UNGER A, CAMERON JS: Carcinoembryonic antigen in tumour-associated membranous nephropathy. *Lancet* (letter) 2:611, 1975
46. BARIÉTY J, DRUET PH, LAGRUE G, SAMARQ P, MILLIEZ P: Les glomerulopathies "extramembraneuses" (GEM). Etude morphologique en microscopie optique electronique et en immunofluorescence. *Path Biol* (Paris) 18:5-32, 1970
47. GÄRTNER HV, WATANABE T, OTT V, ADAM A, BOHLE A, EDEL HH, KLUTHE R, RENNER E, SCHELER F, SCHMULLING RM, SIEBERTH HG: Correlations between morphologic and clinical features in idiopathic perimembranous glomerulonephritis. A study on 403 renal biopsies of 367 patients. *Curr T Pathol* 65:1-29, 1977
48. ROW PG, CAMERON JS, TURNER DR, EVANS DJ, WHITE RHR, OGG CS, CHANTLER C, BROWN CB: Membranous nephropathy. Long-term follow up and association with neoplasia. *Q J Med* 44:207-239, 1975
49. HOPPER J, TREW PA, BIAVA CG: Membranous nephropathy (MN) relative benignity in woman. *Abst Int Congr Nephrol*, Montreal, 1978, p. D2
50. EHRENREICH T, PORUSH JG, CHURG J, GARFINKEL L, GLABMAN S, GOLDSTEIN MH, GRISHMAN E, YUNIS SL: Treatment of idiopathic membranous nephropathy. *N Engl J Med* 295:741-746, 1976
51. KLEINKNECHT C, BROYER M, HABIB R: Steroids for idiopathic membranous nephritis (Letter to the Editor). *N Engl J Med* 296:49, 1977
52. PIERIDES AM, MALASIT P, MORLEY AR, WILKINSON R, ULDALL PR, SEERE DNS: Idiopathic membranous nephropathy. *Q J Med* 46:163-177, 1977
53. CAMERON JS: The treatment of immunological renal disease, in Immunological Renal Disease, edited by ZABRISKIE J, VILLAREAL H, BECKER EL (in press)
54. TOULMIN S: Human understanding. Oxford, Clarendon Press, 1972, p. vi
55. IDELSON BA, SMITHLINE N, SMITH GW, HARRINGTON JT: Prognosis in steroid-treated idiopathic nephrotic syndrome in adults. *Arch Intern Med* 137:891-896, 1977
56. POLLAK VE, PIRANI CL, MUEHRCKE RC, KARK RM: Asymptomatic persistent proteinuria: Studies by renal biopsies. *Guy's Hosp Rep* 107:353, 1958
57. FRANKLIN WA, JENNINGS RB, EARLE DP: Membranous glomerulonephritis: Long-term serial observations on clinical course and morphology. *Kidney Int* 4:36-56, 1973
58. COUSER WG, JERMANOVICH NB, BELOK S, STILMANT MM, HOYER JR: Effect of aminonucleoside nephrosis on immune complex localization in autologous immune complex nephropathy in rats. *J Clin Invest* 61:561-572, 1978
59. OLSEN S, BOHMAN SO, PETERSEN VP: Ultrastructure of the glomerular basement membrane in long term renal allografts

with transplant glomerular disease. *Lab Invest* 30:176–189, 1977

60. STEINMULLER DR, STILMANT MM, IDELSON BA, MONACO AP, SAHYOUN AI, LEWIS EJ, DAVIS RC, COUSER WG: *De novo* development of membranous nephropathy in cadaver renal allografts. *Clin Nephrol* 9:210–218, 1978
61. CROSSON JT, WATHEN L, RAIJ L, ANDERSEN C, ANDERSON R: Recurrence of idiopathic membranous nephropathy in a renal allograft. *Arch Intern Med* 135:1101–1106, 1978
62. RUBIN RJ, PINN VW, BARNES BA, HARRINGTON JT: Recurrent idiopathic membranous glomerulonephritis. *Transplantation* 24:4–9, 1977
63. BACON PA, TRIBE CR, MACKENZIE JC, VERRIER JONES J, CUMMING RH, AMER B: Penicillamine nephropathy in rheumatoid arthritis. A clinical, pathological and immunological study. *Q J Med* 45:661–684, 1976
64. GÄRTNER HV, NEILD GH, BOHLE A, HALLAUER W, HOPPE-SEYLER G, LÜTTGEN FM, SCHOLLMEYER P: Perimembranöse glomerulonephritis nach penicillamin-therapie. Bericht uber 31 fälle. *Klin Wschr* 53:835–837, 1975