Papers and Originals

Membranoproliferative Glomerulonephritis and Persistent Hypocomplementaemia

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Summary: The clinical, laboratory, and histological findings of 50 patients with membranoproliferative glomerulonephritis are described. Three-quarters of the patients, who were mostly older children and young adults, presented clinically with a mixture of "nephritic" and "nephrotic" symptoms; the remaining quarter had no symptoms and were diagnosed after the discovery of proteinuria and microscopic haematuria.

Though this clinical picture may occur in other forms of glomerulonephritis, the patients described here were unified as a group by their glomerular morphological appearance—namely, a combination of mesangial proliferation and capillary wall thickening, mainly due to subendothelial accumulations of mesangial matrix.

In 68% serum C3 (β_{1c}-globulin) levels were reduced initially, while a further 16% subsequently showed a fall to abnormally low levels. All patients had substantial proteinuria, usually of moderately impaired selectivity, and all but one had haematuria in addition. Children frequently presented with an illness resembling acute nephritis, whereas adults usually had a nephrotic syndrome from the start.

In 31 patients, followed for periods of one to eight and a half years, serial measurements of glomerular filtration rate were made. Sixteen have experienced no deterioration of renal function, though their proteinuria continues unchanged. Fifteen have shown progressive deterioration; six of them are still well, six are on regular dialysis treatment, and three have died. Treatment with corticosteroids, azathioprine, or cyclophosphamide, alone or in combination, did not seem to influence the course of the disease, and another two patients died from complications of steroid therapy. The disease usually runs a chronic course and appears to be progressive.

Introduction

The clinical picture of acute post-streptococcal glomerulonephritis is familiar to all; there is an abrupt onset of haematuria and oliguria, often but not always preceded by an upper respiratory infection, and frequently accompanied by transient hypertension and evidence of renal functional impairment. It affects mainly schoolchildren and young adults, and nearly always runs a benign course. The nephrotic syndrome is equally well recognized, though less common. It is characterized by oedema of insidious onset, resulting from massive proteinuria with a reduced serum albumin level. It occurs at any age, but is especially prevalent in pre-school children, when it frequently runs a relapsing course. The division of nephritis into two types roughly corresponding to these clinical descriptions was first suggested by Rayer (1840) and later by Wilks (1853). The idea is appealing because of its simplicity, and it was later revived and developed by Longcope (1936) and Ellis (1942). Though this division has proved useful clinically, it is in fact an over-simplification. As Enticknap and Joiner (1953) pointed out, some patients present with a combination of both "nephritic" and "nephrotic" features. The serum albumin level may be transiently lowered at the onset of acute nephritis, but quickly returns to normal as healing begins. Our experience in both children and adults with renal disease has taught us to be particularly wary of two "mixed" clinical patterns. One is the persistence of proteinuria or the development of a nephrotic syndrome following an apparently ordinary attack of nephritis, and the other is the occurrence of haematuria, with or without hypertension, in a nephrotic patient.

Careful evaluation of renal biopsy material from patients with primary glomerular disease has demonstrated four distinct morphological categories: minimal changes, focal glomerulosclerosis, membranous nephropathy, and proliferative glomerulonephritis (Churg *et al.*, 1970; White *et al.*, 1970). Almost without exception both children and adults with the nephrotic syndrome showing minimal changes on biopsy can be distinguished from those with other lesions by their comparative freedom from additional features, such as haematuria and hypertension (White *et al.*, 1970) and by their highly selective proteinuria and good response to corticosteroid therapy (Cameron, 1968; White *et al.*, 1970). Though a sclerosing process with a focal and segmental distribution was recognized some time ago by Rich (1957), the distinction from focal *proliferative* glomerulonephritis (Heptinstall and Joekes, 1959) and its rather gloomy prognosis have only recently been appreciated (Churg *et al.*, 1970; White, 1970). Membranous nephropathy is characterized by a chronic course (Pollak *et al*, 1968; Forland and Spargo, 1969) and can be recognized by its morphological appearances on both silver-impregnation and electron microscopy (Ehrenreich and Churg, 1968). Proliferative glomerulonephritis was not subdivided in earlier publications (Blainey *et al.*, 1960), but it is now evident that it is a heterogeneous group composed of several fundamentally different conditions (Ogg *et al.*, 1969; Churg *et al.*, 1970; White *et al.*, 1970).

Examination of the clinical aspects, serum complement levels, and renal histological features of patients with proliferative glomerulonephritis whom we have studied during the past seven years has revealed a group that may be distinguished with sufficient consistency to suggest that it forms a clinicopathological entity. The clinical manifestations include both nephritic and nephrotic features, while persistent hypocomplementaemia is a frequent though not constant finding. The whole group is unified by the glomerular appearance seen on renal biopsy, which consists of a unique combination of mesangial proliferation and capillary wall thickening. Such patients have been variously described as cases of

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"chronic latent" or "subacute" nephritis (Addis, 1925, 1948; Bell, 1938) on clinical grounds; "hypocomplementemic persistent glomerulonephritis" (West *et al.*, 1965) on the basis of immunological findings; and "chronic lobular" (Allen, 1955, 1962), "mixed membranous and proliferative" (Kark *et al.*, 1958), "membranoproliferative" (Royer *et al.*, 1962), and "mesangiocapillary" (Churg *et al.*, 1970) glomerulonephritis morphologically.

Though these terms cannot necessarily be regarded as synonymous, we nevertheless believe that they represent incomplete descriptions of the same pathological disorder, for which we have adopted the name membranoproliferative glomerulonephritis (M.P.G.N.). We have observed this lesion in 50 children and adults, and in this paper we summarize the clinicopathological correlations.

Material and Methods

Of the 50 patients only three were referred direct to us by their general practitioners, reflecting the infrequency of the condition compared with uncomplicated forms of acute nephritis and the nephrotic syndrome. The remainder were referred by other paediatricians and physicians as problems of diagnosis or management. The initial clinical and laboratory findings in the majority of patients were therefore those recorded in a number of regional hospitals. Some patients were seen by us soon after they had sought medical attention, but in others proteinuria was discovered or haematuria noted several months before further inquiry was made. In addition to repeating the routine laboratory tests we carried out the investigations listed below.

(*a*) *Differential renal clearance of plasma proteins.* This was performed by the method of Joachim *et al.* (1964) in 24 patients and by that of Cameron and Blandford (1966) in 46.

(*b*) *Serum complement.* (1) The C3 component of complement was measured in all patients as β_{1A}-globulin by a radial diffusion method (Mancini *et al.*, 1965), using commercially available Immunoplates. The mean plasma concentration in healthy adults was found by this method to be $132{\cdot}5 \pm 28{\cdot}5$ mg./100 ml. (Ogg *et al.*, 1968); West *et al.*, (1965) suggested that a level of 90 mg./100 ml. should be accepted as the lower limit of normal. (2) Total haemolytic complement activity C_{H50} was estimated serially in eight patients by Dr. R. A. Thompson (department of experimental pathology, Birmingham University), using a modification of the method of Kabat and Mayer (1961).

(*c*) *The glomerular filtration rate (G.F.R.)* was estimated in 48 patients by either or both of the following methods: (1) *Creatinine clearance,* using either a 24-hour urine sample or duplicated 3-hour collections during water-induced diuresis, the serum and urine creatinine concentrations being measured by AutoAnalyzer technique; and (2) the single injection ^{51}Cr-edetic-acid clearance, as described by Chantler *et al.*, (1969).

(*d*) *Percutaneous renal biopsy* was performed in all patients by standard techniques (Kark and Muehrcke, 1954; White, 1963) and subsequently repeated in 12. Formalin fixation was employed in most cases, but recently alcoholic Bouin's solution has been used in preference. Specimens were processed by routine techniques, sectioned at 2-3 μ thickness and stained with haematoxylin and eosin (H.E.), periodic-acid-Schiff (P.A.S.) and periodic-acid-silver methenamine (P.A.S.M.). Renal tissue from the 11 Birmingham patients was also fixed in glutaraldehyde, post-fixed in osmium and embedded in Epon for electron microscopy; 10 specimens yielded glomeruli.

Renal Morphology

Biopsy sections from all patients were examined by E.F.G. and R.H.R.W. and details of individual features were fully assessed. Glomerular abnormalities such as proliferation and lobularity, and tubular and interstitial changes, were graded 0-3 according to severity. As it is planned to publish a more detailed account of this information separately we propose to limit the description here to a summary of the essential morphological features.

The glomerular tufts are invariably enlarged and show conspicuous mesangial cellular proliferation combined with diffuse capillary wall thickening. The mesangial regions ("lobular stalks") are expanded owing to both the proliferation and a striking increase of fibrils which stain positively with P.A.S. and P.A.S.M. and have the consistency of basement membrane on electron microscopy. When marked these changes give the glomerular tuft a lobulated appearance (Fig. 1). The capillary walls are thickened, and on light microscopy an apparent "duplication" of basement membrane is seen with P.A.S.M. staining, the two layers being separated by predominantly non-argyrophilic material interspersed with delicate P.A.S.M.-positive fibrils (Fig. 5). On electron microscopy this thickening is seen to be composed of a number of structures (Fig. 4); essentially, the true basement membrane is thickened, and between it and the endothelium lining the capillary lumen there is an aggregation of basement membrane-like mesangial fibrils and islands of cytoplasm. Basement membrane and mesangial fibrils stain well with P.A.S.M., unlike cytoplasm, which is non-argyrophilic, and thus the "duplicated" appearance on light microscopy is produced.

Proliferation was present in all the initial biopsy specimens, being moderately severe in most cases. Twenty-seven specimens showed grades 0-1 lobulation, and 23 grades 2-3 lobulation. To simplify the analysis of clinico-pathological correlations we have called the former group "non-lobular" and the latter "lobular." Varying degrees of proliferation, lobulation, and capillary wall thickening may exist together, and in four instances a combination of very mild proliferation with absence of lobularity and marked diffuse capillary wall thickening produced a histological appearance (Fig. 3) which might easily be mistaken for membranous nephropathy. This latter lesion, however, is distinguished by the complete absence of proliferation and by the different morphological characteristics of the capillary wall thickening (Fig. 6). Well-developed crescents were not a prominent feature, though some degree of capsular proliferation or thickening was seen in ten specimens. Similarly, a severe degree of tubular and interstitial involvement was not a general feature of initial biopsies, though slight but definite abnormalities were observed in 17 cases. Aggregations of foam cells were seen in the interstitium in 13 specimens and were observed additionally within the glomerular tufts in one case.

Repeat biopsies were performed in 12 patients at intervals which varied from five months to four years. The degree of proliferation in these specimens was either the same as that in the initial biopsy (five patients) or reduced (seven patients). In none of the repeat biopsy specimens was there any increase in lobularity, and in seven patients it was reduced or absent. Thus some of the repeat biopsies contained glomeruli consisting mainly of finely fibrillar mesangial material with few cells surrounded by some open capillary loops with characteristically thickened walls. There was no consistent increase in the severity of tubular damage or number of totally sclerosed glomeruli in association with these changes, but an increased number of glomeruli showed evidence of damage such as the presence of adhesions, crescents, and thickened Bowman's capsule.

Initial Observations

Clinical Findings

The main clinical findings are summarized in Tables I-III. The condition is found most commonly between the ages of 5

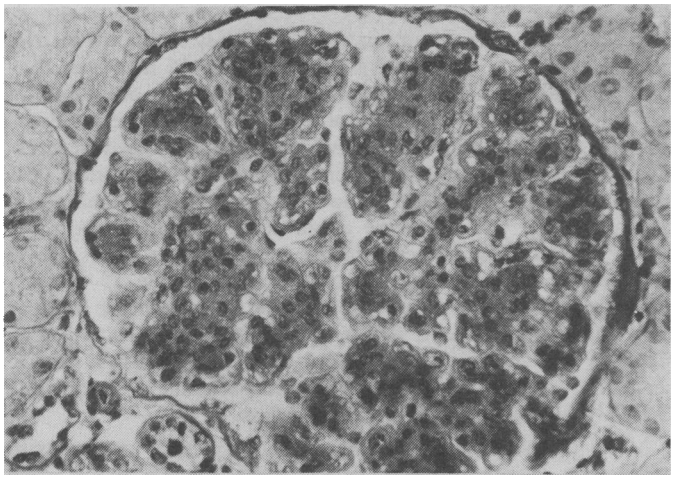

FIG. 1

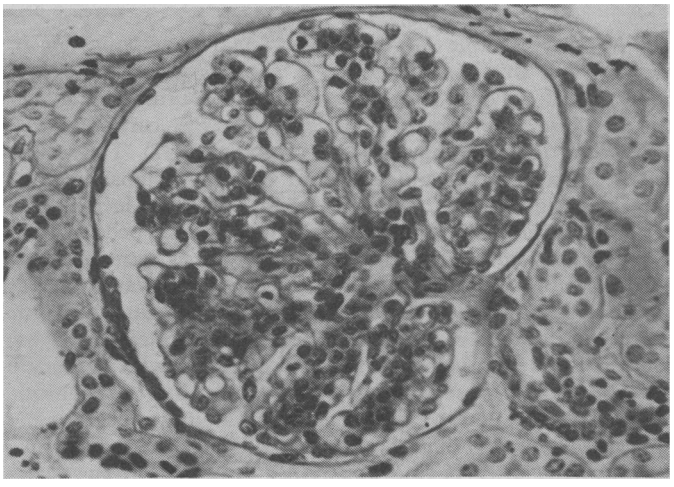

FIG. 2

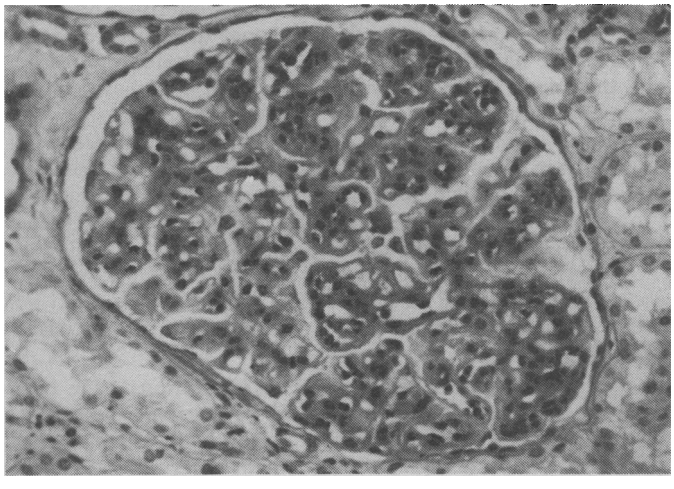

FIG. 3

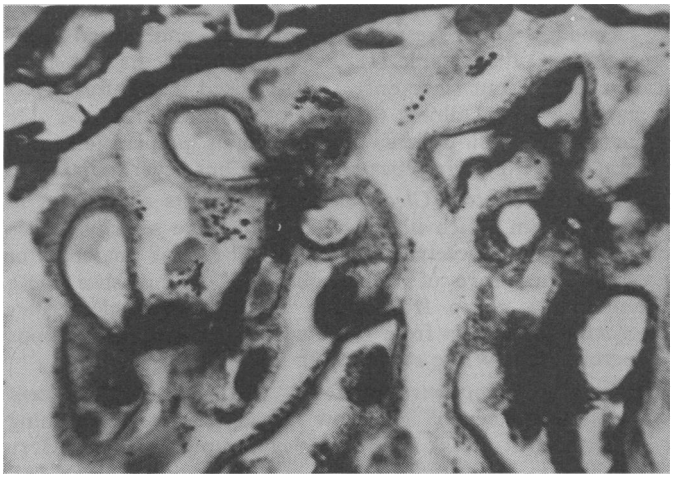

FIG. 4

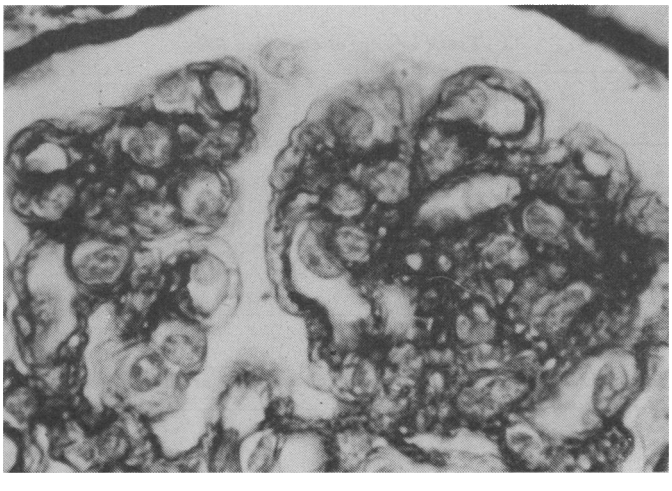

FIG. 5

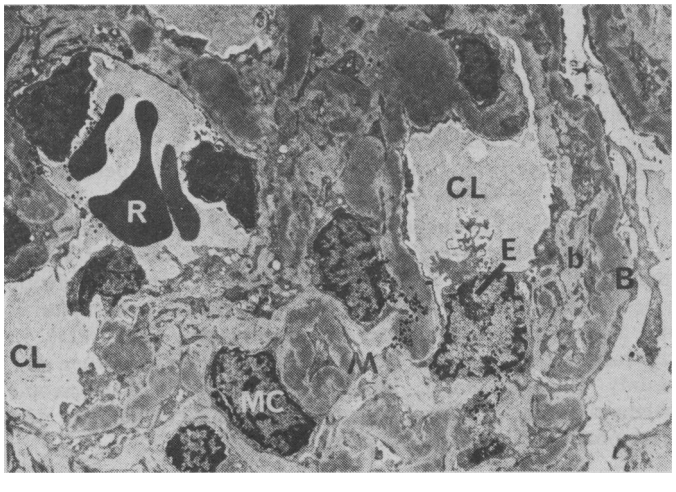

FIG. 6

FIG. 1.—Typical greatly enlarged glomerulus showing lobular form of M.P.G.N. There is marked proliferation of cells and basement-membrane-like fibrils in mesangial regions ("lobular stalks"), with considerable capillary wall thickening. These features have led to narrowing of lumens. (P.A.S. × 274.)

FIG. 2.—Representative glomerulus from case of subsiding poststreptococcal glomerulonephritis. Though lobular architecture of tuft is emphasized by mesangial thickening and proliferation, the glomerulus is not enlarged, while capillaries have widely patent lumens and thin walls. which distinguish lesion from M.P.G.N. (P.A.S. × 547.)

FIG. 3.—This glomerulus shows non-lobular form of M.P.G.N. There is diffuse capillary wall thickening, but in contrast to Fig. 1 mesangial proliferation is mild and lobulation absent, resulting in superficial resemblance to epimembranous nephropathy. Nevertheless, the "spikes" characteristic of this latter lesion on P.A.S.M. staining (Fig. 6) were absent. (P.A.S. × 262.)

FIG. 4.—Electron micrograph illustrating increased mesangium (M) with mesangial cells (MC) and two capillary lumens (CL), one of which contains red blood cells (R) and both lined by endothelial cells and cytoplasm (E). The mesangium has infiltrated the walls of the capillaries between the endothelium and the true basement membrane (B). The components of the thickened capillary walls are best seen separating the lumen of the capillary on the right. They consist of thickening of true basement membrane (B) and subendothelial layers of basement-membrane-like material (b) enclosing islands of cytoplasm. (× 1,864.)

FIG. 5.—High-power magnification showing details of mesangium and capillary walls in M.P.G.N. after silver impregnation. Note striking increase of mesangial cells and basement-membrane-like fibrils. Silver stain is taken up by true capillary basement membrane, which lies at periphery of loops, and by mesangial fibrils which have invaded walls in subendothelial regions, leaving an intermediate clear zone of non-argyrophilic material. This appearance, attributed to "duplication" of basement membrane in earlier publications, is further elucidated in Fig. 4. (P.A.S.M. × 1,097.)

FIG. 6.—High-power magnification of capillary walls in epimembranous nephropathy following silver impregnation. The appearance is quite different from that of M.P.G.N. (Fig. 5), and is characterized by the "spiky" projections from the outer aspect of the basement membrane, which have invaded the subepithelial immune deposits. (P.A.S.M. × 1,100).

and 30 years (Table I). There is a slight predominance of females, and this sex difference is most pronounced around puberty. All patients had substantial proteinuria, and all but one of those in whom urine microscopy was carried out at onset were found to have haematuria (Table II). Two-thirds had oedema at onset and one-third were hypertensive, while half had significantly lowered serum albumin levels. The various combinations of haematuria, proteinuria, oedema, and hypoalbuminaemia can be grouped into four patterns of initial illness (Table III). The nephrotic syndrome was twice as common as any other presentation, while an acute nephritic onset and the discovery of symptomless proteinuria were equally frequent. Most of those presenting with an acute nephritic onset passed almost immediately into a nephrotic phase or were found to have low serum albumin levels at the first estimation; two children, however, developed the nephrotic syndrome after having apparently recovered from "acute nephritis."

A comparatively small proportion of patients gave a history of preceding upper respiratory infection and raised antistreptolysin-O titre (Table II). Twenty-four patients had significant anaemia, but since only 13 had raised blood urea levels during their initial illness this cannot be invoked as the sole explanation. In all patients positive lupus preparations and antinuclear factor were repeatedly sought but never found.

Differential Protein Clearances.—The results are shown in Table IV, from which it can be seen that the selectivity was moderately or severely impaired in the vast majority of patients. The range of values observed distinguishes these patients as a group from those with minimal glomerular abnormalities, but not from those with other structural lesions (Cameron, 1968; White *et al.*, 1970).

Serum Complement.—We defined "persistent hypocomplementaemia" as a low serum C3 level continuing more than eight weeks from onset of symptoms or discovery of proteinuria. The initial results are shown in Table V. All eight children in whom serum C_{H50} activity was measured showed reduced levels; in seven the serum C3 concentrations were also low, but one inexplicably maintained normal levels for four months before showing a fall.

Renal Function.—The results of initial G.F.R. estimations are shown in Table VI. Impairment of renal function was more pronounced in older patients, as might be expected in a progressive condition, and there was a correlation between its severity and the degree of hypertension.

Glomerular Lobularity.—Patients whose glomeruli showed marked lobulation were generally younger at onset of symptoms, mostly female, more likely to have an acute nephritic onset or nephrotic syndrome with macroscopic haematuria, and almost invariably hypocomplementaemic (Table VII). In contrast, those classified as non-lobular were of either sex, mainly older at onset, infrequently exhibited macroscopic haematuria, and included seven out of the eight patients who have not so far become hypocomplementaemic.

Follow-up Observations

Thirty-three patients have been adequately followed up by us for not less than one year, and a further three have died within a year. The clinical course has followed a fairly consistent pattern in most cases. Those with an acute nephritic onset generally continued to have persistent proteinuria and intermittent oedema, associated with hypoproteinaemia. Two children, however, apparently recovered from their "acute nephritis," the urine having become completely normal. They then developed a nephrotic syndrome, after intervals of $2\frac{1}{2}$ and $5\frac{3}{4}$ years. Most of those presenting with a nephrotic syndrome lost their oedema after varying periods, continuing in apparently good health but with persistent proteinuria and often hypertension.

In 31 patients serial estimations of G.F.R. were made over periods of one to eight and a half years. In 16 instances they showed improvement or remained unchanged; only two of these patients had symptoms of more than four years' duration. In the remaining 15 patients the G.F.R. showed progressive deterioration, and in seven the duration of illness was greater than four years. However, since approximately a quarter of the patients were diagnosed following the discovery of symptomless proteinuria the true duration of disease in them could not be reliably estimated. Six of the 15 patients whose renal function has deteriorated now require regular dialysis treatment and a further three have died, leaving only six who are still living normally. From Table VI it can be seen that the prognosis is grave in patients in whom the G.F.R. is 40 ml./min./1·73 sq.m. or less.

Many of the patients in this series have been treated with prednisone or prednisolone, often before referral to us. Two children given intensive therapy during the first three months of illness developed increasingly severe hypertension and died from complications of treatment. In one, the cause of death was massive haemorrhage from a gastric erosion; the other child developed acute cerebral oedema with medullary coning. Cyclophosphamide was used in seven patients, and azathioprine combined with prednisolone in 17. The results of treatment are difficult to evaluate in a disease which generally evolves slowly, but we have based them on the disappearance of oedema, reduction of proteinuria, improvement of serum protein levels, and maintenance or improvement of G.F.R. In Table VIII are shown the results of treatment which, over the relatively short period of observation possible to date, do not reveal convincing evidence that any of these drugs are effective.

Serial estimations of plasma C3 concentrations have been made in 40 patients over periods of up to five years. Most patients have had remarkably constant levels, but nine showed wide fluctuations (Fig. 7). Six of them intermittently came

TABLE I.—*Age at Onset and Sex*

Apparent Age at Onset (Years)	Male	Female	Total
⩽5	0	1	1
5–9	8	6	14
10–14	3	8	11
15–19	4	2	6
20–29	4	8	12
30–49	1	3	4
>50	1	1	2
Total	21	29	50

TABLE II.—*Clinical and Laboratory Observations at Initial Investigation*

Observation	Patients		
	No.		%
Proteinuria	50		100
Haematuria: Microscopic	30/48		63
Haematuria: Visible	17/48		35
			98
Oedema	33		66
Hypertension (diastolic >95 mm. Hg)	16/48		33
History of upper respiratory tract infection	14		28
Antistreptolysin-O titre >200 units/ml.	8/21		38
Serum albumin <2·5 g./100 ml.	26/44		59
Haemoglobin <11 g./100 ml.	24/44		55

TABLE III.—*Clinical Patterns*

Mode of Presentation	No. of Patients	Unusual feature
Nephrotic syndrome	23	Haematuria in 20*
Symptomless proteinuria	12	Haematuria in 11*
Acute nephritic syndrome	12	Proteinuria in all
Recurrent haematuria	3	Proteinuria in all

*In one patient in each group the urine was not tested for blood at onset.

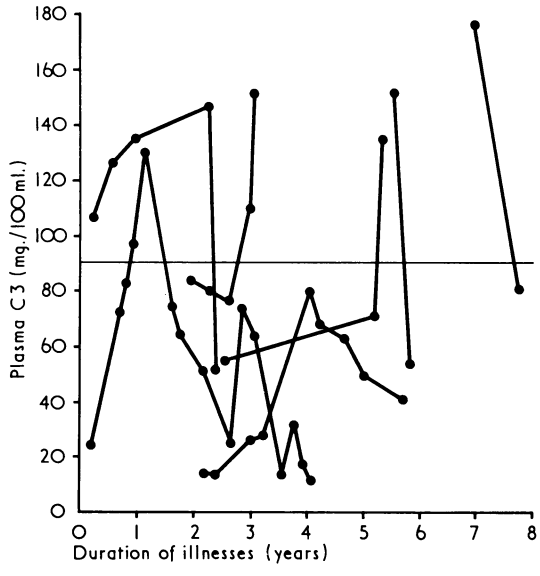

FIG. 7.—Seven patients whose plasma C3 concentrations varied considerably during period of observation. Some of the points on the graph of individual patients have been omitted to afford greater clarity.

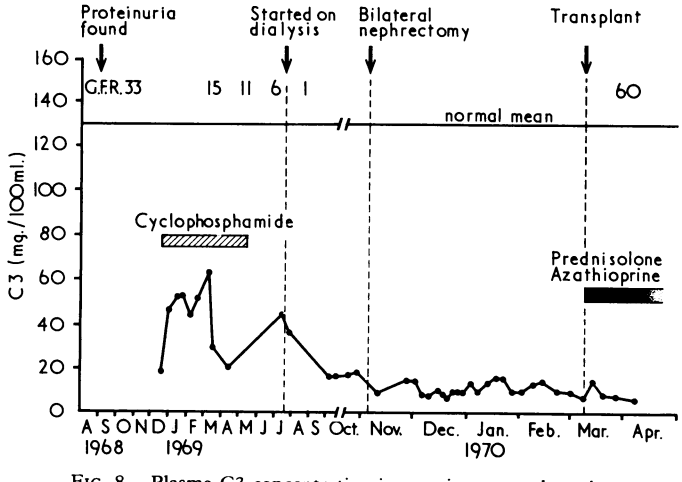

FIG. 8.—Plasma C3 concentration in a patient transplanted after an anephric period on regular haemodialysis. Throughout the whole illness, irrespective of the degree of function and proteinuria exhibited by the patient's own kidneys, or their absence the plasma C3 concentration remained very low.

within the normal range. Of the 16 patients who initially demonstrated normal serum C3 levels, eight subsequently showed a fall. Thus eight patients (16%) have remained normocomplementaemic to date; all but one were adults. In six patients who have been on regular dialysis from 10 to 33 months the serum C3 levels did not change significantly. One patient was subjected to bilateral nephrectomy and subsequently transplanted; the serum C3 concentration has remained low throughout (Fig. 8). Another patient who received a renal homograft showed a fall of his initially normal C3 concentration, not associated with rejection of his graft. A third demonstrated no change in his (normal) C3 concentration; this graft was removed after four weeks because of technical problems, and showed no histological signs of rejection or recurrent disease.

Discussion

The descriptions we have given of the histological appearances and the clinical features of M.P.G.N. are not new. In the past, however, the subject has been tackled from the clinical, immunological, and morphological standpoints, each in isolation, producing a fragmentary and muddled picture of the disease. Moreover, conclusions have been based on comparatively small numbers of patients. The data presented in this paper are based on the study of 50 patients from all three aspects. It has become clear to us that, since the clinical presentation may resemble that of other forms of glomerulonephritis, the condition cannot be satisfactorily defined in clinical terms. The name "persistent hypocomplementemic glomerulonephritis" (West *et al.*, 1965) is also unsatisfactory, since 16% of our patients never showed depressed serum complement activity. We have chosen to define the disease by reference to its morphological characteristics because these distinguish it most clearly from other forms of glomerulonephritis and appear to determine the usually progressive nature of the illness.

Histology

The distinctive glomerular appearance is essentially the result of a combination of capillary wall thickening and mesangial proliferation. The mesangium is expanded owing to an increase of both cellular and fibrillar components, and encroaches on the capillary lumen by infiltrating between the basement membrane and the endothelial lining. The calibre of the lumen thereby becomes progressively reduced while the wall is thickened as a result of the subendothelial aggregations which can be seen on light microscopy, by means of P.A.S.M. stains, and confirmed by electron microscopy. These findings explain the "double" basement membrane described by Jones (1957) and the non-argyrophilic subendothelial deposits observed by West *et al.* (1965). Thus the thickening in M.P.G.N. is essentially *subendothelial,* unlike that in membranous nephropathy, in which the aggregations, interrupted by characteristic "spiky" projections, are between the basement membrane and epithelial cytoplasm—that is, in a subepithelial position. A more accurate descriptive term,

TABLE IV.—*Selectivity of Differential Protein Clearances*

Descriptive Category	Method of Joachim *et al.* (1963) (0)	No. of Patients	Method of Cameron and Blandford (1966) (CIgG/C Transferrin)	No. of Patients
Highly selective ..	>70°	4	0·01–0·04	0
			0·05–0·09	3
Moderately selective	60–69°	12	0·10–0·14	3
			0·15–0·19	0
Poorly selective ..	<60°	8	0·20–0·29	15
			0·30–1·00	16
		24		46

TABLE V.—*Plasma C3 Concentration at First Investigation*

Plasma C3 (mg./100 ml.) ..	0–19	20–49	50–89	>90 (normal)	Total
No. of patients	10	9	15	16 (32%)*	50

*C3 later became less than 90 mg./100 ml. in 8, but was never less than 90 mg./100 ml. in 8 (16%).

TABLE VI.—*Renal Function at Initial Investigation Related to Age at Onset, Blood Pressure, and Subsequent Progress*

	Glomerular Filtration Rate (ml./min./1·73 sq. m.)		
	Normal (>80)	Impaired (40–80)	Severely impaired (<40)
Total number of patients	24	17	7
Age ≤15 years	15	8	0
,, >15 years	9	9	7
Severe hypertension (diastolic B.P. >110 mm. Hg)	1	4	6
Progress* Improved or unchanged ..	14	6	0
Worse	3	3	0
Terminal renal failure†	(2)	2	5

*Includes only those 35 patients with serial G.F.R. measurements over a period of one year or longer.
†Dead, on regular dialysis, or transplanted; both the deaths in the group with "normal" G.F.R. were associated with toxicity from corticosteroid drugs.

epimembranous nephropathy, has been suggested for the latter (White, 1969) and has the additional advantage of eliminating confusion between the terms "membranous" and "membranoproliferative," when in fact the lesion of the capillary wall in each condition is quite distinct. In lupus nephritis true subendothelial deposits are also seen, but they are homogenous masses showing none of the layering of M.P.G.N. while the thickening is accompanied by extremely variable proliferation and occasionally by haematoxyphil bodies.

Proliferation similar in emphasis to that noted in the biopsies we have described is seen in other lesions, especially resolving poststreptococcal glomerulonephritis (Jennings and Earle, 1961) and sometimes Henoch-Schönlein nephropathy, but the thin capillary wall observed on light microscopy (Fig. 2) is quite different from that in M.P.G.N. Similar glomerular appearances have been described in patients with "lobular" glomerulonephritis (Jones, 1957; Allen, 1955, 1962), "mixed membranous and proliferative" glomerulonephritis (Kark *et al.*, 1958; Burch *et al.*, 1962; McGovern, 1964; Churg *et al.*, 1965; Todd and Bouton, 1965; Heptinstall, 1966), and "persistent hypocomplementemic glomerulonephritis" (West *et al.*, 1965). Habib *et al.* (1961), and Royer *et al.* (1962), described two varieties of this lesion which they called "membranoproliferative" and "lobular"; both showed essentially the same capillary wall and proliferative changes, as well as clinical features, and probably correspond to our non-lobular and lobular groups respectively.

Patients in the present series classified as lobular were generally younger and more frequently had an acute nephritic onset, compared with those designated non-lobular. Moreover, comparison of the initial and subsequent findings in the 12 patients who underwent repeat biopsy indicate that proliferation and lobularity tend to lessen in severity with time. The two groups could not be clearly separated on the basis of serum C3 concentrations, although the non-lobular group included all eight patients with persistently normal levels. The division of biopsy specimens into lobular and non-lobular categories, while convenient for the analysis of data, is in fact somewhat artificial, for they showed a spectrum of changes from absent to extreme lobularity. We therefore believe that the lobular and non-lobular forms are not separate entities but variants of the same condition, the extent of lobularity perhaps reflecting the stage or severity of the disease.

Tubular and interstitial changes of a permanent nature were more frequently observed, as would be expected, where glomerular damage was severe, and with increasing duration of illness. There was no clear relationship between the degree of glomerular damage and the G.F.R. value at the time of biopsy, but most patients with severe reduction of G.F.R. showed conspicuous tubular atrophy. A similar relationship between creatinine clearance and morphological tubular changes was observed by Risdon *et al.* (1968).

TABLE VII.—*Relation of Glomerular Lobularity to Age at Onset, Sex, Macroscopic Haematuria, and Serum (Plasma) C3 Concentration*

Glomerular Morphology:	Non-lobular		Lobular	
	No.	%	No.	%
Total number of patients	27		23	
Age at onset ≤15 years	11	44	15	65
Age at onset >15 years	16	56	8	35
Sex Male	14	52	15	65
Sex Female	13	48	8	35
Macroscopic haematuria at onset	5	19	10	44
Plasma C3 <90 mg./100 ml.	15	56	19	83

TABLE VIII.—*Results of Treatment.* (38 *Courses of Treatment in* 28 *Patients*)

Specific Therapy	G.F.R. Before Treatment: Normal >80	Impaired 40–80	Severe <40	Total No. of Treatments	Result: Better	Unchanged	Worse/Dead/On Dialysis
None	4	3	0	7	0	6	1
Steroids alone	7	4	3	14*	2	4	8
Cyclophosphamide	2	3	2	7	1	1	5
Steroids + azathioprine	6	8	3	17†	4	5	8

*11 initial courses; 3 following the other treatments.
†9 initial courses; 8 following corticosteroid therapy alone.

Hypocomplementaemia

It is well known that serum complement activity is reduced at the onset of acute nephritis (Gunn, 1914; Lange *et al.*, 1951) but returns to normal as healing occurs, usually within eight weeks of onset. The third component of complement (C3), identified immunoelectrophoretically as β_{1C}/β_{1A}-globulin (Müller-Eberhard *et al.*, 1960), follows a similar pattern of behaviour (West *et al.*, 1964). Low serum complement levels are also found during the active phase of glomerulonephritis complicating systemic lupus erythematosus (Vaughan *et al.*, 1951; Lange *et al.*, 1960), returning to normal as clinical remission occurs.

Lange *et al.* (1951), however, noted that in several patients with apparent acute nephritis the serum complement levels failed to return to normal. In a later publication (Lange *et al.*, 1960) they reported that these patients, numbering 10 out of 246 investigated, all pursued a chronic course and died from renal failure within less than four years from onset of illness. Unfortunately the histological findings were not recorded. Subsequently West *et al.* (1965), and Gotoff *et al.* (1965), reported persistently low serum β_{1C}-globulin levels in children with chronic and progressive glomerulonephritis. West *et al.* (1965), gave a good description of the glomerular morphological features observed in renal biopsies, drawing special attention to the non-argyrophilic material situated on the inner aspect of the capillary basement membranes, which cause thickening of the capillary walls. At that time they had not observed this particular lesion in patients with normal serum β_{1C}-globulin levels, and designated this condition "hypocomplementemic persistent glomerulonephritis." More recently, however (West and McAdams, 1970), they have observed intermittently normal levels, as a result of which they now concur with our own belief that the condition is best defined morphologically, although they expressed some doubt concerning the choice of the term "membranoproliferative" glomerulonephritis. Michael *et al.* (1969), although finding low serum levels of complement and C3 at some time in all their 22 children with membranoproliferative glomerulonephritis, again concur with the suggestion that a morphological definition of the condition is at present the primary one.

Three main explanations of the persistent hypocomplementaemia need to be considered: diminished synthesis, urinary loss, and increased consumption. Alper and Rosen (1967) provided some evidence of impaired synthesis, but their findings have yet to be confirmed, and this explanation has not found general acceptance. Lagrue *et al.* (1969), have suggested that urinary losses may be significant in some instances. However, the report of persistently low serum C3 levels in two patients with M.P.G.N. whose urine was protein-free (Northway *et al.*, 1969), together with our own observation of continued hypocomplementaemia in six patients who were anuric and maintained on regular dialysis, rules out urinary loss as an important cause. In conditions such as acute poststreptococcal nephritis, lupus glomerulonephritis, and serum sickness, whose activity is reflected by temporary lowering of complement activity (Dixon, 1963), the formation of soluble antigen-antibody complexes and their trapping in the glomeruli is presumably a contributory factor. Although C3 can be detected in the glomeruli by immunofluorescent techniques, both in these conditions and in M.P.G.N. (Gotoff *et*

al., 1965; Michael *et al.*, 1969), this is also true of other forms of glomerulonephritis in which hypocomplementaemia is not a feature (Lachmann *et al.*, 1962). The relationship between serum or plasma C3 concentrations and disease activity is inconsistent in M.P.G.N., as illustrated by some of our own patients and those recently described by West and McAdams (1970). Finally, in one patient the serum level remained low after bilateral nephrectomy prior to transplantation (Fig. 8). Thus, trapping of C3 in the glomeruli is at best an incomplete explanation of the hypocomplementaemia. Evidence of *in vivo* activation of C3 is strong (West *et al.*, 1967) and a circulating factor, such as the C3 lytic factor described by Spitzer *et al.* (1969), seems the more likely explanation at present.

Clinical Features

Recent follow-up studies indicate that post-streptococcal nephritis in children rarely fails to heal, and renal biopsy specimens obtained from those patients with persistent disease activity usually show evidence of more serious renal disease (Edelmann *et al.*, 1964; Lieberman and Donnell, 1965; Perlman *et al.*, 1965). In the older literature patients diagnozed as having "acute nephritis" at onset and showing continued urinary abnormalities were regarded as having passed into the "chronic latent" phase (Addis, 1925, 1948; Bell, 1938). Some of our younger patients would undoubtedly have been similarly labelled because of the clinical resemblance of their initial illness to acute nephritis. However, the occurrence in them of atypical features, such as heavy proteinuria, hypoalbuminaemia, persistent hypertension, and, especially, failure of the C3 level to rise to normal within eight weeks of onset, aroused the suspicion of M.P.G.N. We consider it likely that many of the patients included in the older literature as examples of acute nephritis which became chronic actually had M.P.G.N. The serial measurement of serum C3 concentration is thus a valuable screening procedure in patients with "acute nephritis." We have observed only two children with acute nephritis and diffuse proliferative-exudative glomerulonephritis on biopsy, in whom the plasma C3 level remained low more than eight weeks from onset of haematuria. Both had nephrotic features in addition, and one of them showed typical M.P.G.N. on repeat biopsy a year later; the other has not yet had a repeat biopsy.

It is well known that adult patients with the nephrotic syndrome who exhibit nephritic features—that is, haematuria and hypertension—may show proliferative glomerulonephritis on renal biopsy (Jones, 1957; Kark *et al.*, 1958; Blainey *et al.*, 1960; Lawrence *et al.*, 1963). Although the diagnosis of M.P.G.N. was not used in these publications, some of the illustrations are strongly suggestive. A variable nephrotic course accompanied by microscopic haematuria was also found in seven of the nine children with "glomerulonephrite prolongée" and M.P.G.N. reported by Rover *et al.* (1962). In an earlier publication (Ogg *et al.*, 1968) it was shown that low levels of plasma C3 concentration were rarely encountered, except when the renal biopsy findings were M.P.G.N. diffuse proliferative-exudative glomerulonephritis, or lupus nephritis. Thus it is important to determine these levels in patients with the nephrotic syndrome. The plasma C3 concentration discriminates the patients with M.P.G.N. from other varieties of chronic glomerular disease and from "lipoid nephrosis" much better than the haemolytic complement activity of the serum (Lagrue *et al.*, 1967a). The mean haemolytic complement activity of serum from nephrotic patients with many underlying causes is slightly reduced (Ellis and Walton, 1958; Lange *et al.*, 1960; Lagrue *et al.*, 1967b). In contrast, the C3 concentration in forms of the nephrotic syndrome other than M.P.G.N. is somewhat higher than normal (Ogg *et al.*, 1968).

Recurrent haematuria, while an alarming symptom in childhood, is generally benign (Ayoub and Vernier, 1965; Glasgow *et al.*, 1970). However, three of our patients—all children—presented in this way. One has previously been reported by Glasgow *et al.* (1970), while Arneil *et al.* (1969) mentioned a further patient. In all cases the urine was found to contain substantial amounts of proteinuria between the attacks of haematuria, and although this may occur in other less progressive forms of proliferative glomerulonephritis (Glasgow *et al.*, 1970), the association of these features with low plasma C3 levels suggested the diagnosis of M.P.G.N.

One-quarter of our patients were symptom-free and were investigated on account of proteinuria being found on routine urinalysis. It is impossible to tell whether they had always been symptom-free, for, as Herbert (1952) demonstrated, adults who had suffered from well-documented attacks of acute nephritis during childhood often had no memory of the episode. The degree of renal functional impairment found on initial investigation, and the severity of renal damage observed on biopsy in our series, tended to be greater in older patients, suggesting that in many instances the lesion had existed for months or years before symptoms developed. It seems that M.P.G.N. begins mainly in later childhood and adolescence, and the fact that its peak incidence occurs at an age when patients are arbitrarily referred to either paediatricians or physicians may have hindered its recognition.

Conclusion

The observations which we have made on 31 patients who have been followed up for one to eight and a half years and the three who died in less than one year from onset, together with retrospective data from referring consultants extending over periods of up to ten years, have enabled us to construct a tentative picture of M.P.G.N. The condition is one affecting mainly older children and young adults, adolescent girls being especially prevalent in our series. The illness combines both "nephritic" and "nephrotic" features; persistent hypertension is a frequent finding. In children haematuria and nephrotic oedema are equally common presenting symptoms, whereas in adults the former is unusual. Some patients, however, have neither visible haematuria nor oedema, and the diagnosis is made as a result of investigating symptomless proteinuria. The proteinuria is usually of moderately impaired selectivity. A characteristic feature is hypocomplementaemia, specifically due to the occurrence of persistently low serum β_{1C}-globulin (C3) levels. Although only two-thirds of our patients showed low serum C3 levels on initial investigation, more than four-fifths ultimately did so. The diagnosis of M.P.G.N. finally rests with the demonstration of mesangial proliferation and characteristic capillary wall thickening, on renal biopsy.

The course of the disease is generally chronic, although a minority die after a short illness. Renal function may remain normal for several years, despite the glomerular damage, but tends to decline rapidly during the final one to two years of illness. A few adult patients, however, were found already to have a severely impaired G.F.R. when first investigated, and they deteriorated more rapidly. This latter observation, together with the knowledge that many of the patients studied presented with symptomless proteinuria, leads us to believe that the disease can exist for several years without causing symptoms. Treatment with corticosteroids and cytotoxic drugs has not proved rewarding, and high doses of the former may prove hazardous. As the disease progresses towards renal failure hypertension tends to become a severe problem, even in those patients who are kept alive on regular dialysis treatment. The question of renal transplantation raises further problems. Lesions with somewhat similar morphological characteristics developed in a renal allograft in a patient who had formerly suffered from chronic "lobular" glomerulonephritis (Porter *et al.*, 1968) and, in view of evidence for the existence of a complement-lytic factor in the

blood of patients with M.P.G.N. (Spitzer *et al.*, 1969), it is a matter for speculation whether the disease might be transmitted to homografts (Michael *et al.*, 1969).

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The Enterocyte in Coeliac Disease*

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Conflicting Clinical Observations

Further clinical observations conflict with the theory that coeliac disease may be due to a specific enzyme defect. Firstly, though identical twin studies have usually shown concordance in children a single discordant pair of adult coeliac patients has been described. Secondly, if a patient whose mucosa has been restored to normal by a gluten-free diet is refed gluten the histological response is not always immediate. Thirdly, some individuals who have initially responded to a gluten-free diet may then relapse and sometimes die of their disease. Finally, the histological appearance of the flat mucosa is not specific and may apparently occur in conditions other than those associated with gluten sensitivity (Collins and Isselbacher, 1965; Hindle and Creamer, 1965).

Identical Twin Studies

Before biopsy techniques were available, Ebbs (1956), found concordance in five pairs of identical twins, and MacDonald *et al.* (1965), in the family study already referred to, showed concordance in a pair of male children. Jejunal biopsies were carried out in both these patients. But Hoffman *et al.* (1966) recorded a single pair of discordant adult coeliac twins. The propositus was a woman aged 54 years with steatorrhoea and a flat jejunal mucosa who responded well to a gluten-free diet. Her identical twin, however, whose dietary habits were

* Conclusion of the Oliver-Sharpey lecture delivered at the Royal College of Physicians of London on 26 February 1970. Part I appeared in last week's issue.

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