Plasma C3 and C4 Concentrations in Management of Glomerulonephritis

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Summary

As part of a larger study of serial complement profiles in glomerulonephritis plasma C3 and C4 concentrations were measured using commercially available immunodiffusion plates. A total of 303 samples were obtained from 128 patients suffering from forms of nephritis associated with hypocomplementaemia—namely, lupus nephritis, mesangiocapillary glomerulonephritis (M.C.G.N.), and acute glomerulonephritis.

These simple measurements of C3 and C4 gave clinically useful information. In lupus nephritis C3 and C4 generally correlated and C4 concentrations were more often and more profoundly depressed than C3 concentrations. Neither C3 nor C4 concentrations alone correlated well with the antinuclear factor titre.

In both acute glomerulonephritis and M.C.G.N. the C3 concentrations were frequently lower than 20% of normal (which was never the case in patients with lupus), while the C4 concentration was usually normal and was almost never depressed in the absence of C3 depression. This suggests activation of complement at the C3 level by the "bypass" pathway in acute nephritis as well as in M.C.G.N., though both may be operating in some patients. In acute glomerulonephritis but not in M.C.G.N. C3 concentrations returned to normal within eight to 12 weeks.

The two varieties of M.C.G.N. identified by the site of the deposits in the capillary glomerular walls differed in their C3 levels. In 10 patients with intramembranous dense linear deposits the C3 was always low over very long periods of time, rising in three out of four patients only after transplantation and immunosuppression. Other patients with M.C.G.N., in contrast, often showed normal C3 concentrations. Concentrations of C4 did not differ in either group, being normal in 80% of samples from all types.

Introduction

The complement system consists of 11 components which together form some 10% of circulating plasma protein (*Lancet*, 1972; Ruddy *et al.*, 1972). Its importance in the pathogenesis and management of glomerulonephritis is already established (Cameron, 1972; West *et al.*, 1973), and though the measurement of plasma concentrations rather than rates of turnover has limitations the simplicity of this approach is such that it has been widely adopted in research laboratories (West *et al.*, 1964; Ogg *et al.*, 1968; Lam, 1970; Grenier *et al.*, 1972).

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Three types of glomerulonephritis are associated with consistent changes in the plasma concentrations of complement components—(1) acute glomerulonephritis (A.G.N.) occurring after streptococcal infections, where the concentrations of total haemolytic and C3 complements are lowered, usually returning to normal within two to eight weeks of the attack (Lange *et al.*, 1960; West *et al.*, 1964; Ogg *et al.*, 1968; Cameron, 1970; Grenier *et al.*, 1972); (2) lupus glomerulonephritis, where total haemolytic complement concentrations run a fluctuating course, often varying with disease activity as judged by other measurements or with treatment (Vaughan *et al.*, 1951; Lange *et al.*, 1960; Lange *et al.*, 1965; Lewis *et al.*, 1971); and (3) mesangiocapillary glomerulonephritis (M.C.G.N.; membranoproliferative or "lobular" glomerulonephritis), where many patients show very low complement concentrations, particularly C3, for months or years (West *et al.*, 1964, 1965; Ogg *et al.*, 1968; Cameron *et al.*, 1970; Herdman *et al.*, 1971; Vallota *et al.*, 1972).

Rarer glomerular diseases associated with alterations in complement concentration include the glomerulonephritis associated with subacute bacterial endocarditis (Herdman *et al.*, 1971) and infected juguloatrial shunts for hydrocephalus (Herdman *et al.*, 1971), thrombotic thrombocytopenic purpura (J. S. Cameron and C. S. Ogg, unpublished), and the rare but very interesting syndromes associated with inherited complement abnormalities (Pickering *et al.*, 1970; Holland *et al.*, 1972; Ruddy *et al.*, 1972).

Recently it has been recognized that besides the "classical" activation of complement by the early components C1, C4, and C2 the sequence C3-C9 may be triggered by activation at the C3 stage by an "alternate" or "bypass" pathway. This involves several components, including properdin, glycine-rich - glycoprotein (Gewurtz, 1971; Götze and Muller-Eberhard, 1971; Lachmann and Nichol, 1973), and the C3 breakdown product C3b (Williams *et al.*, 1973 a). When this pathway operates, C3 (but *not* the early components C1, C4, and C2) may be depleted from the plasma. The serum of some patients suffering from M.C.G.N. contains a factor (C3 nephritic factor) which can activate this bypass or alternate pathway (Vallota *et al.*, 1970; Peters *et al.*, 1972; Vallota *et al.*, 1972), as does the serum from some patients with acute glomerulonephritis (Pickering *et al.*, 1969; Williams *et al.*, 1972).

The measurement of different complement components proves useful in distinguishing mechanisms of complement activation and in differentiating the types of nephritis associated with low complement levels in the plasma. In parallel with an extensive serial study of complete complement profiles in M.C.G.N. and acute glomerulonephritis (Williams *et al.*, 1973 b, c) we have measured C3 and C4 plasma concentrations using commercially available immunodiffusion plates to see if these readily available, simple measurements would give useful information to clinicians managing patients with nephritis.

Materials and Methods

PLASMAS

Altogether 303 plasma samples were obtained from 128 patients studied during the period August 1971 to March 1973. Blood was drawn into heparinized, plastic (occasionally glass) containers, separated, and a drop of 1/1,000 sodium azide added; the plasmas were then kept at room temperature for about

24 hours and then frozen. The C3 and C4 concentrations were usually estimated within one to four weeks of receipt.

PATIENTS

Forty-one patients with proteinuria showed the appearances of M.C.G.N. on light microscopy of their renal biopsy specimens, as previously described (Cameron *et al.*, 1970; Cameron *et al.*, 1973 b). Their disease had been present for a few months up to 10 years. A further histological subdivision of 31 of these patients was made—21 with lumpy, "slug-like" subendothelial deposits in the glomerular capillary walls, and 10 with more or less continuous dense deposits within or actually replacing the glomerular capillary basement membrane (Berger and Galle, 1963; Faye and Antoine, 1972; Cameron *et al.*, 1973 b). This distinction was made by light and electron microscopy in 16 patients and by light microscopy alone in 15. During the course of the study four patients, all with intramembranous deposits, were transplanted and placed on azathioprine and prednisolone. Only three other patients were treated during the period of study, one with azathioprine and prednisone alone, and the other two with both these drugs together with dipyridamole and heparin followed by warfarin. All these patients had subendothelial deposits.

Fifty-five patients with a clinical diagnosis of *systemic lupus erythematosus*, all with proteinuria, were studied. All but two had extrarenal manifestations of lupus, and in 32 various forms of glomerulonephritis were seen on renal biopsy. All had been shown repeatedly to be positive for antinuclear factor. Altogether 48 were women and seven were men, one of whom developed lupus after procainamide administration. All but three patients received various amounts and combinations of prednisolone, azathioprine, and cyclophosphamide. One patient was placed on dialysis and one was transplanted during the period of study.

Thirty-two patients (27 children and 5 adults) with a diagnosis of *acute glomerulonephritis* were studied one day to 200 days after the onset of their disease. Only four of these patients underwent biopsy but evidence of streptococcal infection wa present in 28 (raised or rising antistreptolysin O titre or culture); the site of infection was the nasopharynx in all the patients but one, where infected eczema appeared to be the precipitating factor. A typical acute nephritic syndrome was present in all those with haematuria, oliguria, and mild uraemia. All patients showed a diuresis within one week and had completely normal urine when last examined.

LABORATORY TECHNIQUES

Plasma C3 was measured using Hyland Laboratories Immunoplates, as previously described (Ogg *et al.*, 1968).

Plasma C4 was measured using Behringwerke Partigen immunodiffusion plates, as described by the manufacturers.

Antinuclear factors were detected by a standard indirect immunofluorescent technique. Six-micron cryostat sections of rat liver and kidney were used as substrates. Forty-seven serum samples from 24 patients with systemic lupus erythematosus were diluted 1/10, 1/50, 1/250, and 1/1,250 and stained with fluorescein-labelled sheep antihuman immunoglobulin serum.

Results

The plasma C3 is plotted against the plasma C4 in figs. 1 to 3 for the three groups of patients (lupus nephritis, acute glomerulonephritis, and M.C.G.N.). Low plasma C4 concentrations were commonest in the lupus nephritis group, but a C3 concentration of below 20% of reference normal serum (R.N.S.) was not seen in this group. In general C3 and C4 concentrations correlated with one another in lupus nephritis though there was considerable individual variation. In contrast in both the

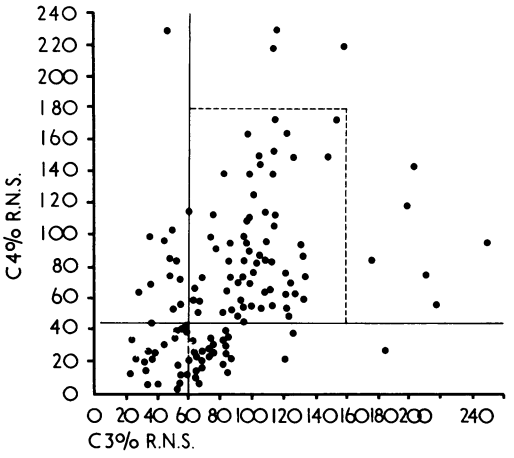

FIG. 1—Plasma C3 and C4 concentrations as percentage of reference normal serum in 136 samples from 55 patients with systemic lupus erythematosus. In figs. 1, 3, and 4 normal limits for C3 and C4 (mean ± 2 S.D. log) are indicated by box.

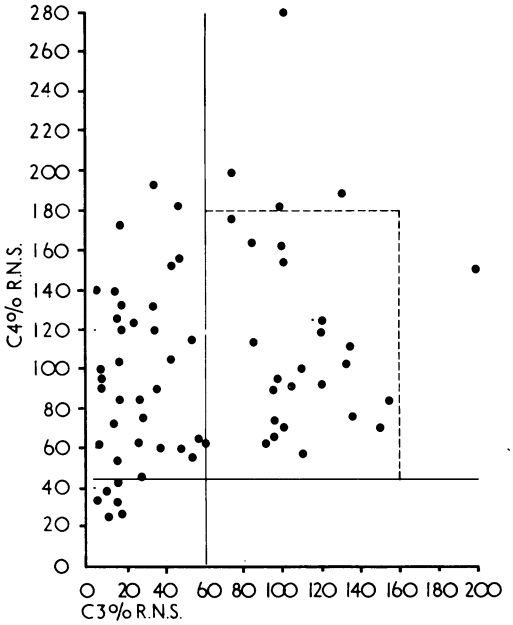

FIG. 2—Plasma C3 and C4 concentrations as percentage of reference normal serum in 66 samples from 32 patients one to 200 days after onset of attack of acute glomerulonephritis.

M.C.G.N. and acute glomerulonephritis groups C3 concentrations below 20% of reference normal serum were common and low C4 concentrations rare. In the patients with acute glomerulonephritis low plasma C4 levels were seen only with very low plasma C3 concentrations. In only one patient in the M.C.G.N. group, and in no patient with acute glomerulonephritis, was the plasma C4 concentration low when the plasma C3 concentration was normal; in lupus this was found in 27 samples from 15 different patients. Few patients with lupus showed low C3 concentrations in the presence of normal C4 concentrations, while this was present in half of the samples from the other two groups. These findings are summarized in table I.

The correlation between antinuclear factor titres and C3 and C4 concentrations was poor (fig. 4). There was a suggestion that the group with low C3 *and* low C4 plasma concentrations were more often strongly positive for antinuclear factor (table II) but the data were insufficient to draw firm conclusions.

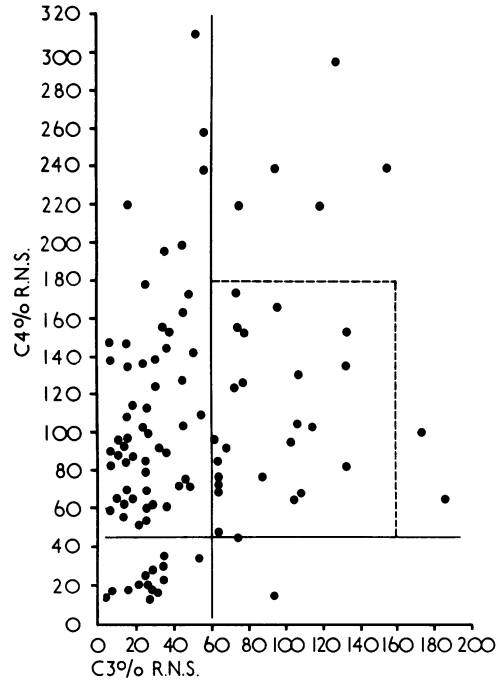

FIG. 3—Plasma C3 and C4 concentrations as percentage of reference normal serum in 101 samples from 41 patients with M.C.G.N. Patients with all types of deposits in glomerular capillary wall are included.

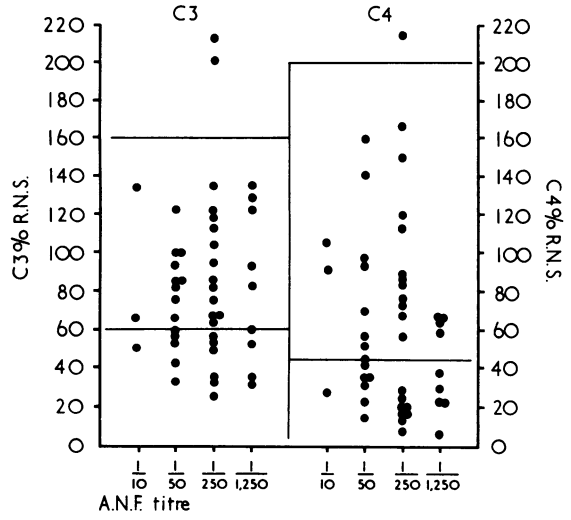

FIG. 4—Correlation between antinuclear factor (A.N.F.) titre and plasma C3 and C4 concentrations.

The serial C3 and C4 concentrations in the patients with acute glomerulonephritis are shown in figs. 5 and 6. As previously reported (West *et al.*, 1964; Gewurtz *et al.*, 1968; Cameron, 1970), the C3 concentrations (fig. 5) were almost always low in the first week or two after the onset of the nephritis. One patient, however, did *not* have a low C3 on the day after onset. Only two patients showed low C3 concentrations more than eight weeks after the attack and none more than 12 weeks. In contrast the C4 concentrations (fig. 6) showed no consistent pattern, the plasma concentrations in most patients remaining normal throughout, even in the early phases of the condition. The few low C4 concentrations, however, were observed mostly in the first few weeks.

The plasma C3 and C4 concentrations in the 10 patients with M.C.G.N. in whom dense intramembranous deposits were identified in the glomerular capillary walls (6 by electron microscopy, 4 by light microscopy) are shown in fig. 7. The consistently low C3 concentrations in this group are striking, as previously reported (Cameron *et al.*, 1973 a, b; Levy *et al.*, 1973), rising to normal levels in three of the four patients transplanted only when they were placed on treatment with

TABLE I—*C3 and C4 Plasma Concentrations in Relation to Diagnosis*

	Lupus (55 Patients)		A.G.N. (32 Patients)		M.C.G.N. (41 Patients)	
	No.	%	No.	%	No.	%
Normal C3, normal C4	75	55	28	42	30	30
Normal C3, low C4 ..	27	20	0	0	2	2
Low C3, low C4 ..	21	15	6	9	14	14
Low C3, normal C4	13	10	32	48	55	54
Total samples	136		66		101	

For the purposes of this table "normal" includes all those values above the lower limit of normal, including those above the upper limit.

TABLE II—*Relation between C3 and C4 Levels and Antinuclear Factor Titre*

	No. of Samples	Antinuclear Factor				
		Neg.	1/10	1/50	1/250	1/1,250
Low C3	17	1	1	5	6	4
Low C4	21	0	1	7	8	5
Low C3, low C4 ..	10	0	0	3	3	4

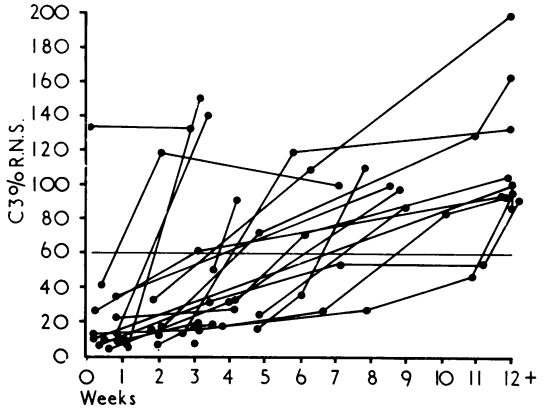

FIG. 5—Serial C3 concentrations after attack in patients with acute glomerulonephritis. Time in weeks refers to time elapsed from clinical onset of condition.

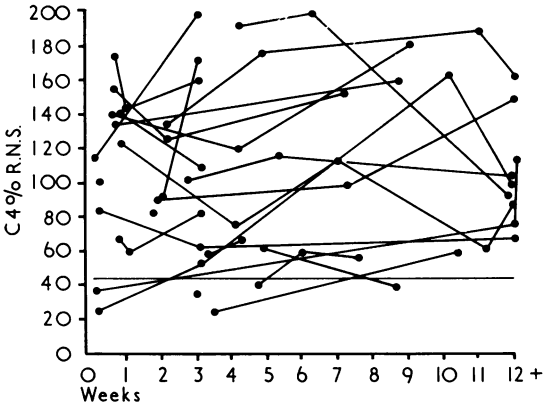

FIG. 6—Serial C4 concentrations after attack in patients with acute glomerulonephritis. Time in weeks refers to time elapsed from clinical onset of condition.

azathioprine and corticosteroids (fig. 8). At the same time their C4 levels also rose, generally to above normal levels. The C3 nephritic factor activity of these patients has been reported elsewhere (Williams *et al.*, 1973 c). A few C4 levels were low before transplantation but most were normal. In contrast in the 21 patients with subendothelial deposits (which may or may not be a homogeneous entity) there was great variation in the C3 levels, about one-third being normal. The C4 concentrations were somewhat more often low than in the dense intramembranous deposit group but were normal in most patients in both groups.

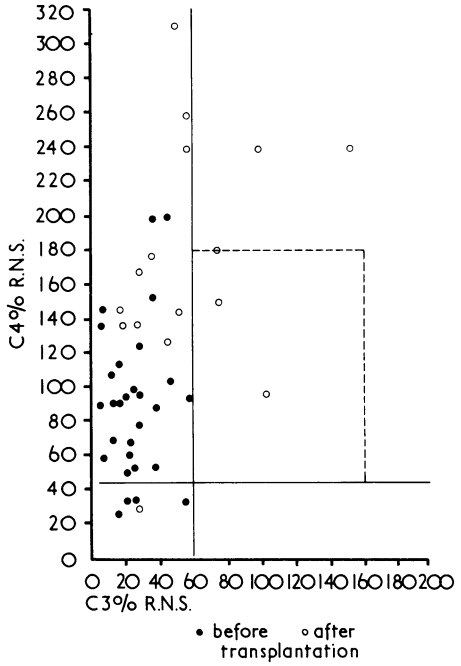

FIG. 7—C3 and C4 concentrations in 10 patients with M.C.G.N. in whom dense intramembranous deposits were identified in basement membranes of glomerular capillary loops, Bowman's capsule, and some tubules. Values obtained in four patients after transplantation and treatment with azathioprine and corticosteroids are indicated. No other patient received any immunosuppressive therapy with either these or other drugs during period of study.

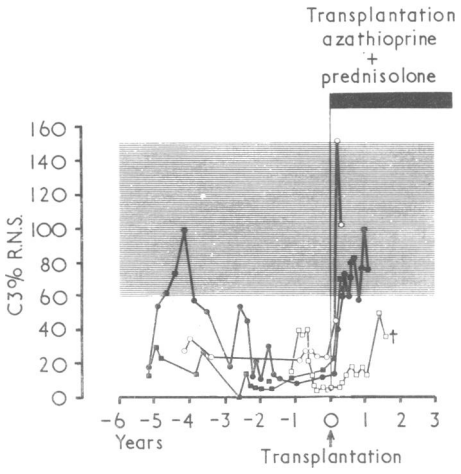

FIG. 8—Serial plasma C3 concentrations in four patients with M.C.G.N. and dense intramembranous deposits who were transplanted and placed on treatment with azathioprine and corticosteroids. Three patients were on dialysis for three to 18 months before transplantation. Fourth patient was transplanted without prior dialysis.

Discussion

The concentration of different complement components in various forms of glomerulonephritis has been described by several workers (Gewurtz *et al.*, 1968; Kohler and ten Bensel, 1969; Lewis *et al.*, 1971). Only the measurement of C3 has gained wide currency. With the description of the C3 bypass system of complement activation (Gewurtz, 1971; Götze and Müller-Eberhard, 1971; Lachmann and Nichol, 1973) it becomes of clinical interest to see whether evidence of "classical" or bypass activation is present in the various forms of nephritis. Normal plasma concentrations of complement components do not, of course, exclude accelerated turnover but depleted concentrations suggest that either accelerated catabolism or depressed synthesis or both may be present. Of the components of the "classical" pathway of C3 activation the easiest to measure is C4. Its measurement together with that of C3 should enhance the understanding of the mechanisms involved and aid the clinical definition of the syndromes of glomerulonephritis. For the past two years we have measured C4 in addition to C3 in all patients with a low C3 and in all patients with clinical or histological features which suggested that some complement abnormality might be present. Almost all these patients with low C3 had one of three conditions—lupus nephritis, M.C.G.N., or early acute nephritis (table III). The only other patients to show a low C3 during this period are indicated in table III, suggesting that C3 is a good screening test for these conditions among patients with renal disease.

TABLE III—*Diagnoses in Patients with Low Plasma C3 Concentration. August 1971 to March 1973*

	No. of Patients
Lupus erythematosus	25
Acute glomerulonephritis	28
M.C.G.N.	23
Others	17
Primary renal:	
Minimal change	1
Membranous	1
Focal glomerulosclerosis	2
Secondary to generalized disorders:	
Vasculitis, unspecified	2
Wegener's granulomatosis	1
Rheumatoid arthritis and arteritis	1
Thrombotic thrombocytopenic purpura	2
No details	7
Total	93 (10%)

Samples studied: 1,380; Patients studied: 915.

Lupus glomerulonephritis is generally considered to be the best established human model of chronic soluble complex renal disease. It is not surprising, therefore, to find that C4 is depleted more than C3 or to an equal degree (fig. 1), since experimental soluble complexes activate the classical pathway. The alternate pathway probably operates as well as a feedback pathway mediated by C3b (Williams *et al.*, 1973 a). This would be difficult to detect in the presence of classical pathway activation unless depletion or increased catabolism of the components of this pathway can be shown (Williams *et al.*, 1973 b). Diagnostically a C3 level of lower than 20% of normal is very unlikely to be the result of lupus nephritis, as shown by the data of Lewis *et al.* (1971). A low C4 level with a normal or less depleted C3 is almost never found in the other two forms of nephritis, which are associated with a low C3 level (table I).

In *M.C.G.N.* the existence of a factor or factors (C3 nephritic factor) capable of activating the alternate pathway is now well recognized (Pickering *et al.*, 1969; Vallota *et al.*, 1970; Peters *et al.*, 1972; Vallota *et al.*, 1972). Concentrations of C4 have generally been reported as normal in the presence of this histological finding (Lewis *et al.*, 1971; Peters *et al.*, 1972) but with the accumulation of more data occasional low C4 concentrations have been observed (Arisz *et al.*, 1972; Levy *et al.*, 1973). Our data support this suggestion. Levy *et al.* (1973) report that low C4 concentrations are never found in patients with M.C.G.N. and dense intramembranous deposits (Berger and

Calle, 1963). Our data show about the same proportion of low C4 concentrations in this group (fig. 7) as in the M.C.G.N. group as a whole (fig. 3), which may indicate some classical pathway activation in some patients. Activation of C3-C9 in this condition, however, is mainly through the bypass as a consequence of C3 nephritic factor activity (Vallota *et al.*, 1970; Peters *et al.*, 1972), with C3b as one limiting cofactor (Williams *et al.*, 1973 a). The rise in C3 and C4 concentrations in the three transplanted patients after beginning azathioprine and corticosteroid treatment (fig. 8) is difficult to interpret. Vallota *et al.* (1971, 1972) reported a rise in C3 concentrations and a fall in C3 nephritic factor activity after treatment with corticosteroids and transplantation but this should not affect C4 concentrations, which became supranormal in our patients. Though C4 concentrations had been normal it is possible there was increased synthesis *and* catabolism of this component; the latter being inhibited in some way by the treatment, leading to a rise above normal levels. On the other hand, C4 is an acute-phase protein and the rise may be a non-specific result of transplantation.

Initially it was assumed that *acute glomerulonephritis* was a soluble complex illness and that the C3-C9 depletion depended on activation of C1, C4, and C2. However, both Gewurtz *et al.* (1968) and Kohler and ten Bensel (1969) found that the early components were very inconstantly depleted in active acute nephritis, as shown in our own data (figs. 3 and 6 and table II), where 60 out of 66 C4 measurements in acute glomerulonephritis were normal. The consistent C3 depression (fig. 5) in the early phases therefore suggests bypass activation as the major route of C3-C9 consumption in acute nephritis, and Williams *et al.* (1972) reported a nephritic factor capable of activating C3 in sera from patients with acute nephritis. Acute nephritis resembles M.C.G.N. much more closely than lupus in the pattern of complement activation. A C3 concentration of less than 20% of normal is often found in both conditions, a low C4 rarely and almost always in association with a low C3. The main distinguishing feature between acute glomerulonephritis and M.C.G.N. so far as complement is concerned is the regularity with which the C3 returns to normal levels in acute glomerulonephritis (fig. 5), whereas in M.C.G.N., especially the variety with dense intramembranous deposits, persistence of low C3 concentrations for months or years is the rule (fig. 8). So far we have followed serial C3 concentrations in 51 patients with acute glomerulonephritis, 32 of whom were included in this study. In all but two of these patients (shown in fig. 5) the C3 concentration had returned to normal by the end of an eight-week period. The persistence of a low C3 beyond this time should make one suspicious that the initial illness was not, in fact, an uncomplicated acute glomerulonephritis. What the relation may be between the clinical entity of acute glomerulonephritis, usually associated with a resolving glomerular histological picture, and the usually progressive M.C.G.N. has still to be worked out. Transition has only rarely been observed (White and Glasgow, 1973) but the similarity in the complement activation is striking.

From these data a policy for investigating patients with glomerulonephritis can be set out. All patients with glomerulonephritis should have the C3 concentration of their plasma or serum estimated. All those with a low C3 or suspected lupus nephritis should have a C4 estimation in addition. All patients with low C3 or C4 concentrations should have serial measurements performed to see if the alterations reverse within a few weeks or persist. Most patients with a persistently low C3 or C4 concentration will need a renal biopsy at some point and should, of course, be screened for lupus. Selected patients will need a full complement profile, including measurement of alternate pathway components and total haemolytic complement. This will require fresh or fresh-frozen serum but the earlier screening can be done by interested laboratories on samples sent by post.

It must be remembered that screening for C3 and C4 will *not* pick up renal disease in association with either C1r (Pickering *et al.*, 1970) or C2 deficiencies (Pickering *et al.*, 1970; Holland *et al.*, 1972) as the measurement of total haemolytic complement will. The numbers of such patients are very small but they are of great interest. Patients with lupus-like conditions but in whom tests for lupus, including antinuclear antibody, are negative should be screened for total haemolytic complement activity even if the C3 is normal, as should patients with membranous nephropathy. The former has been reported in association with C1r deficiency (Moncada *et al.*, 1972) and the latter with C2 deficiency. It is probable that these associations are more common than at present suspected, but the convenience of radial diffusion methods means that for the moment C3 and C4 screening tests are the most efficient methods for samples from scattered hospitals.

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