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Significance of urinary C3 excretion in glomerulonephritis

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SYNOPSIS The third component of complement (C3) was measured in the urine of 98 patients with a variety of renal diseases. Renal biopsy was performed on 83 of the patients and examined by light, electron, and immunofluorescence microscopy. Urinary C3 was detected in cases of membranous glomerulonephritis, mesangiocapillary glomerulonephritis, rapidly progressive glomerulonephritis, and renal amyloidosis. It was not detected in minimal lesion glomerulonephritis; in cases of proliferative glomerulonephritis it was detected only in those showing histological evidence of a progressive lesion. Concentrations were low or undetectable in cases of non-immunological renal diseases. There was a good correlation between urinary C3 concentrations and the deposition of C3 in glomerular capillary walls, as seen by immunofluorescence microscopy, and there was no correlation with the degree or selectivity of proteinuria. Urinary C3 excretion appears to be an accurate indicator of continuing activity of disease. It is suggested that the presence of C3 in urine is due to complement fixation by immune complexes in glomerular capillary walls, and that urinary C3 estimations have potential applications in the study of glomerulonephritis.

The association of complement with renal disease has been known since 1902 when Hedinger described low serum complement in 'acute nephritis'. Many studies have subsequently shown that low serum complement titres are characteristic of acute post-streptococcal glomerulonephritis, mesangiocapillary glomerulonephritis, systemic lupus erythematosus with renal involvement, and nephritis accompanying subacute bacterial endocarditis (Lange *et al*, 1951, 1960; West *et al*, 1964; Gotoff *et al*, 1965; Ogg *et al*, 1968; Gutman *et al*, 1972). This is thought to be due in many cases to utilization of complement by immune complexes (Kohler and Ten Bensel, 1969; Koffler *et al*, 1971), although direct activation of the third component by the alternate pathway is known to occur in some types of glomerulonephritis (Vallota *et al*, 1970). It has also been shown that in many situations there is a correlation between the whole complement titre and the concentration of the third component of complement (C3) (Klemperer *et al*, 1965; Lundh *et al*, 1970).

Since complement is activated during immune reactions, it seems possbile that examination of complement excretion in the urine could give information about renal immune processes. Lange and Wenk (1954) investigated urinary loss of complement as a possible cause of low serum whole complement titres in glomerulonephritis and found that there was no correlation between the urinary complement titre and the serum complement titre, or the degree of proteinuria. Gotoff *et al* (1965) found C3 in the urine of patients with nephrotic syndrome with heavy proteinuria but not in cases of acute or progressive glomerulonephritis. Ogg *et al* (1968) found urinary C3 in only one of 25 patients with low serum C3 concentrations.

Lagrue *et al* (1969) examined complement activity in the urine of 24 patients with glomerulonephritis and found complement components in the urine of nine patients. Complement excretion did not correlate with the degree or selectivity of proteinuria but varied in the four types of glomerulonephritis studied. They concluded that the mechanism of urinary complement excretion remained to be determined and that its frequency and significance should be further studied. Hoq *et al* (1974) studied 15 cases of proliferative glomerulonephritis and 10 cases after renal homotransplantation. Urinary C3 concentrations correlated with urinary concentrations of fibrin degradation products and hetero-

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phile haemagglutinins, were not related to total urinary protein, and gave information on response to treatment and occurrence of rejection. Williams *et al* (1974) studied 123 patients with glomerulonephritis; urinary C3 was detected often in mesangiocapillary and membranous glomerulonephritis and in focal glomerulosclerosis, less frequently in lupus nephritis and a mixed group of cases with proliferative glomerulonephritis, and rarely in minimal lesion glomerulonephritis.

The present study examines the concentration and significance of C3 in the urine of patients with renal disease. Immunofluorescence microscopy has demonstrated the presence of material, consisting of immunoglobulins and complement components, within the glomerular capillary wall in several forms of glomerulonephritis, and it seems likely that deposition of such material could give rise to increased components of complement in the urine. This was investigated by examining (1) the relationship of urinary C3 to the type of glomerulonephritis present, and also urinary C3 concentrations in cases of proteinuria thought not to be due to a complement-fixing glomerular immune reaction; (2) the relationship between urinary C3 concentrations and C3 deposition in glomerular capillary walls detected by immunofluorescence microscopy; (3) the relationship between urinary C3 concentrations and the degree and selectivity of proteinuria.

Material and Methods

PATIENTS STUDIED

The concentration of urinary C3 was measured in 98 patients, 83 of whom underwent renal biopsy. The histological diagnoses are shown in the table. Of the 18 cases of proliferative glomerulonephritis without histological evidence of progression, seven showed an acute, exudative lesion. They were histologically similar to classical poststreptococcal glomerulonephritis, showing diffuse enlargement of tufts, moderate to marked mesangial hypercellularity, and a polymorphonuclear infiltrate. Two of these cases had a proven previous streptococcal infection and a raised ASO titre. In 11 cases there were no polymorphs in the glomeruli, there was slight to moderate hypercellularity of mesangial regions, and these were considered to be cases of mild proliferative glomerulonephritis.

Type of Disease	*No. of Cases*
Proliferative glomerulonephritis	
no histological evidence of progression	18
histological evidence of progression	22
Rapidly progressive glomerulonephritis	6
Membranous glomerulonephritis	15
Mesangiocapillary glomerulonephritis	9
Minimal lesion glomerulonephritis	5
Renal amyloidosis	4
Non-immunological renal disease	12
Urinary tract infection	7

Table *Diagnoses and number of patients of each type*

Twenty-two cases showed enlarged, hyaline, and hypercellular mesangial regions, producing variable degrees of hyalinization of the tufts, and in some cases capsular adhesions and small epithelial crescents were additional features of note. These changes were considered to indicate progression of the disease process resulting in some permanent glomerular damage. They were therefore designated as cases of proliferative glomerulonephritis showing histological evidence of progression.

There were six cases of rapidly progressive glomerulonephritis, the main histological features being florid circumferential epithelial crescents in most glomeruli, compressing the capillary tufts, with little or no mesangial hypercellularity.

Other types of renal disease were also diagnosed histologically according to conventional criteria, except for eight cases of non-immunological renal disease and seven cases of urinary tract infection, which were diagnosed on clinical grounds since a biopsy had not been performed.

HISTOLOGICAL METHODS

All biopsies were examined by light microscopy; electron microscopy was performed in 64 cases and immunofluorescence microscopy in 51. For light microscopy, biopsy material was fixed in corrosive formol saline for 18-24 hours, sectioned at 2 μm, and stained by the haematoxylin and eosin, picro Mallory, periodic-acid Schiff, and Martius scarlet blue methods. For electron microscopy the tissue was fixed in 1% osmium tetroxide, sometimes with preliminary glutaraldehyde fixation. It was dehydrated in graded alcohols and embedded in Araldite or Epon. Sections were cut on a Porter Blum MT2 ultratome at 50 nm, stained with lead citrate and uranyl acetate, and viewed in an AE1 EM6 electron microscope. For immunofluorescence microscopy fresh tissue was 'snap frozen' with Drikold and sectioned at 2 to 3 μm in a cryostat. After fixation with 95% alcohol for 10 minutes, sections were incubated with fluorescein isothiocyanate conjugated (FITC) antihuman serum to C3, IgG, IgA, IgM, and fibrin-fibrinogen (Hoechst). Sections were viewed in a Leitz Ortholux microscope using an HBO 200 lamp with BG12 and BG38 primary filters and a K510 barrier filter.

URINARY C3 AND SELECTIVITY

For C3 estimations 10-30 ml samples of urine were used; where possible these were early morning

specimens. Three to four daily samples were obtained from most patients and the highest urinary C3 concentration found in each patient was used in subsequent analysis. Samples were tested for total protein, using Albustix (Ames), dialysed against running tap water for 8 hours, and left overnight in a solution of polyethylene glycol (Carbowax) at −4°C, which concentrated each sample 10-15 times. Samples were stored at −30°C until they were tested.

C3 concentrations were measured by single radial immunodiffusion; plates were prepared using a modification of the method of Mancini (Mancini *et al*, 1965). 0·3 ml of rabbit antiserum to human β_{1C}-β_{1A} globulin (Hoechst) was used in each 16-well plate. We have not ascertained with which fragments of C3 the antiserum reacts; previous work suggests it may react with intact C3, C3b, and C3c (West *et al*, 1966; Bokisch *et al*, 1969; Ruddy *et al*, 1972). The antiserum was used in immunoelectrophoresis of aged human serum; a single arc was obtained. Four serial dilutions of a pool of 30 normal human sera were included on each plate. The C3 concentration of this pool was measured using standard sera (Travenol Laboratories) and found to be 1·3 g/l. Results of test samples were expressed as mg/l. A single sample was tested on 20 separate occasions and the standard deviation was found to be ±8%. Protein selectivity was estimated by the method of MacLean and Robson (1967).

Results

RELATION OF URINARY C3 TO HISTOLOGY (FIGURE)

Urinary C3 was not detectable in any of the 18 patients with proliferative glomerulonephritis who showed no histological evidence of progression. Of the seven patients in this group with an acute exudative lesion, all had moderate or heavy proteinuria and three showed immunofluorescence to C3 in glomerular capillary walls. Nine of the 11

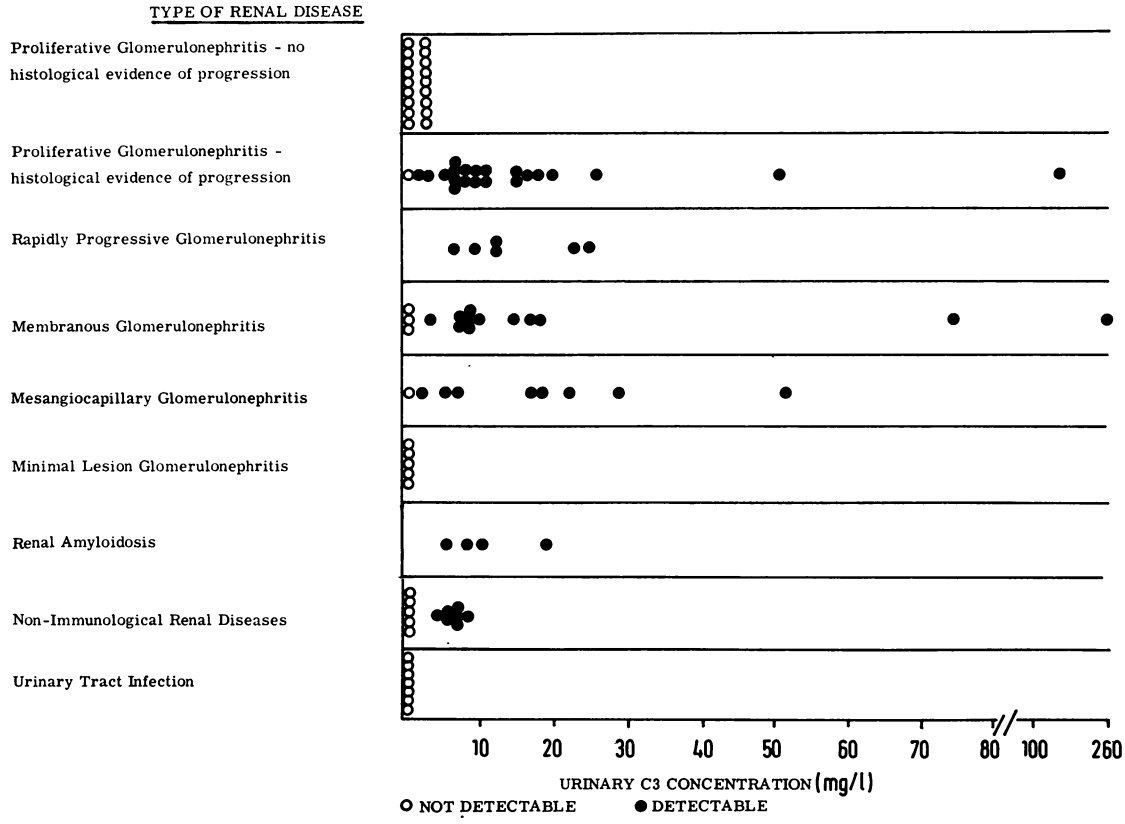

Figure *Maximum concentrations of C3 in urine from patients with renal disease. Conversion: SI to Traditional Units—C3: 1 mg/l = 0·1 mg/100 ml*

patients with mild proliferative glomerulonephritis showed significant proteinuria and one showed some C3 deposition in glomerular capillary walls by immunofluorescence microscopy. In follow-up observations between 13 months and five years after onset of the disease, one of the 18 patients had raised plasma urea and creatinine concentrations, and one had recurrent proteinuria. The other patients were symptom-free, had normal plasma urea and creatinine concentrations, normal blood pressure, and no proteinuria or haematuria.

In contrast, 21 of the 22 cases of progressive proliferative glomerulonephritis showed significant urinary C3 concentrations (median 10 mg/l, range 0-210 mg/l). Of the 12 cases studied by immunofluorescence microscopy, five showed immunofluorescence to C3 in glomerular capillary walls. In follow-up studies between one and 11 years after onset of the disease, all but one of the 22 patients showed one or more of the following abnormalities—raised plasma urea or creatinine concentration, raised blood pressure, persistent proteinuria or haematuria. The patient whose disease clinically resolved had a maximum urinary C3 concentration of 10 mg/l.

The six cases of rapidly progressive glomerulonephritis all showed detectable urinary C3, but, in comparison with the progressive proliferative cases, a smaller range of concentrations was found (median 12 mg/l, range 8-25 mg/l). Four cases showed immunofluorescence to C3 in glomeruli. The greatest range of urinary C3 concentrations was found in the 15 cases of membranous glomerulonephritis (median 8 mg/l, range 0-260 mg/l). No urinary C3 was detected in three of the patients who were in total remission. Urinary C3 was present in 12 patients who had significant proteinuria.

Eight of the nine cases of mesangiocapillary glomerulonephritis showed detectable urinary C3. The median concentration was 17 mg/l and the range 0-52 mg/l. Six of the nine cases showed C3 deposits in glomerular capillary walls by immunofluorescence microscopy.

None of the five cases of minimal lesion glomerulonephritis showed detectable urinary C3, but all had heavy proteinuria. None of the cases showed any C3 deposition by immunofluorescence microscopy.

Four cases of nephrotic syndrome due to amyloid disease proven by renal biopsy showed maximum urinary C3 concentrations of 6 mg/l, 8 mg/l, 10 mg/l, and 18 mg/l.

In the group of non-immunological renal diseases there were cases of hydronephrosis (3), polycystic kidneys (2), analgesic nephropathy (1), chronic pyelonephritis (4), and renal tuberculosis (2). Five of these 12 cases showed no detectable urinary C3; the median value was 5 mg/l and the range 0-8 mg/l. All cases had proteinuria greater than 1 g/l and were considered unlikely to have a glomerular lesion involving C3 fixation.

In seven cases of proven urinary tract infection, no urinary C3 was detected.

Urine samples from eight normal controls showed no urinary C3 even when concentrated up to 50 times.

RELATION OF URINARY C3 TO IMMUNOFLUORESCENCE MICROSCOPY

Fifty-one patients were studied whose renal biopsies had been examined by immunofluorescence microscopy and from whom urine samples had been taken on the morning of, or within two days of, biopsy. Twenty-one of these showed positive immunofluorescence to C3 in glomerular capillary walls; 30 showed no glomerular C3. Of the 21 patients with glomerular C3 deposition, 17 showed detectable urinary C3. The median value was 9 mg/l and the range 0-75 mg/l. Of the 30 patients with no glomerular C3, 20 showed no detectable urinary C3, and the range was 0-20 mg/l. This relationship between positive immunofluorescence to C3 in glomerular capillary walls and urinary C3 concentration is highly significant (Wilcoxon Rank Sum Test $2\alpha < 0{\cdot}01$; Student t test $P < 0{\cdot}001$).

RELATION OF URINARY C3 TO DEGREE OF PROTEINURIA

A sample of fresh urine from 82 patients was tested for total protein. The correlation between this value and the C3 concentration in each sample was examined. A one-way analysis of variance showed no significant relationship ($F = 2{\cdot}0695$; for 5% significance F must be $> 5{\cdot}67$). Six patients had detectable urinary C3 in the absence of proteinuria; 15 patients had proteinuria greater than 3 g/l but no detectable urinary C3.

RELATION OF URINARY C3 TO SELECTIVITY OF PROTEINURIA

The urinary C3 concentration and the total protein concentration were determined in single urine samples from 24 patients, 12 of whom had selective proteinuria ($k_2 < 1{\cdot}3$) and 12 of whom had non-selective proteinuria ($k_2 > 2{\cdot}3$). All samples had a total protein concentration of over 1 g/l. The urinary C3 concentrations in the patients with selective and non-selective proteinuria were compared using a Wilcoxon Rank Sum Test; this showed that there was no significant difference in urinary C3 excretion between the two groups ($2\alpha = > 0{\cdot}10$).

Discussion

C3 was the most suitable component for this study for four reasons: (1) it correlates well with whole complement levels in many situations; (2) it is involved in both the classical and the alternate pathways of complement activation; (3) a single complex of the earlier complement components can activate a large number of C3 molecules, so that changes in C3 concentration are more likely to be detectable than changes in the levels of earlier components; and (4) a suitable commercial antiserum is available. Although it would be of interest to know which fragments of C3 are present in urine and in what proportions, it is considered that, in this type of study, valid use may be made of an antiserum reacting with two or more of the major fragments.

C3 has been detected in the urine of 59 of the 98 cases of renal disease studied. This is a higher proportion than has previously been reported. Lagrue *et al* (1969) found urinary C3 in nine of 24 cases, Gotoff *et al* (1965) in nine of 32 cases, and Ogg *et al* (1968) in one of 25 hypocomplementaemic patients.

Of the three possible sources of urinary C3—(1) filtration from plasma, with other plasma proteins, through a damaged glomerular capillary basement membrane, (2) complement activation due to general inflammation within the urinary tract, (3) complement activation by immune complexes situated on the glomerular capillary basement membrane—we favour the third possibility.

There is no correlation between the urinary C3 concentration and the total amount of protein in the urine. Detectable C3 may be present when proteinuria is absent, while in some patients with very heavy proteinuria (up to 10 g/l) no urinary C3 is detected. There is no relationship between urinary C3 concentration and the selectivity of proteinuria. Since C3 is a large molecule (mol wt 180 000), higher concentrations of C3 in the urine would be expected in cases of non-selective than selective proteinuria if urinary C3 arose by simple filtration from plasma, and this does not in fact occur. Since urinary C3 concentrations are not related to the degree or selectivity of proteinuria, it is unlikely that C3 in urine is derived to a significant extent from simple filtration through the glomerular capillary basement membrane. We have also found that urinary C3 is not detected in cases of proven urinary tract infection. It therefore seems likely that the third possible source of urinary C3, ie, complement activation by immune complexes on the glomerular capillary basement membrane, is the most significant. Supporting evidence for this is provided by the close relationship between the detection of C3 in glomerular capillary walls by immunofluorescence microscopy and the detection of C3 in the urine. Since complement deposition in glomerular capillary walls is due to immune processes, this relationship suggests a similar origin for urinary C3. This view is also supported by the absence of urinary C3 in five cases with heavy proteinuria due to minimal lesion glomerulonephritis, in which glomerular deposition of immunoglobulins or complement cannot be detected by available methods, and by the observation that urinary C3 is undetectable or present in low concentrations in patients with proteinuria associated with non-immunological diseases such as polycystic renal disease or hydronephrosis. The detection of urinary C3 in four cases of nephrotic syndrome due to amyloid disease is compatible with this theory. It has been shown that amyloid in some cases contains fragments of immunoglobulins (Glenner *et al*, 1971a, b), and it has been suggested that a specific immunological mechanism is involved in the pathogenesis (Isobe and Osserman, 1974), possibly involving complement (Lachmann *et al*, 1962; Franklin and Zucker-Franklin, 1972).

Urinary C3 excretion was found to bear some relationship to histological diagnosis. No urinary C3 was detected in cases of non-progressive proliferative glomerulonephritis and minimal lesion glomerulonephritis; low or undetectable concentrations were found in cases of non-immunological renal disease. Detectable urinary C3 concentrations were, however, found in almost all cases of progressive proliferative and rapidly progressive, membranous and mesangiocapillary glomerulonephritis, and in renal amyloidosis. The greatest range of concentrations was observed in progressive proliferative and in membranous glomerulonephritis. The absence of urinary C3 in cases of minimal lesion glomerulonephritis and non-immunological renal diseases might be due to the absence of complement-fixing immune complexes in the glomeruli of these cases. In proliferative glomerulonephritis, immune complexes are frequently involved in the pathogenesis. Four of our cases of non-progressive proliferative glomerulonephritis showed deposits of immunoglobulin and C3 in glomerular capillary walls but had no urinary C3; a possible explanation is that urinary C3 excretion is very closely related in time to glomerular disease activity. If, as we have suggested above, urinary C3 arises from complement fixation by glomerular immune complexes, one would expect urinary C3 excretion to coincide with periods of active deposition of immune complexes in glomerular capillary walls. In the four cases mentioned above, the C3 deposits seen by immunofluorescence microscopy may have been derived

from a previous episode of complement-fixing glomerular disease activity during which urinary C3 was present, but which had ceased by the time of biopsy, as had urinary C3 excretion. Our results give further evidence for this close relationship between urinary C3 concentration and current disease activity. In renal lesions characterized by continuing disease activity, such as progressive proliferative or membranous glomerulonephritis, urinary C3 was an almost constant finding. Significantly, the three cases of membranous glomerulonephritis with no urinary C3 were found to be in clinical remission, whereas the highest concentrations were found in patients with progressing disease. Long-term serial urine and serum C3 estimations were performed in a patient with proliferative glomerulonephritis associated with influenza virus A infection and in a patient with malignancy-associated nephrotic syndrome, in both of whom the disease eventually resolved. Both initially had low serum C3 and high urinary C3 concentrations. In both cases, urinary C3 ceased to be detectable at a very early stage, preceding by several weeks the return of serum C3 concentrations to normal and the disappearance of proteinuria. These findings suggest that urinary C3 estimations may provide a sensitive index of current glomerular disease activity.

Urinary C3 estimation has many potential applications. It may be used in the diagnosis of renal disease, as an indicator of current glomerular disease activity, to assess patients' response to therapy, and to study the natural history of different types of glomerulonephritis.

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