PRELIMINARY COMMUNICATION

Urinary Excretion of C3 Antigen in Glomerulonephritis

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Summary

C3 and fibrin degradation products (F.D.P.) have been measured in early morning urine samples from 38 normal people and 123 patients with glomerulonephritis. Normal urine contained less than 0·3 μg of either antigen per ml. C3 and F.D.P. were both detected in the urine of many patients with glomerulonephritis. Levels above 1 μg/ml were exceptional in patients with "minimal change," and the highest excretion of both antigens occurred in mesangiocapillary glomerulonephritis, membranous nephropathy, and focal glomerulosclerosis.

Both C3 and F.D.P. excretion showed considerable variation with time, with parellel fluctuations in the two antigens. These fluctuations did not depend on the total protein leakage and suggest that the complement and clotting sequence are closely related in these glomerular disorders.

Introduction

Many workers have commented on the intraglomerular deposition of fibrin in glomerulonephritis (Vassali and McCluskey, 1964, 1971; Humair *et al.*, 1969; Morel-Maroger *et al.*, 1972; Davidson *et al.*, 1973) and on the value of urine fibrin-fibrinogen degradation products (F.D.P.) in the study of these diseases (Vermylen *et al.*, 1970; Clarkson *et al.*, 1971).

Intraglomerular deposition of several complement components, particularly C3, has also been shown in many patients with glomerulonephritis. The measurement of C3 excretion in the urine of patients with glomerulonephritis could therefore provide comparable information to that obtained by measurement of urinary F.D.P. We report our preliminary observations in normal patients and subjects with glomerulonephritis using a sensitive haemagglutination inhibition assay for the semiquantification of C3 together with measurements of F.D.P. excretion on the same samples.

Subjects and Methods

Altogether 38 healthy individuals and 123 patients with various forms of glomerulonephritis were studied. All the patients underwent renal biopsy and the glomerular lesions were examined by light microscopy of paraffin-embedded materials and 1-μm sections embedded in Araldite. The appearances were classified according to the descriptions of (Churg *et al.* (1970) and Kincaid-Smith *et al.* (1973).

Urine samples were obtained on at least five mornings (usually seven) and collected in universal containers containing sodium azide. Samples from outpatients were sent to the laboratory at the end of the week's collection. Each was dialysed for six hours against tap water and concentrated using polyethylene glycol. Samples were then either assayed immediately or stored at −20°C and assayed later.

The C3 concentration was measured by a red cell haemagglutination inhibition assay using C3-coated red cells and monospecific anti-C3 serum (Williams *et al.*, 1974). In urine samples concentrated with polyethylene glycol this assay underestimates by 50% the C3 concentrations obtained by radial immunodiffusion against the same antiserum. F.D.P. were measured by a tanned red cell haemagglutination inhibition assay (Clarkson *et al.*, 1971). The concentration of both proteins was expressed in μg/ml by reference to standards run concurrently. All samples were run in ignorance of the clinical or pathological details of the patient.

Results

The excretion of both F.D.P. and C3 was less than 0·3 μg/ml in all specimens from the 38 normal subjects studied. The number of patients with each form of glomerulonephritis and the proportion of urine samples falling within five arbitrarily chosen concentration ranges (< 0·3, 0·3-1, > 1-10, > 10-100, and > 100 ug/ml) are shown in fig. 1.

Only 1% of the samples from patients classified as having minimal changes in the biopsy specimen showed concentrations of C3 or F.D.P. above 1 μg/ml. Excretion of C3 above 10 μg/ml was virtually confined to three histological groups—mesangiocapillary glomerulonephritis, membranous nephropathy, and focal glomerulosclerosis (segmental hyalinosis). In these groups a high proportion of samples showed concentrations in this range—21%, 41%, and 44% respectively. In contrast the excretion of F.D.P. reached more than 10 μg/ml in only 2%, 5%, and 7% of samples from these three groups.

Surprisingly the excretion of C3 in the 22 patients with lupus nephritis exceeded 10 μg/ml in only three out of 150 samples studied. These patients showed all types of histology and all types of clinical course, from quiescence to active, rapidly progressive renal disease with decline in renal function; almost all were on some form of immunosuppressive treatment at the time of study. A mixed group of patients with proliferative glomerulonephritis had urinary C3 and F.D.P. concentrations of over 10 μg/ml in only 11% and 3% of the samples respectively.

Serial observations of C3 in 10 patients with lupus nephritis and proliferative glomerulonephritis showed marked fluctuations in C3 excretion from day to day, fluctuations which paralleled those of the F.D.P. (fig. 2). These fluctuations were independent of the protein content of the sample.

Discussion

Available evidence suggests that activation of the coagulation system contributes in part to the inflammation which occurs in both experimental and human glomerulonephritis. Both fibrin (Vassali and McCluskey, 1964, 1971; Humair *et al.*, 1969)

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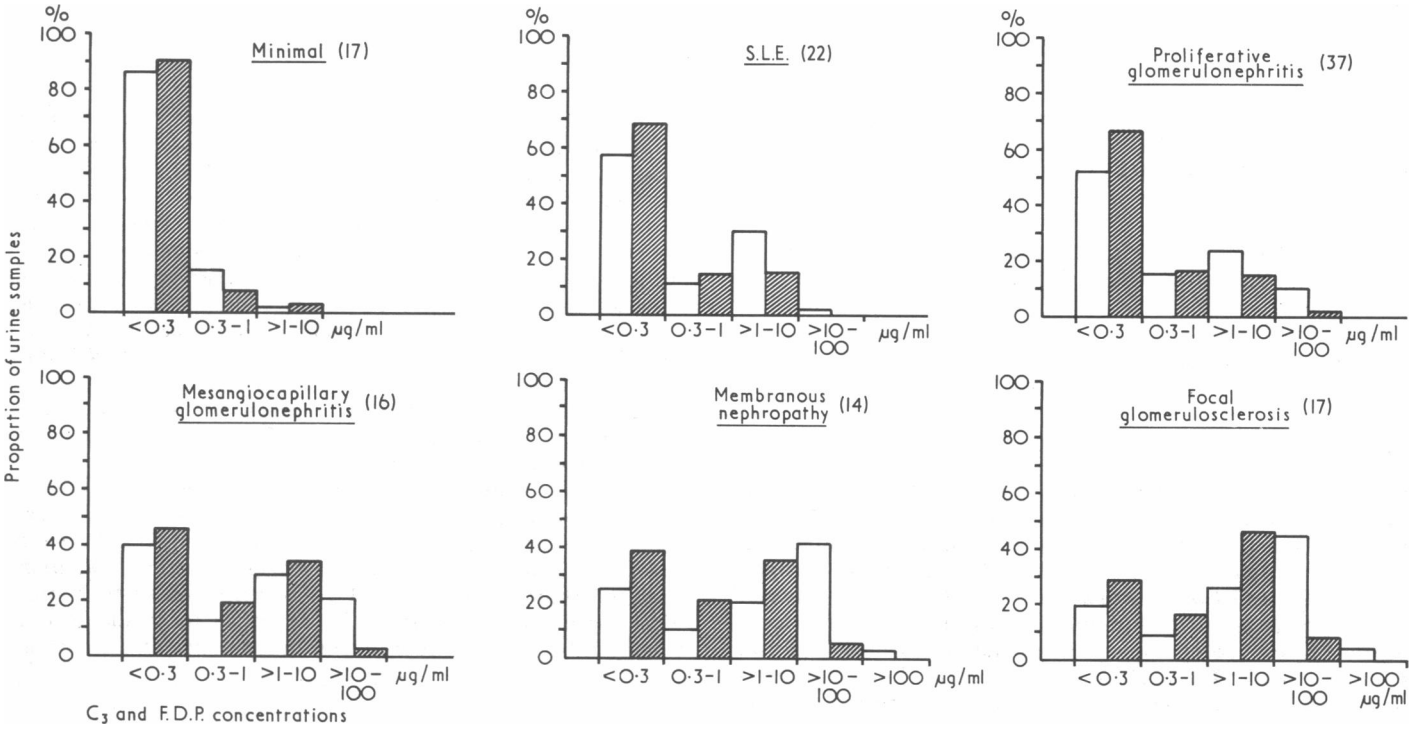

FIG. 1—Concentrations of C3 (open areas) and F.D.P. (hatched areas in urine samples from patients with glomerulonephritis. For each histological group the number of patients studied is indicated.

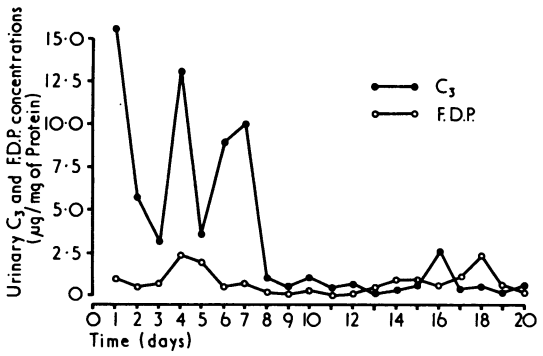

FIG. 2—Serial C3 and F.D.P. urine concentrations in consecutive early-morning specimens from one patient with proliferative nephritis.

and platelets (Wardle, 1972; Clark *et al.*, 1974) are involved but the former has received more study to date. The evidence indicates that measurements of fibrin and F.D.P. in the urine correlate with renal histology, with activity of the condition as judged clinically and histologically, and with intraglomerular deposition of fibrin as shown by immunofluorescence studies (Clarkson *et al.*, 1971; Davidson *et al.*, 1973). It is not clear, however, whether the bulk of the F.D.P. excreted in proliferative glomerular disease represents the result of lysis of intraglomerular fibrin, with only minor contributions from filtered fibrinogen, or whether the urinary F.D.P. concentration values merely reflect the total protein excretion (Naish *et al.*, 1974). Measurements of serum F.D.P. have not proved so useful as correlation with histology, activity, and prognosis was less precise.

Another factor producing inflammation in many forms of experimental glomerulonephritis is complement localization within the glomerulus. The evidence that complement is responsible for the inflammation and damage occurring in the many forms of human glomerulonephritis is circumstantial and depends on the demonstration of proteins of the complement sequence, particularly C3, in the glomeruli of patients with nephritis. The development of a sensitive assay for C3 enabled us to examine the urine of a number of patients with glomerulonephritis for the presence of this protein.

Interesting correlations with the renal biopsy appearances have already emerged. Patients with minimal changes in their renal biopsy specimens show little excretion of C3; conversely, those diseases associated with high urine concentrations of C3 (mesangiocapillary glomerulonephritis, membranous nephropathy, and focal glomerulosclerosis) are all conditions in which C3 is consistently present in the capillary walls rather than in the mesangium (Morel-Maroger *et al.*, 1972; Berger *et al.*, 1974; Verroust *et al.*, 1974). This is also true of some patients with lupus nephritis and proliferative glomerulonephritis, but the C3 concentration in the urine of these patients was not so high. Measurement of urinary C3 can therefore help to predict the possible histological type of glomerulonephritis in patients with the nephrotic syndrome. If the level of C3 is above 1 µg/ml the histological changes are unlikely to be those of minimal change. The correlations between the distribution of C3 seen in the glomeruli, the selectivity of the proteinuria, and the excretion of C3 are now being studied and will be reported elsewhere.

Urinary C3 concentration has been measured as part of the assessment of glomerular permeability using differential protein clearances. These studies indicate that C3 may be found in the urine of patients with proteinuria and suggest that its concentration is determined by its molecular weight, the permeability of the glomerular basement membrane, and reabsorption by the tubules. Differences in the quantities of C3 and F.D.P. noted in the urine samples examined presumably reflect the molecular weight differences between these two proteins. The contribution to the urinary C3 of C3 filtered as part of the heavy and relatively non-selective proteinuria commonly found in patients with glomerulonephritis remains to be determined. The appreciable fluctuations in urinary C3 levels shown in fig. 2 were independent of the excretion of protein into the urine. Possibly these fluctuations represent antigenic fragments of C3 which are either released after the intraglomerular deposition of immune-complex-bound C3 or generated in the urine by proteolysis of filtered C3. The fluctuation in C3 was similar to that

already reported for F.D.P. and again the rise of C3 and F.D.P. excretion tended to coincide.

Until now little attention has been given to the excretion of C3 in the urine of patients with glomerulonephritis. Indeed the published reports (Lange and Wenk, 1954; Lagrue *et al.*, 1969) have been concerned with establishing that the low serum C3 concentrations have not been produced by excessive urinary C3 loss. Recently C3 has been shown to be present in the urine of patients with proliferative glomerulonephritis and after renal transplantation using the relatively insensitive radial immunodiffusion assay (Hoq *et al.*, 1974). Our preliminary results indicate that C3 excretion occurs often in patients with glomerulonephritis and suggest that further studies may be useful.

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References

Berger, J., Noel, L. M., and Yaneva, H. (1974). *Advances in Nephrology*, 4, 87.
Churg, J., Habib, R., and White, R.H.R. (1970). *Lancet*, 1, 1299.
Clark, W. F., *et al.* (1974). *Kidney International*. In press.
Clarkson, A. R., *et al.* (1971). *British Medical Journal*, 3, 447.
Davidson, A. M., *et al.* (1973). *Journal of Clinical Pathology*, 26, 102.
Hoq, M. S., *et al.* (1974). *British Medical Journal*, 2, 535.
Humair, L., Potter, E. V., and Kwaan, H. C. (1969). *Journal of Laboratory and Clinical Medicine*, 74, 60.
Kincaid-Smith, P., Matthew, T. H., and Becker, E. L. (editors) (1973). *Glomerulonephritis*. New York, Wiley.
Lagrue, G., Brécy, H., and Hartmann, L. (1969). *Revue Française D' etudes Cliniqueset Biologiques*, 14, 346.
Lange, K., and Wenk, E. J. (1954). *American Journal of the Medical Sciences*, 228, 448.
Morel-Maroger, L., Leathem, A., and Richet, G. (1972). *American Journal of Medicine*, 53, 170.
Naish, P., Evans, D. J., and Peters, D. K. (1974). *British Medical Journal*, 1, 544.
Vassali, P., and McCluskey, R. T. (1964). *Annals of the New York Academy of Sciences*, 116, 747.
Vassali, P., and McCluskey, R. T. (1971). *Advances in Nephrology*, 1, 47.
Vermylen, J., *et al.* (1970). *Revue Européenne d'Etudes Cliniques et Biologiques*, 15, 979.
Verroust, P. J., *et al.* (1974). *Journal of Clinical Investigation* 53, 77.
Wardle, N. (1972). *Journal of the Royal College of Physicians of London*. 7, 5.
Williams, B. D., *et al.* (1974). *Journal of Immunological Methods*. In press.

MEDICAL MEMORANDA

Hypertension with Dissecting Abdominal Aortic Aneurysm

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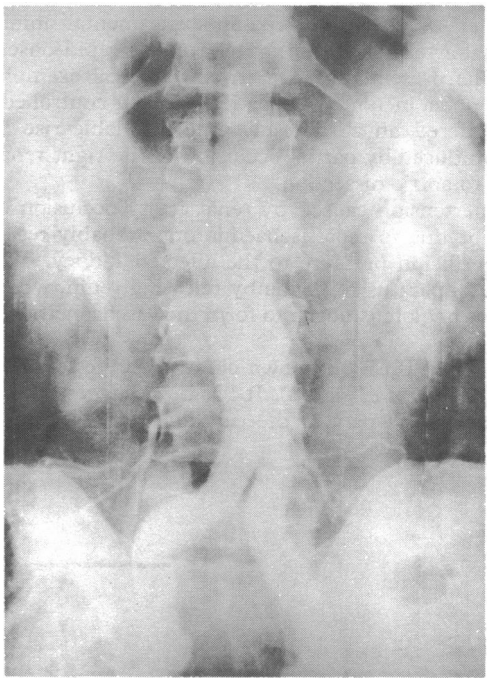
A 46-year-old bricklayer presented with a three-hour history of sharp upper abdominal pain. The onset was sudden and occurred while he was seated watching television. The pain was midline and extended from the lower sternum to the umbilicus; there was no radiation. He was given 5 mg intramuscular diamorphine and had no further pain. The referring doctor noted slight epigastric tenderness but on arrival at hospital there were no abnormal signs in the abdomen. Both femoral pulses were normal and no bruit was audible. The heart rate was 100/min and the blood pressure 185/155 mm Hg. A hypertensive retinopathy with arteriovenous nipping was noted but the apex beat was not displaced. An E.C.G. was normal.

The patient had been found to be hypertensive three years previously and at that time an intravenous pyelogram was normal, blood urea was 43 mg/100 ml, and urinary catecholamine excretion was within normal limits at 20 μg/24 hr. His blood pressure had been maintained at 160/100 mm Hg supine and 150/90 mm Hg standing on 80 mg debrisoquine daily. He had taken 20 mg debrisoquine two hours before admission but four hours after arrival his blood pressure was 220/140 mm Hg, and by eight hours it had risen to 300/180 mm Hg. He was nursed in a head-raised position and in addition to debrisoquine 20 mg six-hourly was treated with subcutaneous injections of pentolinium every two hours, initially 2.5 mg and increasing to 15 mg. This regimen was continued for 60 hours but the diastolic blood pressure never fell below 130 mm Hg. He remained symptom-free throughout. A sinus tachycardia of 150/min then developed and 24 hours later he was first noted to have a diminished left femoral pulse with a bruit over the upper abdomen. A satisfactory urinary output was maintained; however, the urine was found to contain some red cells. A right transfemoral aortogram (see fig.) confirmed a dissection just below the renal artery on the right side extending down to the aortic bifurcation. There was evidence of involvement of the right renal artery; the left renal artery appeared to be normal. It was felt that absolute indications for surgical intervention were present in view of uncontrollable hypertension with evidence of major branch occlusion.

At operation the radiological findings were confirmed though there was some retrograde dissection for a distance of 1-2 cm above the level of the renal artery. The right kidney appeared to be adequately



Aortogram showing aortic dissection affecting right renal artery.

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