somewhat higher degree of deviation (fig. 7), indicating that those who use the method must be properly trained. None of the five most divergent results led to a wrong insulin dosage. The clinicians found that results could be obtained two to three hours quicker by the Dextrostix-Eyetone method than by the method to which they were accustomed.

Discussion

Blood glucose levels above 400 mg/100 ml are outside the range measured by the Dextrostix-Eyetone method. This limits its clinical use, especially in the treatment of diabetic coma. On the other hand, the accuracy of the Eyetone instrument in measuring the levels of blood sugar commonly found in diabetics is highly satisfactory. Furthermore, packed cell volumes in the range of 30-50% had little effect on the accuracy of the method, a finding which is contrary to that of Kutter (1973). Nor did we find the differences in performance of the Eyetone instruments that Junker and Ditzel (1972) found in the Dextrostix Reflectance Meter instruments.

Compared with the Dextrostix Reflectance Meter instrument the Eyetone calibrates at two different blood glucose levels instead of one, an improvement which had often been suggested by those using the Dextrostix Reflectance Meter (Scherstén, 1971; Kühl, 1973). After we had found that the best results were obtained when the calibration points were set at 40 and 280 mg/100 ml, and the three instruments were adjusted to that setting, each one measured blood glucose concentrations in the range 10-400 mg/100 ml with equal accuracy. The differences observed between the regression equations at centres 1 and 3 when using the same calibration points of 40 and 300 mg/100 ml can therefore be explained by the difference in the reference methods used and not by variance in the Eyetone instruments. The original calibration strips set at 50 and 400 mg/100 ml have now been altered by the manufacturer to equate with our findings. When the new strips were matched against the strips we had used all three instruments gave readings of 40 and 290 mg/100 ml, which were thus in near agreement with our results. Furthermore, when the instrument was checked against a standard control glucose solution of 105 mg/100 ml the

results were fully acceptable. Using this standard control solution of glucose we also studied the day-to-day variance of the method over a period of three months and found a coefficient of variation around 5%. This indicates a high degree of functional stability of the instrument. Nevertheless, a similar study using a stronger glucose solution of, say, 350 mg/100 ml is desirable.

Our results with the Dextrostix-Eyetone method of blood sugar measurement show that it represents a quick and reliable alternative to a conventional laboratory method, especially for use outside hospital. Adequate training in the technique is essential, and the performance of each instrument should be checked at regular intervals.

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Self-induced Glomerulonephritis

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Summary

A patient is described who developed renal failure due to a severe proliferative glomerulonephritis with hypo-complementaemia and cryoglobulinaemia while repeatedly injecting herself with diphtheria, pertussis, and tetanus vaccine (D.P.T. vaccine). After stopping the antigen administration there was recovery of renal function,

complement values returned to normal, and cryoglobulin could no longer be recovered from the sera. The pathogenesis of the glomerulonephritis is discussed.

Introduction

In experimental animals the generation of circulating antigen-antibody complexes by the repeated administration of serum protein antigens, such as bovine albumin, has been shown to produce glomerular disease lesions closely resembling human chronic glomerular disease (Dixon *et al.*, 1961; Germuth *et al.*, 1957). Immunofluorescent studies of renal biopsy material have shown that the glomerular deposits of immunoglobulin and complement are found in most human nephritides, suggesting that these are mediated by immune complexes (Berger *et al.*, 1971; McCluskey, 1971). It is only rarely possible, however, to show circulating antigen-antibody complexes or to identify the antigenic component of the complex in the glomerular deposits. We therefore thought it would be of interest to report a patient

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in whom repeated immunization with a vaccine containing diphtheria, pertussis, and tetanus antigens (D.P.T. vaccine) was apparently responsible for the development of a severe proliferative glomerulonephritis.

Case Report

The patient, a 33-year-old nurse, presented in December 1969 with an influenza-like illness associated with fever and red indurated lesions on her right leg. In March 1970 she was admitted to hospital with cellulitis of the right leg. She was treated with antibiotics and discharged, only to be readmitted in June 1970 with a further crop of indurated red lesions around the right knee. She was febrile and on investigation found to have hypocomplementaemia, microscopic haematuria, cryoglobulinaemia, and impaired renal function. Biopsy of the skin lesion showed fat and bacteria in the centre with polymorphonuclear neutrophil infiltrate around neighbouring arterioles.

In November 1970 she was transferred to Hammersmith Hospital, where examination showed several healing indurated lesions around her right knee, blood pressure of 150/90 mm Hg, and a haemic mid-systolic murmur. The creatinine clearance was 33 ml/min, there was proteinuria of 0.1 g/24 hr, and the urine contained red cells and red cell casts. C3 concentration was 40% of normal. Blood cultures were sterile. Renal biopsy showed an acute diffuse lobular proliferative glomerulonephritis with neutrophil aggregation (fig. 1). Studies using immunofluorescent antisera showed weak interrupted linear deposition of C3 (fig. 2) but no immunoglobulin deposits. Occasional

subendothelial deposits were found by electronmicroscopy (fig. 3). Tests for antinuclear factor were negative. She was treated with heparin for one month then prednisolone 60 mg/day, which was reduced to 20 mg/day by the time she was discharged in March 1971. She was admitted three times over the next 12 months for various reasons, including pleurisy, arthralgia, and haematuria. Cyclophosphamide and azathioprine were given as shown in fig. 4. A second renal biopsy in January 1972 showed no significant change.

FIG. 3—Electronmicroscopic appearances of first biopsy specimen showing subendothelial deposit (arrowed).

FIG. 1—Histological appearances of first renal biopsy specimen showing mesangial proliferation and mild excess of polymorphs. (H. and E. $\times 315$.)

FIG. 2—Immunofluorescence of first biopsy specimen showing interrupted linear deposits of C3. ($\times 250$.)

By March 1972 the creatinine clearance had fallen to 11 ml/min, and hypocomplementaemia and cryoglobulinaemia persisted. Steroids and cyclophosphamide were stopped and she was discharged, only to be readmitted 21 days later with gross generalized oedema, heavy proteinuria (up to 11 g/24 hr; see fig. 4), and further deterioration in renal function. Treatment with peritoneal dialysis was started. Two weeks later she had two generalized seizures. Search of her bedside locker revealed phials of D.P.T. vaccine and used needles and syringes. On further questioning she admitted injecting herself over the previous four years. She became oliguric after the fits and she was treated with prednisolone 60 mg/day and by plasmapheresis in which three litres of plasma were exchanged on two occasions. A third renal biopsy (July 1972) again showed diffuse exudative glomerulonephritis on light microscopy, but on this occasion immunofluorescent studies showed heavy granular deposits of C3, IgG, and IgA in the glomeruli

FIG. 4—Clinical course of patient. H=Heparin. C=Cyclophosphamide (2 mg/kg/24 hr). A=Azathioprine (2 mg/kg/24 hr).

FIG. 5—Immunofluorescence of third biopsy showing heavy granular deposits of C3. ($\times 150$.)

(fig. 5). Renal function gradually improved and the steroid dosage was decreased over the next eight months. By August 1973 the creatinine clearance had risen to 64 ml/min, C3 was 100% of normal, and cryoglobulinaemia could no longer be detected though proteinuria (4 g/24 hr) persisted (see fig. 4). A fourth renal biopsy in August 1973 showed diffuse chronic glomerulonephritis with hyalinization of parts of some glomeruli but with much reduced neutrophil infiltration and reduced amounts of C3 and no immunoglobulins. There was no evidence of amyloid.

The patient claimed that she had injected herself with 2 ml of D.P.T. vaccine about every two months for about four years. The red raised lesions around the right knee and on the forearm had developed at the injection sites. A detailed psychiatric assessment, which we do not propose to publish, disclosed features of a hysterical and depressive illness.

Immunological Studies

Complement Studies.—Measurement of the concentration of serum C3, C4, C1q, glycine-rich β -glycoprotein, cobra factor convertase, and properdin and the detection of C3 breakdown products were made as previously described (Williams *et al.*, 1973). Catabolism of radiolabelled C3 was studied as described by Charlesworth *et al.* (1974). The C1q precipitation test was performed according to the method of Agnello *et al.* (1970).

Antibody Titres.—Antibody titres to tetanus were measured by neutralization of toxin assayed in mice and to diphtheria by neutralization of toxin assayed in guinea-pigs (*British Pharmacopoeia*, 1973). Antibody titres to pertussis were measured by direct bacterial agglutination.

Cryoglobulin Studies.—A 20-ml sample of blood was taken into a warm syringe and allowed to clot at 37°C and the serum removed and allowed to stand at 4°C for 48 hours. The precipitate was separated by centrifugation at 4°C and then washed three times in phosphate buffered saline. It was then redissolved by incubating at 37°C. Protein concentration of the cryoglobulin was measured by the Folin technique. The constituents of the cryoglobulin were identified by dissolving the washed cryoglobulin at pH 3.2 and using immunoelectrophoresis and specific antisera. 125 I-labelled tetanus and diphtheria toxoids were layered on cryostat sections of the renal biopsy specimens, and after washing the sections were submitted to autoradiography.

Results

The sequential concentrations of complement components are shown in the table. The C1q precipitation test was positive on

Serum Complement Levels in Patients with Self-induced Glomerulonephritis. Values expressed as Percentages of Pooled Normal Human Serum

Date	C3	C4	C1q	G.B.G.	Co.F.	Properdin
Dec. 71 ..	30	55	11	66	100	N.D.
June 72 ..	10	100	70	55	30	N.D.
Dec. 72 ..	11	33	50	37	45	60
May 73 ..	65	100	50	100	100	60
Oct. 73 ..	95	125	55	100	N.D.	60
Normal values (mean $\pm$ 2 S.D.)	68-116	50-210	60-136	40-210	65-115	40-210

G.B.G. = Glycine-rich β -glycoprotein.

Co.F. = Cobra-factor convertase.

N.D. = Not done.

sera taken in July 1972. The fractional catabolic rate of C3 was increased with a $t_{1/2}$ of 47 hours (normal 68-81 hours). C3d was found in the serum in July 1972. Antibody titres to the diphtheria and pertussis are shown in fig. 6. Antibody titres to *Bordetella pertussis* were very high (1/256,000; comparable to those obtained in hyperimmunized animals) and fell to 1/2,000 by July 1973. Protein concentration of the cryoglobulin was 80 mg/100 ml serum in July 1972. The cryoglobulin contained IgG, IgM, and C3. Specific uptake of tetanus or diphtheria toxoids by cryostat sections of the renal biopsies could not be shown.

FIG. 6—Antibody titres to tetanus and diphtheria antigens during course of patient's illness.

Discussion

Our patient developed a severe progressive proliferative glomerulonephritis probably caused by repeated immunization with D.P.T. vaccine. When this was discontinued substantial recovery of renal function occurred, cryoglobulins disappeared, and serum complement returned to normal. There is one other report (Bishop *et al.*, 1966) of a patient given pertussis vaccine at weekly intervals for seven weeks who after the eighth injection some eight weeks later developed fever, arthropathy, lymphadenopathy, and proliferative glomerulonephritis. He died of renal failure three months later and necropsy showed a widespread healing vasculitis.

Repeated administration of antigen is a well established method of producing immune-complex glomerulonephritis in rabbits (Dixon *et al.*, 1961), and the development of disease in our patient after repeated injections of vaccine suggests a similar pathogenetic mechanism. This interpretation was supported by the serological evidence of circulating immune complexes: hypocomplementaemia with evidence of activation of the

classical pathway (Schur and Sandson, 1968), persistent cryoglobulinaemia, and the positive result of the precipitation test between the patient's serum and purified C1q (Agnello *et al.*, 1970). The immunofluorescent findings of interrupted linear deposits of C3 without immunoglobulin in the first two biopsies were unusual, however. These appearances have been described in mild poststreptococcal glomerulonephritis (Fish *et al.*, 1970) and in the dense-deposit type of mesangiocapillary glomerulonephritis (Germuth and Rodriguez, 1973), both regarded as examples of immune-complex nephritis. The third biopsy showed granular deposits of C3 and immunoglobulin, which are typical of immune-complex glomerular disease. It is interesting that deposits of C3 were still present one year after the last injection of D.P.T. vaccine. Attempts to show binding of radiolabelled diphtheria and tetanus antigens in the glomeruli were unsuccessful.

In certain respects the findings in this patient resemble essential mixed cryoglobulinaemia (Meltzer *et al.*, 1966), and similar proliferative glomerulonephritis has been described in this syndrome (Verroust *et al.*, 1971). Cryoglobulins in this patient, however, though present in high concentration were unlikely to have been directly responsible for glomerular injury since IgM could not be detected in the deposits. Furthermore the immunofluorescent findings in the earlier biopsies were unlike those so far described in essential mixed cryoglobulinaemia (Verroust *et al.*, 1971). Cryoglobulins of this type have been found in numerous diseases including some forms of glomerulonephritis (Adam *et al.*, 1973; McIntosh *et al.*, 1970), systemic lupus erythematosus (Hanaver and Christian, 1967), and various chronic infections—for example, infective endocarditis (Lerner *et al.*, 1947). It is therefore not surprising that cryoglobulinaemia should result from hyperimmunization with a vaccine containing bacterial antigens. Indeed, Bokisch *et al.* (1972), showed that immunization of rabbits with group A or C streptococcal antigen led to the production of mixed (19S, 7S) cryoglobulins. Cryoglobulinaemia in these conditions seems to be a reflection of prolonged antigen stimulation and can be regarded as a useful marker for circulating immune complexes (Kunkel, 1971). Apart from anti-IgG activity, however, few specific antibodies have so far been identified in cryoprecipitates (Grey and Kohler, 1973). In our patient the disappearance of cryoglobulins when the vaccine was discontinued also suggests that they resulted from prolonged antigenic stimulation.

Though there are striking similarities in the development of immune-complex nephritis between our patient and experimental animals there are a number of discrepant features, especially the quantity and frequency of antigen administration. In 2-3 kg rabbits daily injections of 0.5-50 mg bovine albumin containing 0.08 mg of nitrogen were used (Dixon *et al.*, 1961), whereas in our 55-kg patient it seems that injection containing about 20 mg of nitrogen every two months was sufficient. In rabbits immunization regimens generating antigen-antibody complexes in moderate antigen excess result in glomerulonephritis (Dixon *et al.*, 1961; Germuth *et al.*, 1957). In our

patient all measurements showed high titres of free antibody to tetanus, diphtheria, and pertussis antigens. Though immune complexes formed in moderate antibody excess can also cause glomerular damage (Germuth *et al.*, 1972) it is possible that a different antigen-antibody system, not reflected in the serological tests, was responsible; the vaccine contains numerous antigens. It is also possible that the adjuvant effect of *B. pertussis* (Kind, 1953) may have caused antibody production to exogenous or endogenous antigens unrelated to the vaccine, thus leading to immune-complex disease.

Over the course of two years this patient was given a variety of therapeutic regimens—anticoagulants, steroids, cyclophosphamides, and azathioprine—all without demonstrable beneficial effect. Withdrawal of antigen, however, led to a striking clinical remission. This also occurs after treatment of patients with renal involvement associated with subacute infective endocarditis (Boulton-Jones *et al.*, 1974), another immune-complex nephritis. The clinical importance of research aimed at identifying the responsible antigen or antigens in this type of glomerulonephritis is again stressed.

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