Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

of male and female blood-donors. Their serum-bilirubin data were skewed but women with a bilirubin >0·7 mg/dl (12·0 μmol/l) and men with a bilirubin >1·4 mg/dl (24·0 μmol/l) were claimed to constitute a second, minor population who, Owens and Evans suggested, had Gilbert's syndrome; if so about 6% of the population would have this disorder. They found two distinct linear sections when their data were plotted on normal-probability as distinct from log-probability paper. Our data plotted on normal-probability paper yielded a smooth logarithmic curve with no evidence of bimodality. This difference could be due to sampling error in earlier study; the upper end of Owens and Evans' distribution contains very few data points. In fact their data can be accommodated by a single log-normal distribution, thus weakening their case for bimodality.

The men with a bilirubin >25 μmol/l who were recalled had the usual criteria of Gilbert's syndrome—i.e., their serum-bilirubin rose when they fasted,[12 13] the bilirubin was mainly unconjugated, and gross hæmolysis was excluded. The minor hæmolysis demonstrated in some patients with Gilbert's syndrome is not enough to account for the hyperbilirubinæmia.[14]

It is not surprising that the repeat bilirubins were slightly different from the first ones. In patients with a raised bilirubin the concentration does vary and the patients had not fasted before the second examination.

Is Gilbert's syndrome a disease or merely an extreme expression of normality? A low uridine-diphosphate glucuronyl transferase,[15] altered bilirubin kinetics,[4] slightly reduced red-blood-cell survival,[14] or even a genetic predisposition[3] do not mean that it represents a disease state. One would expect to find differences in factors which control bilirubin concentration in a group of people with a high bilirubin if they are compared with those with a lower bilirubin. Were it feasible to measure them in a large normal population these factors could also be expected to have a skew distribution.

The frequency of symptoms in patients with Gilbert's disease probably reflects the way they were diagnosed—i.e., by blood tests done to investigate vague lassitude or discomfort. Our recalled patients had no such symptoms.

We suggest that "constitutional hyperbilirubinæmia" be used in preference to "Gilbert's disease" so that the patient can be reassured that the condition produces no symptoms and so that unnecessary investigations will not be ordered during any future non-associated illness in which the bilirubin may be found to be further increased. Certainly, treatment with phenobarbitone[16] to lower the bilirubin and diminish vague symptoms seems misplaced.

Failure to recognise that the distribution of bilirubin concentration is skewed and the subsequent misapplication of gaussian statistical techniques can yield a misleadingly low upper limit of normal. An upper limit of 14 μmol/l (or 0·8 mg/dl) seems too low. The 98th percentiles in our population were 19 μmol/l (1·1 mg/dl) for females and 25 μmol/l (1·5 mg/dl) for males, and these values more realistically define the upper limit of "normality". Even on these strict criteria 2% of our screened men had abnormal bilirubin concentrations; almost all of them will have had no hepatocellular disease, but they would, in the past, have been diagnosed as having Gilbert's disease.

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RAISED URINARY FIBRIN-DEGRADATION PRODUCTS, COMPLEMENT, AND IgG DURING AN INFLUENZA-LIKE ILLNESS

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Summary Urine from eight normal controls in whom an influenza-like illness developed contained high concentrations of fibrin-degradation products (F.D.P.), IgG, and C_3. The study was carried out when influenza A was prevalent in the community. However, a wide range of serological investigations revealed no evidence for influenza A or other viruses. The infection may have been caused by other viruses which produce upper-respiratory-tract infections and which are not readily diagnosed by serology. Urinary fibrin-degradation products are a well-known marker of glomerulonephritic activity and viral antigens may have induced an immune-complex glomerulonephritis in the 8 controls in whom an influenza-like disease developed. A larger normal population should be investigated during a virus epidemic.

Introduction

THE immunological basis for glomerulonephritis is well established and immunofluorescence studies have shown immunoglobulins in the glomeruli of patients with glomerulonephritis. Immunoglobulins are produced in response to an antigen, but the nature of the antigen is often obscure and its demonstration difficult. However, numerous bacterial antigens (especially streptococci) may cause glomerular damage through immunological mechanisms. Chronic viral infection of animals with lymphocytic choriomeningitis,[1] the agent of aleutian dis-

ease of mink,[2 3] Coxsackie B4,[4] adenovirus,[5] and other viruses[6] can result in immune-complex nephritis. In man the role of viruses in the ætiology of glomerulonephritis has been studied chiefly in cases or renal disease developing after viral illnesses caused by echovirus 9,[7] Coxsackie B,[8] and varicella infections.[9] Raised titres of anti-Epstein-Barr have been demonstrated in patients with systemic lupus erythematosus glomerulonephritis, and parainfluenza type-3 antibody titres were raised in patients with immune-complex glomerulonephritis.[10] Intravascular coagulation with renal involvement has been reported in patients with influenza-A virus infection[11 12] and in a case of Goodpasture's syndrome after influenza-A_2 virus infection.[13]

Urine fibrin-degradation products are an accurate marker of the activity of the glomerulonephritic process.[14] In the present study high concentrations of urine fibrin-degradation products and immunoglobulins suggested that an immunological process consistent with a subclinical attack of glomerulonephritis had occurred during an epidemic of an influenza-like disease.

Patients and Methods

Sixteen normal healthy adult volunteers agreed to take part in an investigation to define the normal concentration of urine fibrin-degradation products (F.D.P.) for our laboratory. 20 ml of urine was collected daily in the early morning for a month, dialysed against tap-water, concentrated in polyethylene glycol at 4°C overnight, and stored at −18°C until assay.

F.D.P. Assay

The tanned red-cell hæmagglutination-inhibition immunoassay[15] was used with the following modifications. The immunoassay was performed in the microtitre system (Cooke Engineering Co.) and all reagents, fibrinogen standards, and sensitised cells were from the same batch. Three concentrations of human fibrinogens (Kabi Pharmaceuticals Ltd., lots no. 5 and 175) were included in each assay. The concentration of "clottable" protein in these standards was estimated by the method of Ratnoff and Menzie.[16] The mean sensitivity of the assay calculated with these values was 0·35 mg/l. The concentration of antisera was 1/40 000 (Burroughs Wellcome lot K 24482). The antigen/antibody incubation period was 4 h at 20°C. The incubation period after the addition of the sensitised cells was 18 h (overnight) at 20°C. Human group-O-negative, glutaraldehyde-fixed, fibrinogen (10 μg/ml) (Kabi Pharmaceuticals Ltd., lot no. 58175) sensitised cells were used.[17] The plates were read by two observers who agreed to within half a well.

IgG, IgM, and C_3 were measured by the Mancini technique.[18] Because of the limited amount of urine available for analysis, urine was examined for protein content and blood by 'Labstix' only.

During the investigation clinical evidence of an influenza-like syndrome with acute upper-respiratory-tract symptoms (cough, sore throat, runny nose), general malaise, and arthralgia developed in eight controls. Lymphadenopathy developed in one, and three were absent from work for 2 or 3 days. During the study there had been an epidemic of influenza in the community caused by parainfluenza A and B viruses. Blood was taken for virus studies during the attack and one month later, and serum was investigated for antibodies to a wide range of viruses. Complement-fixing antibody titres to the following antigens were determined: influenza A, B; parainfluenza type 1; adenovirus; chlamydia group B; *Coxiella burneti; Mycoplasma pneumoniæ,* and respiratory syncytial virus. Antibodies to Coxsackie B1-6 viruses were measured by a metabolic inhibition test. Serum was tested for heterophil Paul Bunnell antibody and streptolysin O antibody. During the influenza-like illness blood was also taken to measure serum-F.D.P., blood-urea, serum-creatinine, platelets, reticulocyte-count, haptoglobulins, prothrombin index, and thrombin-time and a blood-film was studied for fragmented cells or burr cells to detect any evidence of intravascular coagulation.

Results

Urinary F.D.P., IgG, and C_3 concentrations are shown in two of the controls who had an influenza-like illness (fig. 1). The mean urine F.D.P. concentration during the illness in eight controls was 2·37 mg/l (S.D. ±12·63) and this was significantly higher than the mean concentration in those controls who did not have the illness (0·10±0·14 mg/l) (Mann Whitney test, U=10, P <0·01) (fig. 2).

IgG was found at some time in the urine of all the controls who had the influenza-like illness and there was

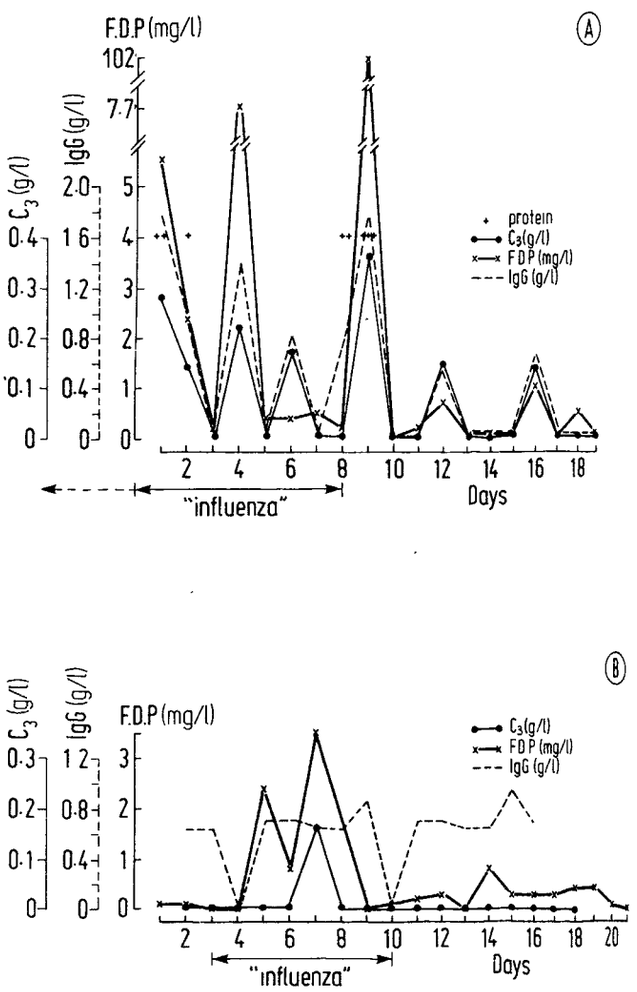

Fig. 1—Daily urinary F.D.P., C_3, IgG, and protein excretion in 2 normal controls with influenza-like illness.

a good correlation between IgG and urinary F.D.P. excretion (r=0·40). C_3 was detected in the urine of three of the eight patients with the illness and was associated with peak F.D.P. excretion. IgG and C_3 were not detected at any time in the urine of those controls who remained well throughout the study. There was no IgM in any of the urine samples from the controls.

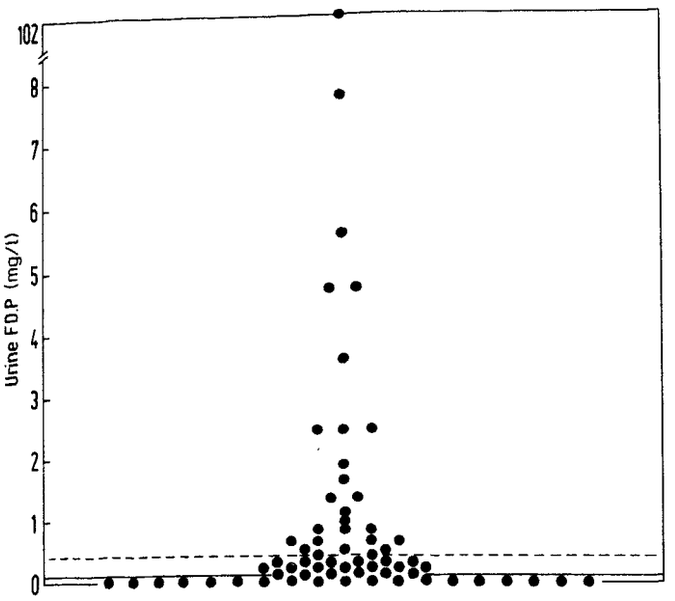

Fig. 2—Urinary F. D. P. excretion during an influenza-like illness in 8 normal controls.

The mean value (±2 S.D.) in normal healthy individuals is shown.

Proteinuria was found in only two controls (30 and 300 mg/dl), but the method used to measure urinary protein was poor, there being insufficient urine to carry out a detailed protein estimation by the biuret method. Blood not associated with menstruation was detected by microscopy in two consecutive urine samples from one woman during the illness. Blood urea and creatinine were not raised in any of the controls at any time.

Serum antibody titres to the antigens tested were not raised in any of the eight controls. Throat and nasal swabs were not taken . The Paul Bunnell test for heterophil antibody was negative in all eight cases. Routine bacteriological cultures were negative and the anti-streptolysin O titre was not raised in any of the eight controls.

The results of all hæmatological investigations for disseminated intravascular coagulation were within normal limits.

Discussion

Urine F.D.P., complement, and IgG were raised in eight of sixteen adult controls studied. In all eight, the urinary changes were associated with an influenza-like illness. There were no changes in the urine of the other eight controls who remained well throughout the study.

The possibility that a virus was responsible for the influenza-like illness was investigated. The infection occurred at the beginning of April, 1976, when influenza-A infection was prevalent in the area.[19] However, in the eight controls affected there was no serological evidence for influenza-A infection or for infection by a wide range of other viruses. However, the infection may have been caused by viruses—e.g. rhinovirus, echovirus, coronavirus, all known to cause upper respiratory infection—which are not readily diagnosed by serology. Although there was no laboratory confirmation of viral infection, the clinical illness with upper-respiratory-tract symptoms, general malaise, fever, and arthralgia was compatible with such an infection.

The excretion of F.D.P., complement, and IgG in the urine is associated with glomerulonephritis.[20] Immune complexes consisting of viral antigen and antibody may have been responsible for a glomerular nephritic process in the eight controls in whom urinary fibrin-degradation products, IgG, and complement were raised.

In man, as in laboratory animals, immune-complex disease due to deposition of viral antigen/antibody complexes in glomeruli may be of importance, since Coxsackie-B antigen[8] and Australia antigen[21 22] have been observed in glomeruli of patients with nephritis. Others have used explant cultures in an attempt to isolate viruses directly from renal tissue obtained either at necropsy or by diagnostic biopsy.[23 24] Cytomegalovirus, adenovirus, measles, varicella, and Coxsackie virus B1 have been isolated from infant kidneys obtained at necropsy, but not from kidneys of older patients.[23] Particles with the characteristics of coronaviruses have been seen in the kidneys of patients with endemic (Balkan) nephropathy.[25] C-type R.N.A. viruses have been implicated in the ætiology of systemic lupus erythematosus,[26] and kidneys from patients with lupus nephropathy have been found to contain antigens related to C-type virus from human cells (HEL-12 virus).[27]

Urine fibrin-degradation products, complement, immunoglobulin, and protein excretion should be measured prospectively and detailed virus studies should be carried out during an epidemic of influenza or other viral infection in a larger normal population.

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