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CRYOGLOBULINAEMIA IN HUMAN RENAL DISEASES
A STUDY OF SEVENTY-SIX CASES

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SUMMARY

The frequency of cryoglobulinaemia was statistically significant in acute proliferative endocapillary glomerulonephritis, in membrano-proliferative glomerulonephritis and in lupus glomerulonephritis. They all have in common glomerular cellular proliferation and glomerular deposits of C3. The cryoglobulins in most of these cases contain IgG and IgM. Except for acute proliferative endocapillary glomerulonephritis, a rather good correlation was observed between the cryoglobulin constituents and the immunoglobulins present in the glomerular tuft. Rheumatoid factor was very rarely found in the serum of these patients.

A cryoglobulin characterized by the presence of the three immunoglobulins was also found in cirrhosis of the liver with or without proven glomerulonephritis.

INTRODUCTION

A comparative study of experimental glomerulonephritis and human glomerulonephritis utilizing immunomorphological techniques, suggests that immune complexes are the cause of many human glomerular diseases (Dixon, 1968; Bariety & Druet, 1971). The definite proof would be the identification of antigen and antibody in these complexes and in the kidney.

Lerner & Watson (1947) gave the name of cryoglobulins to serum globulins which precipitate in the cold. Cryoglobulins can be monoclonal or mixed. Lo Spalluto (1962), Peetom and van Loghem Langereis (1965), Costanzi *et al.* (1965), demonstrated that the constituents of mixed cryoglobulins are IgG and IgM, and that the latter has an anti-IgG activity. Meltzer & Franklin (1966), Meltzer *et al.* (1966) strongly suggested that in certain circumstances, immune complexes are cryoglobulins which can be deposited in the glomerulus and induce a glomerulonephritis.

Cryoglobulins have been described in certain diseases, in particular lupus erythematosus (Christian, Hatfield & Chase, 1963), in which the presence of cryoglobulinaemia is directly correlated to renal disease.

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It was thus logical to investigate the presence of cryoglobulins in the glomerulopathies which can be caused by immune complexes. Grupe (1968), McIntosh *et al.* (1970), McIntosh, Kulvinskas & Kaufmann (1971) have shown that cryoprecipitates are frequently found in acute gomerulonephritis whether of streptococcal origin or not, but they did not demonstrate its significance.

Our work was designed to study the frequency of cryoglobulin in various human glomerulopathies, their composition and the comparison with the intraglomerular deposits revealed by immunohistochemical methods, the level of serum complement and the presence of rheumatoid factor.

PATIENTS AND METHODS

Patients

Between January 1970 and June 1972, 907 tests were carried out for cryoglobulin on 597 patients. These patients had a primary renal disease or an illness which could be complicated with renal pathology. Two hundred patients with arteriosclerosis obliterans of the lower extremities were used as controls. This group was chosen because a glomerular disease is not usually associated with this frequently encountered disease.

Cryoglobulins

A 20-ml blood sample was placed immediately in an incubator at 37°C until clot formation. Serum was then removed, centrifuged, divided in two samples of 5 ml each and stored with Merseptyl (1/10000) at +4°C. The sample was examined every 3 days during a period of 15 days for the appearance of a precipitate. To verify that it was indeed a cryoprecipitate, a sample was heated to 37°C at which point it redissolved. Once the cryoglobulin was demonstrated, a 60-ml blood sample was drawn for further study. 185 analysis were carried out on 42 patients with a cryoglobulinaemia and 267 on 100 patients without cryoprecipitate. Repeated experiments were performed in order to determine the fate of a cryoglobulin or to confirm its absence.

Analysis of cryoglobulins

The cryoglobulin was spun at 9,000 rev/min for 10 min at +4°C in an MSE high speed 18 centrifuge. The cryoprecipitate was resuspended in PBS at +4°C, homogeneized in a blender and centrifuged again. This procedure was repeated until the optical density of the supernatant at 280 nm was 0. The cryoglobulin was then dissolved at 37°C in acetate buffer, pH 5·5, 0·1 M. The protein concentration was determined by the modified Biuret method (Uriel, 1961). We attempted to obtain a final concentration of 10 mg/ml, but in most samples, due to the small amount of cryoglobulin, the final concentration was only 3–6 mg/ml. The cryoglobulin was left overnight at 37°C, in the presence of cysteine (0·05 M final). Immunoelectrophoresis (Grabar & Williams, 1953) was carried out using horse anti-whole human serum (Pasteur Institute) and anti-human heavy chain specific sera (anti-γ, α, μ) and anti-C3. Each time, different quantities of the same concentration of cryoglobulin was studied by immunodiffusion at 37°C using the same monospecific sera and anti-human albumin serum to verify the purity of the preparation.

Immune sera

Immune sera used were commercially available (CDTS, Bois Guillaume, France) for

γ, α and μ anti-sera, and Hyland Laboratory for human C3 anti-serum). Sheep and rabbit anti-human IgG, IgA, IgM and albumin sera were also prepared in the laboratory (Bariety *et al.*, 1971). Each immunoglobulin anti-serum was made heavy chain specific using glutaraldehyde immunoadsorbents according to Avrameas and Ternynck (1969). Anti-human IgG was adsorbed on a BSA and human IgG Fab copolymer. Human IgG Fab was obtained from human IgG (Pentex) with papain (Porter, 1959). Anti-human IgA and IgM were adsorbed on an immunoadsorbent prepared from patients sera lacking IgA and IgM respectively. The specificity of sera was controlled by immunoelectrophoresis and immunodiffusion

Anti-cryoglobulin sera

Thirty Wistar rats were immunized with a different cryoglobulin from thirty patients. 200 ng of cryoglobulin mixed with Freund's complete adjuvant were injected in the footpads. A 200-ng booster injection was administered i.p. a month later on 3 consecutive days. Rats were bled by cardiac puncture a week later. Rat sera were adsorbed on a copolymer of BSA and human IgG Fab immunoadsorbent, tested by immunoelectrophoresis and immunodiffusion with human IgG Fab, albumin, purified IgG, IgA and IgM at various concentrations.

Other immunological studies

504 patients were studied for complement. Either CH 50 (Lagrue *et al.*, 1967) or, and C3 by the method of radial immunodiffusion (Mancini, Carbonara & Heremans, 1965). IgG, IgA and IgM were measured in the same manner as C3 and normal values were obtained utilizing healthy volunteers.

Serum electrophoresis and immunoelectrophoresis, L.E. test and ASO were performed in most patients, latex test and Waaler-Rose reaction in 417 cases and the sero-reaction for syphilis in 227. Anti-nuclear antibodies were carried out by indirect immunofluorescence and anti-thyroglobulin antibodies by passive haemagglutination in 339 patients.

Immunohistochemistry

Renal biopsies were studied by light microscopy and immunohistochemistry (immunofluorescence and immunoperoxidase).

The classification used has been described elsewhere (Bariety and Druet, 1971). For immunoperoxidase, immune sera anti-IgG, IgA, IgM and fibrinogen were obtained and purified with immunoadsorbents as described above and labelled with peroxidase (Avrameas, 1969). For immunofluorescence studies, the same sera and an anti-human C3 serum were purchased from Hyland Laboratory. Results observed with both techniques were similar and expressed as positive (+) or traces (Tr).

RESULTS

Frequency

Results are reported in Tables 1 and 2. The frequency of cryoglobulinaemia has been determined for primary and secondary glomerular diseases. Diseases without proved glomerulopathies were not included in this statistical study. Cirrhosis of the liver with or without glomerulonephritis were also excluded because the relationship between cirrhosis and glomerular disease has not been definitely established.

The occurrence of cryoglobulin in gomerulopathies is significantly higher ($P<0{\cdot}001$) than

TABLE 1. Occurrence of cryoglobulin in glomerulonephritis available for statistical study

Diagnosis	Number	Cryoglobulin
Group I		
Membranous glomerulopathies	35	2
GN with mesangial deposits	80	1
Minimal glomerular changes	81	1
Focal hyalinosis	27	0
Group II		
Acute endocapillary proliferative GN	22*	6†
Membrano-proliferative GN	72	23
Group III		
Lupus nephritis	13	12

* Ten acute streptococcal GN.
† Five acute streptococcal GN.
GN: Glomerulonephritis.

in the control group of patients with arteriosclerosis obliterans of the lower extremities. In Table 1 are reported the groups of glomerular diseases with enough cases for statistical study. The groups with a smaller number of cases are in Table 2. There is a great difference between the various groups of Table 1. (a) A cryoglobulin is relatively rare in the first group (membranous glomerulopathies, glomerulonephritis with mesangial deposits, minimal glomerular changes and focal hyalinosis). This group is statistically homogenous. (b) A

TABLE 2. Occurrence of cryoglobulin in diseases not available for statistical study and in control group

Diagnosis	Number	Cryoglobulin
Primary glomerulonephritis		
Extra and endocapillary proliferative GN	5*	2*
Extra capillary proliferative GN	6	0
Glomerulosclerosis	9	0
Focal proliferative GN	6	0
Miscellaneous glomerulonephritis†	37	1‡
Cirrhosis of the liver with GN	10	7
Cirrhosis of the liver without proved GN	23	5
Lupus without proved GN	12	2
Scleroderma without proved GN	25	5
Mixed essential cryoglobulinaemia	2	2
Monoclonal gammapathies	4	1
Interstitial nephritis and nephroangiosclerosis	31	0
Unclassified nephritis	98	5§
Control group (arteritis)	200	1

* Two streptococcal GN.
† Ten diabetic nephropathies, eleven toxaemia, seven renal amyloidosis, two periarteritis nodosa, seven Schonlein-Henoch purpura.
‡ Schonlein-Henoch purpura.
§ One endocarditis, four chronic renal insufficiency.
GN: Glomerulonephritis.

cryoprecipitate is found more frequently in the second group (acute endocapillary proliferative glomerulonephritis and membrano-proliferative glomerulonephritis). The frequency is significantly greater ($P<0{\cdot}001$) than in the preceding group. This second group is also homogenous. (c) A cryoglobulin is found very frequently in lupus with morphological renal involvement. The frequency is significantly greater than in the two preceding groups ($P<0{\cdot}001$).

A few points must be emphasized. Five cryoprecipitates were found among ten acute streptococcal proliferative glomerulonephritis. Among the five negative cases, one transient cryoglobulin was noted but it could not be typed and two other patients were studied only 4 months after the disease had begun. On the other hand, only one cryoglobulin has been identified among twelve acute proliferative glomerulonephritis without evidence of streptococcal infection. Another one was noted but could not be analysed because it was transitory. In nine of the remaining ten cases, the cryoglobulin was looked for at least 2 months after the onset of the disease.

In the twenty-five lupus patients studied: (a) thirteen had a proved glomerular disease (nine membrano-proliferative glomerulonephritis, four membranous glomerulonephritis) and twelve of these had a cryoglobulinaemia; (b) four patients had a normal kidney biopsy without cryoglobulin; (c) among the eight remaining patients, without morphological study, five cryoglobulins were found (only two were analysed) and four of the latter had clinical evidence of renal involvement (Table 3).

TABLE 3. Disseminated lupus erythematosus with and without cryoglobulinaemia

Cryoglobulin	Cases	Decreased complement	Positive RF	Membrano-proliferative GN	Membranous glomerulopathies	Normal kidney	Renal biopsy not done
+	17*	12	1/14	9	3	0	5†
−	8	1	1/07	0	1	4	3

* Fourteen cryoglobulins typed.
† Four patients with clinical renal involvement.
RF: Rheumatoid factor; GN: glomerulonephritis.

Scleroderma and cirrhosis of the liver, both diseases in which the kidney may be affected, but usually not studied for cryoglobulin, were included in this survey. A cryoprecipitate was present in five out of twenty-five patients with scleroderma without clinical evidence of renal involvement. Among ten cirrhotics with glomerulonephritis, seven had a cryoglobulinaemia. For this reason, twenty-three patients with cirrhosis of the liver without evidence of renal involvement were studied. In this latter group, five cryoglobulins were found (Table 2).

In the other groups, very few cryoprecipitates could be demonstrated. However, in five cases of extra and endocapillary proliferative glomerulonephritis, two were associated with a streptococcal infection and they both had a cryoglobulin. Two mixed essential cryoglobulinaemia without renal involvement were studied. Finally, among ninety-eight patients, with unclassified nephritis, five cryoglobulins were found. One case was due to endocarditis and four had a chronic renal insufficiency (Table 2).

The average quantity of cryoprotein found in the four major groups was similar, between 15 and 25 mg/100 ml.

Characterization of the protein present in the cryoglobulin

Seventy-six cryoglobulins were typed (Table 4). Fifty-one contained IgG and IgM (67%),

TABLE 4. Proteins found in seventy-six cryoprecipitates.

Cryoglobulin	Total	Group I	Group II	Lupus*	Cirrhosis*	Scleroderma	Others
IgG-IgM	45	4	18	10	1	5	7†
IgG-IgM-C3	6	—	5	—	—	—	1‡
IgG	5	—	1	3	—	—	1§
IgG-C3	3	—	3	—	—	—	—
IgG-IgM-IgA	15	—	2	1	9	—	3¶
IgG-IgM-IgA-C3	1	—	—	—	1	—	—
IgG-IgA	1	—	—	—	1	—	—

* With or without nephritis.

† One endo- and extra-capillary proliferative GN, one Schonlein-Henoch purpura, two mixed essential cryoglobulins, three unclassified nephritis.

‡ Endo- and extra-capillary proliferative GN.

§ Monoclonal gammapathy.

¶ One arteritis, one unclassified nephritis.

and six of these also had C3. In sixteen cases (21%) IgG, IgA and IgM were found with C3 in one case. Ten of these patients had cirrhosis of the liver. IgG was found, as the only immunoglobulin in eight cases (10·5%), associated three times with C3. One contained IgG and IgA. It must be emphasized that, with anti-cryoglobulin sera, IgM was demonstrated 11 times although the previous immunochemical analysis revealed only IgG. In the eight cases where IgG was found alone, the cryoprecipitate was not available for immunization.

Cryoglobulin and immunohistochemistry

In fifty-two cases, a comparison between the constituents of the cryoglobulin and the proteins found in the glomerular deposits was carried out (Table 5).

In six acute endocapillary proliferative glomerulonephritis (cases 1–6), although IgG and IgM sometimes associated with C3 are present in the cryoglobulin, C3 is most often the only protein found in the glomerular tuft. Similar results were observed for two cases of extra and endocapillary proliferative glomerulonephritis (cases 44, 45). It is interesting to note that in the only case of acute proliferative glomerulonephritis without evidence of streptococcal infection, but with a high level of rheumatoid factor, both IgG and IgM were found in the cryoglobulin and in the glomerular tuft (case 6).

In twenty-three primary membrano-proliferative glomerulonephritis (cases 7–29) there is a rather good correlation between the immunoglobulins found in the cryoprecipitate and those present in the glomerular tuft. On the other hand, it is very interesting to note that C3 is always present in the glomerular tuft, but rarely found in the cryoglobulin. The proteins found in the glomerular tuft were the same whether a cryoglobulinaemia was present or absent.

Among the thirteen lupus patients with a proved glomerular disease, eight (cases 30–37) had immunohistochemistry (six membrano-proliferative glomerulonephritis and two membranous glomerulopathies). The observations were essentially the same as those of

primary membrano-proliferative glomerulonephritis. However, in addition, IgA is found often in the glomerular tuft but only in one cryoglobulin.

The results were similar for glomerular diseases associated with cirrhosis of the liver (four membrano-proliferative glomerulonephritis, one proliferative glomerulonephritis and one glomerulonephritis with mesangial deposits). However, in these cases (38–43), IgA was found in the cryoglobulin as well as in the glomerular tuft.

In the above cases immunoglobulins and C3 were fixed only on the capillary walls, except in the case with a high rheumatoid factor level in which the lumen as well as the walls was labelled.

In the other groups, due to the small number of cases, it was impossible to make a correlation. In the two cases of mixed essential cryoglobulinaemia with a high level of rheumatoid factor, the glomerulus was normal.

Cryoglobulin and various antibodies

Sixteen positive serological reactions for syphilis were found among 277 patients. Among 374 patients without evidence of lupus or scleroderma, the titre of antinuclear antibodies was lower than 1/100 in twenty-eight cases, and higher in four cases. The titre of antithyroglobulin antibodies was equal or lower than 1/1000 in nineteen cases, and greater in one case. No correlation could be made between the presence of these antibodies and a cryoglobulinaemia.

Cryoglobulin and immunoglobulin level

There was no statistical difference between the serum level of IgG, IgA or IgM whether a cryoglobulin was present or absent (Table 6). As is well known, the IgA level was increased in cirrhosis of the liver.

Cryoglobulin and rheumatoid factor activity

An anti-γ-globulin activity has been looked for in the serum of 417 patients. If one considers only high concentrations (Waaler-Rose $\geqslant 1/50$ or Latex $\geqslant 1/80$), positive reactions were found in one primary membranoproliferative glomerulonephritis, one acute proliferative glomerulonephritis, both with mixed cryoglobulinaemia, in two lupus erythematosus, one of which had a cryoglobulin, one cirrhosis of the liver with cryoglobulin and glomerular disease, one rheumatoid arthritis with amyloidosis but without cryoprecipitate and two mixed essential cryoglobulinaemias.

No anti-γ-globulin activity was found in six isolated cryoglobulins. The reaction was done at room temperature.

Cryoglobulin and complement

Normal values for CH 50 were: 96 ± 28 (2 SD) units/ml ($n = 25$) and for C3: $1{\cdot}32 \pm 0{\cdot}66$ (2 SD) mg/100 ml ($n = 46$).

CH 50 and or C3 was decreased in eight of twenty-one acute endocapillary proliferative glomerulonephritis and in eighteen of seventy membrano-proliferative glomerulonephritis, whether a cryoglobulin was present or absent. Among the thirteen lupus patients with glomerular disease, all had a decreased complement level and twelve had a positive cryoglobulin (Table 3).

In the remaining 388 patients studied for complement, the level was decreased in twelve

TABLE 5. Comparison between the constituents of cryoglobulins and the proteins found in glomerular tufts

Cases	Diagnosis	IgG		IgM		C_3		IgA	
		CG	IHC	CG	IHC	CG	IHC	CG	IHC
1	Acute endocapillary proliferative GN (St)	+	+	+	0	0	+	0	0
2	Acute endocapillary proliferative GN (St)	+	+	+	0	+	+	0	0
3	Acute endocapillary proliferative GN (St)	+	0	+	0	+	+	0	0
4	Acute endocapillary proliferative GN (St)	+	0	+	0	0	+	0	0
5	Acute endocapillary proliferative GN (St)	+	0	0	0	+	+	0	0
6	Acute endocapillary proliferative GN (N. St)	+	+	+	+	+	+	0	0
		6/6	3/6	5/6	1/6	4/6	6/6	0/6	0/6
7	Membrano-proliferative GN	+	+	+	Tr	0	+	+	+
8	Membrano-proliferative GN	+	+	+	0	0	+	0	+
9	Membrano-proliferative GN	+	+	0	+	+	+	0	0
10	Membrano-proliferative GN	+	Tr	+	Tr	0	+	0	Tr
11	Membrano-proliferative GN	+	+	+	Tr	0	+	0	0
12	Membrano-proliferative GN	+	+	+	Tr	0	+	0	0
13	Membrano-proliferative GN	+	+	+	+	0	+	0	0
14	Membrano-proliferative GN	+	+	+	Tr	+	+	0	0
15	Membrano-proliferative GN	+	Tr	+	+	0	+	0	0
16	Membrano-proliferative GN	+	+	+	Tr	0	+	0	0
17	Membrano-proliferative GN	+	+	+	+	0	+	0	0
18	Membrano-proliferative GN	+	+	+	+	0	+	0	0
19	Membrano-proliferative GN	+	+	+	+	0	+	0	+
20	Membrano-proliferative GN	+	+	+	+	0	+	+	Tr
21	Membrano-proliferative GN	+	0	0	Tr	+	+	0	0
22	Membrano-proliferative GN	+	+	+	Tr	0	+	0	+
23	Membrano-proliferative GN	+	+	+	0	0	+	0	0
24	Membrano-proliferative GN	+	+	+	0	0	+	0	0
25	Membrano-proliferative GN	+	+	+	Tr	0	+	0	0
26	Membrano-proliferative GN	+	Tr	0	0	0	+	0	0
27	Membrano-proliferative GN	+	Tr	+	Tr	0	+	0	0
28	Membrano-proliferative GN	+	+	+	+	0	+	0	0
29	Membrano-proliferative GN	+	0	+	0	+	+	0	0
		23/23	21/23	20/23	18/23	4/23	23/23	2/23	6/23

cases (two extra- and endo-capillary proliferative glomerulonephritis, three cirrhosis of the liver with glomerulopathy, one cirrhosis of the liver without evidence of renal involvement, five unclassified nephritis and one scleroderma).

Clinical data

The classical clinical signs described in mixed essential cryoglobulinaemia were found only in five of seventy-six patients.

The severity of the renal disease was judged by the presence or absence of renal insufficiency hypertension or nephrotic syndrome. In the group of acute endocapillary proliferative glomerulonephritis 66% of the cases with a cryoglobulin had a renal insufficiency compared with 18% of those with no cryoprecipitate. However, twelve of sixteen patients without cryoglobulinaemia were seen for the first time more than 2 months after the beginning of their disease. In acute endo and extracapillary proliferative glomerulonephritis and in membranoproliferative glomerulonephritis, the renal insufficiency was the same whether a cryoglobulin was present or absent.

TABLE 5 *cont.*

Cases	Diagnosis	IgG		IgM		C_3		IgA	
		CG	IHC	CG	IHC	CG	IHG	CG	IHC
30	Membranous glomerulopathy (lupus)	+	+	+	+	0	Tr	0	+
31	Membranous glomerulopathy (lupus)	+	+	+	+	0	+	0	0
32	Membrano-proliferative GN (lupus)	+	+	+	+	0	+	0	0
33	Membrano-proliferative GN (lupus)	+	+	0	+	0	+	0	Tr
34	Membrano-proliferative GN (lupus)	+	+	+	+	0	Tr	0	Tr
35	Membrano-proliferative GN (lupus)	+	+	+	+	0	+	0	Tr
36	Membrano-proliferative GN (lupus)	+	+	+	+	0	+	+	0
37	Membrano-proliferative GN (lupus)	+	+	+	+	0	+	0	Tr
		8/8	8/8	7/8	8/8	0/8	8/8	1/8	5/8
38	Membrano-proliferative GN (cirrhosis of the liver)	+	Tr	+	+	0	+	+	+
39	Membrano-proliferative GN (cirrhosis of the liver)	+	Tr	+	+	0	+	+	Tr
40	Membrano-proliferative GN (cirrhosis of the liver)	+	0	+	Tr	0	+	0	+
41	Membrano-proliferative GN (cirrhosis of the liver)	+	+	+	+	0	+	+	0
42	GN with mesangial deposits (cirrhosis of the liver)	+	+	+	0	0	+	+	+
43	Acute endocapillary proliferative GN (cirrhosis of the liver)	+	+	0	Tr	0	+	+	Tr
		6/6	5/6	5/6	5/6	0/6	6/6	5/6	5/6
44	Extra- and endo-capillary proliferative GN	+	0	+	0	0	+	0	0
45	Extra- and endo-capillary proliferative GN	+	0	+	0	+	+	0	0
46	Membranous glomerulopathy	+	+	+	+	0	+	0	0
47	Membranous glomerulopathy	+	+	+	+	0	+	0	+
48	GN with mesangial deposits	+	+	+	Tr	0	+	0	+
49	Schonlein-Henoch purpura	+	+	+	0	0	+	0	+
50	Minimal glomerular changes	+	0	+	0	0	0	0	0
51	Mixed essential cryoglobulinaemia	+	0	+	0	0	0	0	0
52	Mixed essential cryoglobulinaemia	+	0	+	0	0	0	0	0

IHC: Immunohistochemistry. St: Streptococcal. N. St: Non Streptococcal. GN: Glomerulonephritis.

The evolution of the disease was followed by cryoglobulin, clinical signs and repeated renal biopsies. In membrano-proliferative glomerulonephritis, eighteen patients had repeated studies for a period of 6–31 months.

(a) In eleven patients with a repeatedly positive cryoglobulin the disease progressed in four cases, two cases had a remission and no change was observed in five. Two cases in this group (one of which had a remission) had repeated renal biopsies without any immunomorphological changes.

(b) Twice the cryoprecipitate was not found on subsequent examination. One case had persistent immunomorphological lesions.

(c) Three cases were negative on the first examination but were found positive on subsequent studies. In these cases there was no changes in the clinical status.

In the group of acute streptococcal glomerulonephritis the illness was not modified by the presence of a cryoglobulin which persisted 3–20 months after the onset of the disease.

TABLE 6. Serum immunoglobulin levels in four categories with or without cryoglobulinaemia

	Normal controls	Lupus Cryoglobulin		Acute endocapillary proliferative GN Cryoglobulin		Membrano-proliferative GN Cryoglobulin		Cirrhosis of the liver Cryoglobulin	
		+	−	+	−	+	−	+	−
IgG	9·34 ± 1·9*	9·95 ± 5·23	12·30 ± 8·90	9·76 ± 5·56	11·24 ± 2·23	7·44 ± 2·83	9·20 ± 2·59	12·50 ± 4·46	13·80 ± 7·16
	0·18†	1·45	3·98	2·49	1	0·53	0·72	1·29	2·39
	n: 42‡	n: 13	n: 5	n: 5	n: 5	n: 29	n: 13	n: 12	n: 9
IgA	1·85 ± 0·8	3·75 ± 2·19	3·42 ± 3·39	2·92 ± 0·73	3·03 ± 1·05	3·33 ± 1·86	2·88 ± 1·05	9·18 ± 2·33	8·08 ± 3·80
	0·14	0·61	1·52	0·33	0·47	0·35	0·30	0·64	1·27
	n: 34	n: 13	n: 5	n: 5	n: 5	n: 29	n: 12	n: 12	n: 9
IgM	0·90 ± 0·35	1·42 ± 0·93	1·40 ± 1·07	1·21 ± 0·26	2·26 ± 2·02	1·48 ± 0·41	1·38 ± 2·59	2·08 ± 1·53	2·50 ± 1·47
	0·08	0·26	0·48	0·12	1·01	0·07	0·72	0·46	0·49
	n: 21	n: 13	n: 5	n: 5	n: 4	n: 30	n: 13	n: 11	n: 9

* Mean ± SD mg/100 ml.
† Standard error of the mean.
‡ Number of determinations.
GN: Glomerulonephritis.

Likewise this was even more evident in the two cases of acute streptococcal endo- and extracapillary proliferative glomerulonephritis which recovered spontaneously in spite of the persistence of a cryoglobulin. In one of these cases a renal biopsy was done and no immunoglobulins or C3 were further observed.

In lupus erythematosus, one cryoglobulin disappeared during the course of the disease as well as the clinical symptoms of renal involvement.

DISCUSSION

Cryoglobulins are rarely found in normal subjects (Lerner, Barnum & Watson, 1947; Wager *et al.*, 1968; McIntosh *et al.*, 1970; Klein *et al.*, 1972). This fact was confirmed by this study. However, Cream (1972), recently reported a frequency of 38% in normal subjects with a titre of 80 ng/ml or less. In the various groups reported in this paper the average quantity was approximately twice or more the amount found by Cream (1972) in normal subjects.

The results concerning acute streptococcal proliferative glomerulonephritis are in agreement with those reported by others. The frequency is comparable: 50% in this series, 36% (Grupe, 1968) and 78% (McIntosh *et al.*, 1970). The quantity is the same, the absence of correlation between the level of immunoglobulin, or complement and the absence of rheumatoid factor are also found in this work. On the other hand, we could not correlate the severity of the glomerulonephritis to the presence of a cryoglobulin. The major difference concerns the constituents of the cryoprecipitate which we have almost always found to include IgM.

Another difference concerns the acute non-streptococcal proliferative glomerulonephritis. McIntosh *et al.* (1970) found a cryoglobulin in four of four cases but in this investigation only one was detected in a series of twelve patients. This discrepancy may be explained by a difference in the morphological classification of glomerular diseases. On the other hand, in this study, the cryoglobulin was investigated late, after the beginning of the disease.

In primary membrano-proliferative glomerulonephritis, contrasting with acute endocapillary proliferative glomerulonephritis a correlation was found between the immunoglobulins detected in the cryoglobulin and those present in the glomerular tuft.

The results concerning lupus erythematosus agree with those reported in the literature, in particular for the relationship between glomerular disease, cryoglobulin and low complement level (Christian *et al.*, 1963; Hanauer & Christian, 1967; Mustakallio *et al.*, 1967; Statsny & Ziff, 1969).

The presence of a cryoglobulin in cirrhosis of the liver has been rarely reported or has not been found significantly different from a control group (Lerner *et al.*, 1947; Griffiths & Gilchrist, 1953). Jori and Buonanno (1972), reported mixed IgG–IgM cryoglobulinaemia or cryoglobulins consisting only of IgG in cirrhosis. In this work, the three immunoglobulins were most often found in the cryoglobulin as well as in the glomerular tuft.

An important point to be discussed is the role of complement. It has been shown that cryoglobulin has an anti-complementary effect *in vitro* (Balazs & Frohlich, 1966; Wager *et al.*, 1968; Statsny & Ziff, 1969; Marcus & Townes, 1971). It is striking to note that glomerulonephritis with cryoglobulinaemia are proliferative with C3 deposits in the glomerular tufts. On the other hand, complement components are rarely found in the cryoprecipitate, except in the case of acute endocapillary proliferative glomerulonephritis (Grupe, 1968; McIntosh *et al.*, 1970). C3 is found inconsistently in the cryoprecipitate of

lupus erythematosus (Hanauer & Christian, 1967; Statsny & Ziff, 1969). It is probable that one or more of the constituents of complement attach themselves to the cryoglobulin but they cannot be detected, probably due to the methods used. This has been shown by Hanauer & Christian (1967) who have demonstrated anti-C3 and C4 antibodies in anti-cryoglobulin lupus sera. Moreover, C1q is also present and is necessary for cryoprecipitation (Christian *et al.*, 1963; Agnello *et al.*, 1971).

The fundamental point to be discussed is the role of cryoglobulinemia in the pathogenesis of glomerulonephritis. To answer this question, one would have to characterize the antigen and the antibody in the cryoglobulin and in the glomerular tuft. This is partially known for mixed essential cryoglobulinaemia, lupus nephritis and acute streptococcal glomerulonephritis.

In mixed essential cryoglobulinaemia, the presence of monoclonal IgM anti-IgG has been demonstrated (McKenzie *et al.*, 1968; Klein *et al.*, 1972). The disease can be complicated with glomerular disease with IgG and IgM glomerular deposits (Meltzer *et al.*, 1966; Golde & Epstein, 1968; Klein *et al.*, 1968; Feizi & Gitlin, 1969; Mathison *et al.*, 1971; Morel-Maroger & Mery, 1972).

In lupus nephritis, two facts have been well established. First of all, the glomerular deposits contain DNA (Koffler, Schur & Kunkel, 1967) and anti-DNA antibodies (Koffler *et al.*, 1967; Andres *et al.*, 1970). But the presence of DNA in the cryoprecipitate (Bluestone *et al.*, 1970) is a controversial point (Hanauer & Christian, 1967; Statsny & Ziff, 1969). Second, IgM with anti-IgG activity has been identified in the cryoglobulin as well as in the glomerular tuft (Agnello *et al.*, 1971). Cryoglobulins have also been found in NZB mice (Hijmans *et al.*, 1969).

In streptococcal glomerulonephritis, a streptococcal antigen is present in the glomerular deposits (Treser *et al.*, 1970; Zabriskie, 1971), but it could not be detected in the cryoglobulin (McIntosh *et al.*, 1971). In addition, as far as we know, anti-IgG activity has not been reported. It is important to note that Davie *et al.* (1968) immunized rabbits, with C streptococcus and found anti-streptococcal antibodies in mixed cryoglobulin, and anti-IgG antibodies in the serum (Bokisch, Bernstein & Krause, 1972).

Finally, most of the cryoglobulinaemia encountered in glomerulonephritis are mixed. They resemble group III of Brouet *et al.*, 1972. It is probable that, as in lupus erythematosus, leprosy (Bonomo & Dammacco, 1971), infectious mononucleosis, cytomegalovirus infection (Wager *et al.*, 1968; Kantor *et al.*, 1970), and normal subjects (Cream, 1972), one of the constituents of the cryoglobulin has an anti-IgG activity which could be characterized only on the isolated cryoglobulin at +4°C. In this hypothesis, the cryoglobulin could contain an antibody anti-pre-existing immune complexes and be in part responsible for some glomerulonephritis. On the other hand, since IgM is not found in the glomerular tuft of acute endocapillary proliferative glomerulonephritis, it is also possible that, in such cases, cryoglobulin is unrelated to the glomerular disease, but a parallel phenomenon.

The presence of IgA in some cryoprecipitates, especially in cirrhosis of the liver, brings up the question of the role of IgA. A contamination could not always be excluded. It is also possible that IgA has an anti-IgG activity as shown previously (Wager, Mustakallio & Rasanen, 1968; Whitsed & Penny, 1971).

Most of studies to date suggest that cryoglobulinaemia is an immune manifestation.

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