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Experimental immune glomerulonephritis induced in the rabbit with streptococcal vaccine

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SUMMARY

Heavy C3 glomerular deposits were observed in rabbits injected intravenously with C5 streptococcal vaccine. Immunoglobulin deposits appeared later in a few rabbits. Although some data favour the presence of circulating immune complexes during the course of this glomerulonephritis, no evidence for their initiating role could be demonstrated. Streptococcal components are known to activate the alternative pathway of complement. It is suggested that complexes made of streptococcal components and activated C3 might deposit in glomerular tufts.

INTRODUCTION

Some human glomerulonephritides (GN) are characterized by the presence of C_3 glomerular deposits without demonstrable immunoglobulins. This has mainly been observed in some acute endocapillary proliferative glomerulonephritides (Bariety & Druet, 1971; Morel-Maroger, Leathem & Richet, 1972) and in some membrano-proliferative glomerulonephritides (Verroust *et al.*, 1974a). This fact suggests that other mechanisms, besides antiglomerular basement membrane antibodies and circulating immune complexes (Dixon, 1968; Wilson & Dixon, 1976), may be operative in the mediation of glomerulonephritis. One possibility could be the activation of the alternative pathway of complement. However, inulin and zymosan which are known to activate the alternative pathway have not been found to be nephritogenic in rabbits (Verroust, Wilson & Dixon, 1974b).

Many bacteria, such as *Streptococcus*, are able to activate the alternative pathway of complement (Tauber, Polley & Zabriskie, 1976). Furthermore, some streptococcal strains are responsible for human glomerulonephritis. Although these agents have been used to induce an experimental glomerulonephritis (Lindberg, Vosti & Raffel, 1967; Becker & Murphy, 1968; Vosti *et al.*, 1970; Markowitz *et al.*, 1971; McIntosh, Kaufman & Kulvinskas, 1971a), its mechanism has not been clearly established.

The aim of this study was to investigate the glomerular consequences of repeated injections of streptococcal vaccine in the rabbit.

MATERIAL AND METHODS

Animals. Male and female New Zealand White (NZW) rabbits, 2 kg body weight, were purchased from Evic Ceba (Montrouge, France).

Experimental procedure. Rabbits were injected intravenously (i.v.) with C5 streptococcal vaccine (C5SV). The vaccine treated with pepsin and inactivated with 0·17% formaldehyde in phosphate buffered saline (PBS), was obtained from the Pasteur Institute (Garches, France); its optical density at 450 nm was 8·50. Rabbits were divided into three groups depending on the amount of C5SV injected.

Group I: thirteen rabbits (six males and seven females) were injected i.v. with C5SV three times a week for 2 weeks according to Herd & Spragg, (1972). They received 0·5 ml at each injection during the first week and 1 ml during the second

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week. An open wedge biopsy was performed in six rabbits on day 5 after the first C5SV injection and in nine rabbits on day 8. The thirteen rabbits were killed on day 15.

Group II: thirteen rabbits (seven males and six females) were injected .i.v. with C5SV three times a week for 4 weeks. They received 0·5 ml at each injection during the first week, 1 ml during the second, 1·5 ml during the third and 2 ml during the fourth week. An open wedge biopsy was performed on day 15 after the first injection in all thirteen rabbits, on day 45 in nine of them and on day 60 in seven.

Group III: twenty-one rabbits (eleven males and ten females) were used as controls. Seven rabbits (group IIIa) were not injected but were maintained for the same period as the C5SV-injected rabbits in the animal house. Nine rabbits (group IIIb) were injected i.v. with PBS three times a week for 4 weeks. The volume of PBS injected was the same as the volume of C5SV used in group II rabbits. Five rabbits (group IIIc) were injected i.v. with PBS just as group IIIb rabbits except that the PBS contained 0·17% formaldehyde. A renal biopsy was performed on day 8 after the first injection in the group IIIc rabbits and on day 15 in all control animals.

Antisera. Goat and sheep anti-rabbit IgG and anti-rat IgG antisera were prepared as described elsewhere (Bellon & Druet, 1974). The IgG fraction isolated from these antisera was fluoresceinated as already described (Bellon, Sapin & Druet, 1975).

Anti-whole normal rabbit serum (NRS) antisera were prepared in ten Wistar rats. Each rat was injected intradermally (i.d.) in the thoracic wall with 5 mg of lyophilized NRS in 1 ml of PBS emulsified in the same volume of Freund's complete adjuvant. One month later each rat was injected intramuscularly for 3 consecutive days with 5 mg NRS in 1 ml PBS. They were then bled out and their serum was tested by immunoelectrophoretic analysis against NRS.

Anti-rabbit C3 antiserum was raised in F_1 hybrids (Fisher × August) rats, according to Mardiney & Müller-Eberhard (1965). The antisera obtained were pooled and absorbed on normal rat IgG insolubilized with glutaraldehyde (Avrameas & Ternynck, 1969). The antiserum, when tested by Ouchterlony analysis against NRS, gave only one precipitation line. This antiserum cross-reacted with a goat anti-human C3 antiserum (Hyland, Travenol Laboratories, Costa Mesa, California). Both antisera revealed rabbit and human C3.

The anti-C5SV antiserum was obtained from group I and II rabbits which had received C5SV. The sera which had the highest agglutinating titre (see below) were selected. IgG was isolated from these sera and fluoresceinated as above. As a control, IgG from group III rabbits without anti-C5SV antibodies was isolated and fluoresceinated in the same way.

Because of an antigenic relationship between rabbit and human IgM, fluoresceinated goat anti-human IgM was used (Hyland, Travenol Laboratories, Costa Mesa, California).

Immunomorphology. Kidney cryostat sections were stained with the flouresceinated sheep anti-rabbit IgG, goat anti-human IgM and rabbit anti-C5SV antisera. The presence of C3 was detected by indirect immunofluorescence using the rat anti-rabbit C3 antiserum, which was then revealed with the fluoresceinated anti-rat IgG antiserum. The later reagent was also used alone as a control.

Forty-two renal samples were studied by light microscopy. They were fixed in Bouin's solution.

CH50 level, anti-C5SV antibodies and circulating immune complexes. A blood sample was collected from each rabbit before C5SV injection by puncture of the central artery of the ear. Aliquots were frozen at −50°C. Haemolytic activity was determined according to Mayer (1961). Normal values were obtained from the forty-seven rabbits before the beginning of the experiment. Anti-C5SV agglutinating antibodies were measured as described by Herd (1973a) and mercaptoethanol treatment was carried out on every sample (Herd, 1973b). Circulating immune complexes (IC) were sought using the C1q binding assay (C1q BA) (Nydegger *et al.*, 1974). As a control for the detection of IC, sera from rabbits with acute serum sickness (Wilson & Dixon, 1976) were also studied together.

Cryoglobulinaemia. Investigations for cryoglobulinaemia were performed weekly and when the animals were killed as described previously (Davie *et al.*, 1968; Herd, 1973a, b). Cryoglobulins were isolated and tested by immunoelectrophoretic analysis with the rat anti-whole NRS antiserum.

Other studies. Urinary proteins were measured every day for 4 weeks using the biuret method and haematuria was sought using the Sangur test (Boehringer Mannheim, Germany). The serum creatinine level was determined once a week for 4 weeks. Normal values were obtained from ten rabbits before the beginning of the experiment.

RESULTS

During the whole experiment, the rabbits looked well, had no apparent signs of disease, and their weight did not change. Two rabbits died after kidney biopsy.

Immunofluorescence studies

Groups I and II. Kidney. (a) Day 5 and day 8: six rabbits from group I had a kidney biopsy on day 5. A granular straining was observed in mesangial areas in five of them with anti-C3 antiserum. A similar but less intense fixation was observed in two with anti-IgM antiserum and in one with anti-IgG antiserum. On day 8, similar results were obtained in the same six rabbits. Three other rabbits from the same group had their first kidney biopsy performed on day 8. All three had granular staining in mesangial

areas with anti-C3 antiserum and one of them showed a similar but less intense fixation with anti-IgG antiserum (Table 1).

(b) On day 15, all twenty-six rabbits of groups I and II (except one in group II) had heavy granular staining, mainly mesangial but sometimes also parietal, with anti-C3 antiserum (Fig. 1). Occasionally, there was granular staining with anti-IgM (fourteen out of twenty-two) and IgG (five out of twenty-six) antisera. The staining with anti-IgM (Fig. 2) and IgG antisera was always less intense than with anti-C3 antiserum.

(c) On day 45, 15 days after the last C5SV injection, the granular staining had decreased, but was still detectable in seven out of nine rabbits with anti-C3 antiserum.

(d) On day 60, 30 days after the last C5SV injection, only two out of seven rabbits had a weak granular staining of the glomeruli with anti-C3 antiserum.

TABLE 1. Immunofluorescent study of rabbit kidney samples at different times of the experiment

		Day of biopsy									
		Group I			Group II			Group III			
	Intensity of							a	b	c	
Antiserum used	fluorescence	5	8	15	15	45	60	15	15	8	15
Anti-C3	+ or ++	1	4	10	13	5	1	0	0	0	0
	±	4	4	2	0	2	1	2	1	1	2
	−	1	1	1	0	2	5	5	8	4	3
Anti-IgM	+ or ++	0	n.t.	4	8	0	0	0	0	n.t.	n.t.
Anti-IgM	±	2	n.t.	2	0	0	0	0	0	n.t.	n.t.
	−	4	n.t.	3	5	5	7	3	5	n.t.	n.t.
Anti-IgG	+ or ++	0	2	1	1	0	0	0	0	0	0
Anti-IgG	±	1	0	3	0	0	0	0	0	0	0
	−	5	7	9	12	9	3	7	9	5	5
Anti-C5SV	+ ot ++	0	0	n.t.	0	0	0	0	n.t.	n.t.	n.t.
	±	0	0	n.t.	0	0	0	0			
	−	6	9	n.t.	5	5	4	3			

Group I and II rabbit kidney samples were also tested with fluoresceinated anti-C5SV antiserum (six on day 5, nine on day 8, five on day 15, five on day 45 and four on day 60). No fixation was ever observed.

Liver. On day 15, three group I rabbit liver samples were tested. Coccus-like structures, either grouped or isolated in hepatic sinusoides, were stained with anti-C5SV antiserum (Fig. 3) and to a lesser extent with anti-rabbit IgG antiserum. No labelling was observed when using anti-C3 and anti-IgM antisera.

Group III a,b,c. Kidney. In group III, four rabbits out of twenty-one had a weak granular staining of some glomeruli with anti-C3 antiserum but this staining was always much less intense than in C5SV-injected rabbits. In some controls and C5SV-injected rabbits, a weak staining of the tubular basement membrane with anti-C3 antiserum could be seen. Neither anti-IgG antiserum, anti-IgM antiserum nor anti-C5SV stained control rabbit glomeruli. None of the antisera fixed to the arteries of control and C5SV injected rabbits.

Liver. Eight control rabbit liver samples out of twenty-one were tested. No fixation was observed with anti-C3 anti-IgG, anti-IgM and anti-C5SV antisera.

Light microscopy

Four group I and thirteen group II kidney samples were examined on day 15. Glomerular lesions were marked in eight cases and mild in five cases. These lesions were mainly mesangial cellular proliferation

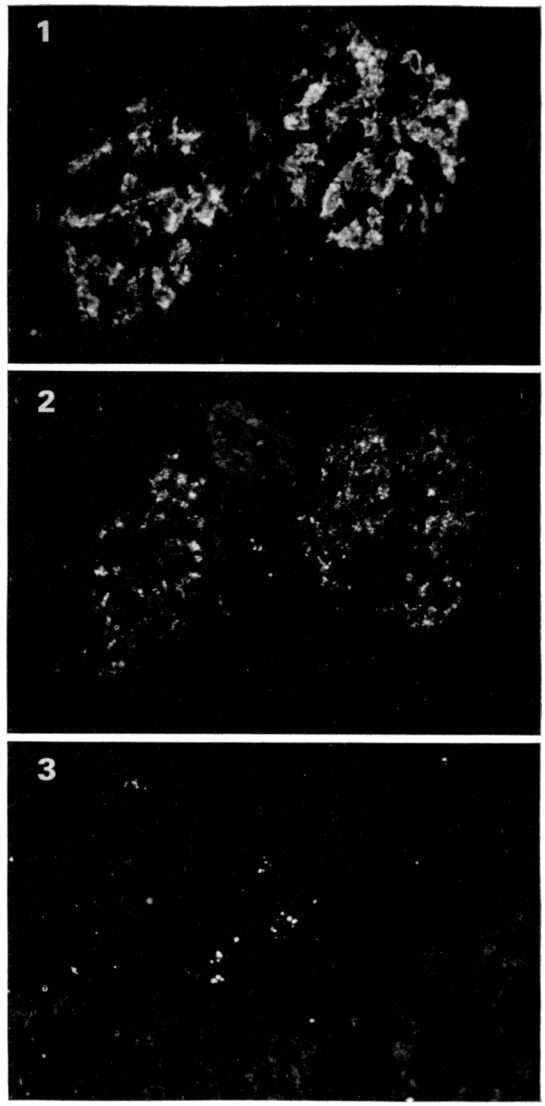

FIG. 1. Kidney of group II rabbit stained with unlabelled rat anti-rabbit C3 antiserum revealed with fluoresceinated sheep anti-rat IgG antiserum. Heavy granular staining is localized to mesangial areas. (Magnification × 300.)

FIG. 2. Kidney of group II rabbit stained with fluoresceinated goat anti-human IgM antiserum. Granular staining is located to mesangial areas. (Magnification × 250.)

FIG. 3. Liver of group I rabbit stained with fluoresceinated rabbit anti-C5SV antiserum. Coccus-like structures are seen in the sinusoides. (Magnification × 200.)

without thickening of the glomerular capillary wall. Polymorphonuclear cells, over five per glomerulus were sometimes present. Occasional fibrinoid deposits were seen in mesangial areas. Renal arteries were most often normal and in some cases, vacuoles were seen in smooth muscle cells. On day 45, these lesions were no longer observed. Seventeen group III kidney samples were tested and none had lesions on day 15 or on day 45.

Serum CH50 level

The mean normal value was calculated after fifty-seven measurements in forty-seven normal NZW

rabbits and was 18·70±6·42 u. In the thirteen group I rabbits, the CH50 level fell around day 4, reached a nadir value around day 10 and remained low until the animals were killed (day 15). In all group II rabbits, the CH50 level fell as in group I and returned to the normal value around day 20. In fourteen of the twenty-one control rabbits, the CH50 level was followed up as in C5SV-injected animals. In all of them, the CH50 level remained within the normal range during the whole of the experiment (Fig. 4a & b).

Agglutinating anti-C5SV antibodies

Before the first C5SV injection, only one rabbit out of forty-seven had agglutinating anti-C5SV antibodies. During C5SV injections, agglutinating anti-C5SV antibodies appeared around day 6 in all twenty-five rabbits which had no antibodies before injection and reached a maximum (titre from 1/4096 to 1/8192) around day 12. In three rabbits, a rise in IgM antibodies was observed between day 2 and day 6, with a maximum on day 3. No agglutinating antibodies were detected in group III rabbits during the whole experiment (Fig. 4c & d).

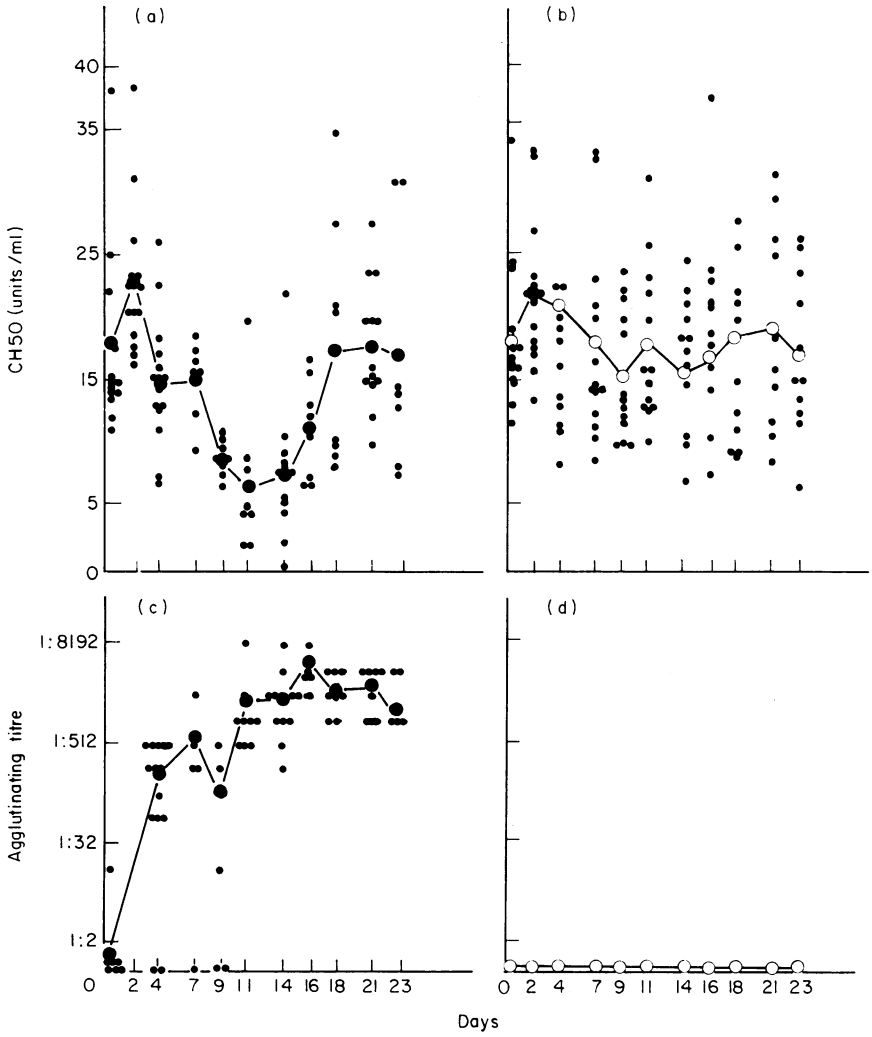

FIG. 4. (a) Evolution of the CH50 level of the thirteen group II rabbits, and (b) fourteen rabbits from group III. (c) Evolution of the agglutinating anti-C5SV antibody titre of the thirteen group II rabbits, and (d) and fourteen rabbits from group III.

Cryoglobulinaemia

Cryoglobulinaemia was detected in all C5SV-injected rabbits as early as the first week. Immunoelectrophoretic analysis showed that these cryoglobulins consisted of IgG and IgM. Cryoglobulinaemia was no longer detected 30 days after the last C5SV injection.

Detection of IC by C1q binding assay

No IC were detected in C5SV-injected or control rabbits, using the C1q BA which was performed three times a week during the whole experiment. However, IC were easily detected in the serum of three rabbits with acute serum sickness induced with bovine serum albumin.

Clinical follow-up

In ten rabbits (five C5SV-injected and five controls) none had proteinuria, haematuria or an increased serum creatinine level.

DISCUSSION

Several manifestations were observed in NZW rabbits injected intravenously with C5SV. (1) A mild proliferative glomerulonephritis with mesangial C3 deposits from day 5. These deposits were found in most of the rabbits on day 15 and disappeared 1 month after the last C5SV injection. IgG glomerular deposits were very rarely detected and significant IgM deposits were found on day 15 in only twelve out of the twenty-six rabbits injected with C5SV. (2) Streptococcal components were detected coated with IgG, and not with C3 in the liver (probably in the reticuloendothelial system), but not in the glomerular tufts. (3) The CH50 level decreased transiently during the second week of the experiments. The C1q BA was continuously negative but a mixed cryoglobulin was detected in these rabbits.

Immune glomerular injury in man has been shown to be mediated either by anti-glomerular basement membrane (GBM) antibodies or by the deposition of circulating immune complexes (IC) (Dixon, 1968). The fact that glomerular injury could, in some instances, be unrelated to the presence of ICs was suggested by the finding of some human glomerulonephritis (GN) with G3 deposits only (Bariety & Druet, 1971; Morel-Maroger *et al.*, 1972; Verroust *et al.*, 1974a). Activation of the alternative pathway of complement could be another operative mechanism.

Experimental granular glomerular deposits induced with various antigens have always been shown to be due to IC (Wilson & Dixon, 1976). A recent experiment was designed to induce in rabbits glomerular deposits unrelated to the presence of ICs, by using either inulin or zymosan (Verroust *et al.*, 1974b). These agents are known to activate the alternative pathway of complement (Götze & Müller-Eberhard, 1976). Under these conditions, complement was activated *in vivo*, but no evidence for the nephritogenicity of this process could be ascertained. In the experimental model reported here, the major finding was the presence of mesangial C3 deposits with occasional IgG and IgM deposits. This suggests that the C3 deposition in the mesangial areas was the initial major event. It has recently been shown that streptococcal components are able to activate the alternative pathway of complement (Tauber *et al.*, 1976). A streptococcal membrane protein was suspected to be responsible for this activation. According to this hypothesis, the complement system could be activated by streptococcal components in the circulation and both would then be trapped in the mesangial areas. Another possibility could be that streptococcal components are first located in the mesangium and then activate complement *in situ*. A similar mechanism has recently been suggested for an *in situ* formation of IC (Izui, Lambert & Miescher, 1976). The expected fixation in kidney of the anti-C5SV antiserum was not observed at any time during the experiment, although the antiserum used was able to reveal streptococcal components, as it did in the liver. The absence of mesangial fixation could be related to: (1) the absence of streptococcal component in the kidney; (2) the presence of different streptococcal components in the kidney and the liver and the failure of the antiserum to detect the former; (3) a steric hindrance of streptococcus covered with C3 molecules. No data could be obtained which favoured any one of these hypothesis.

Other workers have tried to induce an immune-type GN using streptococcal components with different experimental procedures. Immunoglobulins and C3 were deposited mainly in mesangial areas. It was concluded that either IC (Lindberg *et al.*, 1967; Vosti *et al.*, 1970; McIntosh *et al.*, 1971a) or a cross-reactivity with a GBM antigen (Markowitz *et al.*, 1971) were involved. A role for IC cannot be excluded from the present experiments and IgM mesangial staining could be related to the secondary deposition of cryglobulins. McIntosh *et al.* have also shown that *streptococcus* was able to alter human IgG *in vitro* (McIntosh, Kulvinskas & Kaufman, 1971b). They have also shown that non-specific autologous IgG previously incubated *in vitro* with streptococcus and then intravenously injected alone was able to induce C3 and IgG mesangial glomerular deposits in the rabbit (McIntosh *et al.*, 1972). The involvement of similar mechanisms could not be ruled out in the present experiments. However, the fact that IgG was very rarely detected is an important argument against a major role for IC containing IgG (McIntosh *et al.*, 1971a) or for altered IgG (McIntosh *et al.*, 1972). It must be stressed that experimental procedures were different as alive streptococci were used in McIntosh's studies (McIntosh *et al.*, 1971a, 1972) and killed streptococci in the present one. It is therefore quite possible that streptococcal-induced immune-type GN might be relevant to several non-exclusive mechanisms.

The C1q BA was negative in all the rabbits during the whole study. This finding could mean either that there were no circulating IC or that these IC were not detected by this test. Three data favour the later hypothesis: (1) a mixed cryoglobulin (IgM–IgG) was found in the present study, as previously described by others (Davie *et al.*, 1968; Herd, 1973a, b); (2) the finding of streptococcal IgG-coated component in the liver would appear to argue in favour of the existence of IC; (3) the fall in the CH50 provides the last piece of evidence and can be explained in different ways: either it fell because of the mixed cryoglobulin acting as IC, or as a result of a strong activation of the alternative pathway of complement, or because of a decrease in synthesis (Charlesworth *et al.*, 1974). These mechanisms are not exclusive. In the present experiments, the fact that the lowering of CH50 was transient and began at a time when circulating anti-C5SV antibodies were detected suggests that the decrease in CH50 was at least in part related to the presence of IC.

In conclusion, several arguments support the existence of circulating IC in our model, but no proof could be obtained for an initiating role for these IC. On the other hand, data have been presented suggesting that besides other mechanisms described by different workers, activation of the alternative pathway of complement by streptococcal components could be responsible for the early presence of C3 mesangial deposits together with a mild proliferative GN.

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REFERENCES

AVRAMEAS, S. & TERNYNCK, T. (1969) The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry*, **6**, 53.

BARIETY, J. & DRUET, P. (1971) Résultats de l'immunohistochimie de 589 biopsies rénales (transplants exclus). *Ann. Med. Int.* **122**, 63.

BECKER, C.G. & MURPHY, G.E. (1968) The experimental induction of glomerulonephritis like that in man by infection with group A streptococci. *J. exp. Med.* **127**, 1.

BELLON, B. & DRUET, P. (1974) Essai d'explication du phénomène de prozone en immunofluorescence et immunoperoxydase. *Ann. Immunol. (Inst. Pasteur)*, **125C**, 871

BELLON, B., SAPIN, C. & DRUET, P. (1975) Comparaison de la sensibilité des techniques d'immunofluorescence et d'immunoperoxydase en méthode directe et indirecte. *Ann. Immunol. (Inst. Pasteur)*, **126C**, 15.

CHARLESWORTH, J.A., WILLIAMS, D.G., SHERINGTON, E., LACHMANN, P.J. & PETERS, D.K. (1974) Metabolic studies of the third component of complement and the glycine-rich β-glycoprotein in patients with hypocomplementemia. *J. clin. Invest.* **53**, 1578.

DAVIE, J.M., OSTERLAND, C.K., MILLER, E.J. & KRAUSE, R.M. (1968) Immune cryoglobulins in rabbit streptococcal antiserum. *J. Immunol.* **100**, 814.

DIXON, F.J. (1968) The pathogenesis of glomerulonephritis. *Amer. J. Med.* **44**, 493.

GÖTZE, O. & MÜLLER-EBERHARD, H.J. (1976) The alternative pathway of complement activation. *Adv. Immunol.* **24**, 1.

HERD, Z.L. (1973a) Antiglobulins and cryoglobulins in rabbits producing homogeneous streptococcal antibodies. *Immunology*, **25**, 923.

HERD, Z.L. (1973b) Experimental cryoglobulinaemia. Production and properties of streptococcus-induced rabbit cryoglobulins. *Immunology*, **25**, 931.

HERD, Z.L. & SPRAGG, J. (1972) The occurrence of homogeneous antibodies and antigenic selection of K-light chains to streptococcal carbohydrates in monash rabbits. *Aust. J. exp. Biol. Med. Sci.* **50**, 225.

IZUI, S., LAMBERT, P.H. & MIESCHER, P.A. (1976) *In vitro*

demonstration of a particular affinity of glomerular basement membrane and collagen for DNA. A possible basis for a local formation of DNA-anti-DNA complexes in systemic lupus erythematosus. *J. exp. Med.* **144**, 428.

LINDBERG, L.H., VOSTI, K.L. & RAFFEL, S. (1967) Experimental streptococcal glomerulonephritis in rats. *J. Immunol.* **98**, 1231.

MCINTOSH, R.M., KAUFMAN, D.B. & KULVINSKAS, C. (1971a) Glomerular lesions induced in rabbits by type 12 group A β-haemolytic streptococcus: study of pathogenic mechanisms and role of cryoglobulins. *Exp. Med. Microbiol.* **23**, 277.

MCINTOSH, R.M., KULVINSKAS, C. & KAUFMAN, D.B. (1971b) Alteration of the chemical composition of human immunoglobulin G by streptococcus pyogenes. *J. Med. Microbiol.* **4**, 535.

MCINTOSH, R.M., KAUFMANN, D.B., MCINTOSH, J.R. & GRISWOLD, W. (1972) Glomerular lesions produced by autologous serum and autologous IgG modified by treatment with a culture of a β-haemolytic streptococcus. *J. Med. Microbiol.* **5**, 1.

MARDINEY, M.R., JR & MÜLLER-EBERHARD, H.J. (1965) Mouse β_1C-globulin: production of antiserum and characterization in the complement reaction. *J. Immunol.* **94**, 877.

MARKOWITZ, A.S., HORN, D., ASERON, C., NOVAK, R. & BATTIFORA, H.A. (1971) Streptococcal related glomerulonephritis. III. Glomerulonephritis in rhesus monkeys immunologically induced both actively and passively with a soluble fraction from nephritogenic streptococcal protoplasmic membranes. *J. Immunol.* **107**, 504.

MAYER, M.M. (1961) Complement and complement fixation. *Experimental Immunochemistry* (ed. by E.A. Kabat & M.M. Mayer), p. 133. Thomas, Springfield.

MOREL-MAROGER, L., LEATHEM, A. & RICHET, G. (1972) Glomerular abnormalities in non-systemic diseases. Relationship between findings by light microscopy and immunofluorescence in 433 renal biopsy specimens. *Amer. J. Med.* **53**, 170.

NYDEGGER, U.E., LAMBERT, P., GERBER, H. & MIESCHER, P.A. (1974) Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen. Quantitation by binding to radio-labelled C1q. *J. clin. Invest.* **54**, 297.

TAUBER, J.W., POLLEY, M.J. & ZABRISKIE, J.B. (1976) Non specific complement activation by streptococcal structures. II. Properdin-independent initiation of the alternate pathway. *J. exp. Med.* **143**, 1352.

VERROUST, P.J., WILSON, C.B., COOPER, N.R., EDINGTON, T.S. & DIXON, F.J. (1974a) Glomerular complement components in human glomerulonephritis. *J. clin. Invest.* **53**, 77.

VERROUST, P.J., WILSON, C.B. & DIXON, F.J. (1974b) Lack of nephritogenicity of systemic activation of the alternate complement pathway. *Kidney Int.* **6**, 157.

VOSTI, K.L., LINDBERG, L.H., KOSEK, J.C. & RAFFEL, S. (1970) Experimental streptococcal glomerulonephritis: longitudinal study of a laboratory model resembling human acute poststreptococcal glomerulonephritis. *J. Infect. Dis.* **122**, 249.

WILSON, C.B. & DIXON, F.J. (1976) The renal response to immunological injury. *The Kidney* (ed. by B.M. Brenner & F.C. Rector), p. 838. Saunders, Philadelphia.