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ACUTE SERUM SICKNESS NEPHRITIS IN THE RABBIT

AN IMMUNE DEPOSIT DISEASE

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Evidence has accumulated implicating antigen–antibody complexes as mediators of tissue damage in Arthus reactions,[1] acute experimental serum sickness nephritis,[2] chronic experimental glomerulonephritis [3] and poststreptococcal glomerulonephritis in man.[4,5] Observations by Rich and Gregory [6] and subsequent studies by others,[7–9] have shown that proliferative glomerulonephritis occurs in rabbits following the administration of large doses of foreign proteins. By immunofluorescent analysis it was first shown that during serum sickness immunoglobulin G (IgG) is deposited in glomeruli,[10] along the capillary wall and that antigen and immunoglobulin localization was finely granular.[2] Features of the glomerular ultrastructure at the onset of the disease include endothelial proliferation and rare electron-dense basement membrane lesions.[11]

Dixon, Feldman and Vazquez [3] produced chronic nephritis by repeated injections of antigen, using a form of experimental glomerulonephritis based on prolonged exposure to circulating antigen–antibody complexes; antigen and IgG were usually found in discrete, granular

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TABLE I

RABBIT KIDNEY IN ACUTE SERUM SICKNESS NEPHRITIS INDUCED BY PRIMARY IMMUNE RESPONSE

Rabbit number	Elimination BSA (day)	Kidney biopsy (day)	Fluorescent microscopy * IgG	Fluorescent microscopy * BSA	Light microscopy †
Group I					
62	13	12	0	0	2–3+
		14	2+	1+	2+
		16	3+	0	1–2+
		21	3+	0	1+
		28	1+	0	1+
63	14	7	0	0	—
		14	0	0	1–2+
		16	1+	0	2+
		23	1+	0	1+
		27	0	0	1+
		36	0	0	0
68	12	11	0	0	0
		13	1+	0	1+
		24	1+	0	1+
		31	0	0	—
69	12	10	0	0	±
		14	1+	0	2+
		17	3+	2+	2+
		24	2+	0	1+
		28	1–2+	0	±
		31	1+	0	—
73	16	2	0	0	—
		16	2+	0	2+
		17	±–1+	0	1+
		22	0	0	2+
74	17	7	2+	±–1+	—
		15	0	0	—
		17, 20, 24	0	0	0, ±, —
59	17	0, 15, 21	0	0	0, 1+, 1+
		30	0	0	0
61	13	0, 10, 14	0	0	0
		20, 31	—	—	±, 0
64	16	9, 15	—	—	0, 0
		20	0	0	0
65	15	4	±	±	0
		13, 16, 24, 27	0	0	±, ±, 1+, 1+
71	17	4, 13, 20, 27	0	0	—, ±, 1–2+, 1+
75	15	9, 16	0	0	0, 0

* The intensity and amount of fluorescence are graded as 0, negative; ±, minimal; 1+, 2+, and 3+.

† Grading of the lesions by light microscopy was done without knowledge of the day of antigen elimination or the findings by fluorescent microscopy. Grade 0, negative; ±, minimal; 1+, 2+, 3+; —, biopsy was not examined by light microscopy.

deposits occasionally associated with linear deposition of IgG along the basement membrane. Here electron-dense masses were found on the epithelial side of the basement membrane. These masses were subsequently shown by Andres and associates [12] to contain both antigen and host IgG.

Acute poststreptococcal glomerulonephritis in man has features similar to those of serum sickness in rabbits.[4,13] Immunofluorescent studies have shown that in acute poststreptococcal glomerulonephritis, IgG and complement are deposited in nodules along the basement membrane,[5] a similar distribution to that seen by Dixon and co-workers in experimental chronic glomerulonephritis.[3] The ultrastructural changes consist of endothelial swelling, narrowing of the capillary lumen, and formation of discrete masses of electron-dense material within and along the epithelial surface of the glomerular basement membrane.[4,5,14-20]

Having observed the pathologic parallels between acute hemorrhagic nephritis in man and experimental chronic serum sickness nephritis,[3] and having noted the disparities between the lesions of the clinical disease and those in the acute serum sickness model, it seemed important to restudy acute serum sickness by immunofluorescent methods and electron microscopy, emphasizing the later lesions of acute serum sickness nephritis which had not been fully considered in earlier work.

Here we report a study of acute experimental serum sickness nephritis produced by a single dose of bovine serum albumin. Morphologically the glomerular lesions were shown to be virtually identical to those found in human poststreptococcal glomerulonephritis. The early lesion, as previously described, was featured by endothelial proliferation, and the older lesion by large discrete nodules of IgG and $beta_{1C}$ globulin; these appeared by electron microscopy as electron-dense masses along the epithelial side of the basement membrane.

MATERIAL AND METHODS

Induction of Serum Sickness. Albino rabbits weighing 1 to 2 kg were obtained from a local breeder, fed Purina® Rabbit Pellets and water *ad libitum*. Serum sickness nephritis was induced in 24 rabbits (Tables I and II) by a single intravenous dose of bovine serum albumin (BSA) (Armour Pharmaceutical Company, Kankakee, Ill.) equivalent to 250 mg per kg body weight. In 15 additional rabbits (Table III) nephritis was studied during the secondary immune response; primary immunization was achieved using BSA, 250 mg per kg as above. The animals were rested from 5 to 9 weeks before the second dose of BSA (250 mg per kg) was administered. Seven of the latter group received BSA labeled with ^{131}I to follow antigen elimination from the circulation.[21] To determine the level of soluble antigen–antibody complexes in these animals the ammonium sulfate fractionation method of Farr [22] was employed. Elimination of BSA from the circulation in all other animals was monitored by capillary tube precipitin reactions using hyperimmune rabbit anti-BSA serum.

Open Renal Biopsy. One-hundred and thirty-two serial open renal biopsy pro-

TABLE II

RABBIT KIDNEY IN ACUTE SERUM SICKNESS NEPHRITIS INDUCED BY PRIMARY IMMUNE RESPONSE

Rabbit number	Elimination BSA (day)	Kidney biopsy (day)	Fluorescent microscopy *			Light microscopy †
			IgG	BSA	$beta_{1C}$	
Group II						
213	12	12	1+	±–1+	±	3+
		17	1–2+	±–1+	3+	2+
		19	1–2+	0	2–3+	2–3+
		25	±	0	2+	—
214	10	10	1–2+	1+	±	2+
		13	±–1+	0	2+	1–2+
		16	1+	0	2–3+	3+
		24	0	0	2+	—
215	11	11	±	0	0	2+
		13	1+	±–1+	2+	—
		18	1+	±	2+	1–2+
		25	±–1+	0	1–2+	0
217	13	17	2+	1+	2+	2–3+
		23	±	0	2+	—
		30	0	0	1–2+	—
218	10	10	1+	±	0	2–3+
		13	2–3+	1–2+	2–3+	2–3+
		17	3+	1+	3–4+	2+
		24	1–2+	0	3–4+	—
479	10	10	±	±	0	1–2+
		13	±–1+	±	1+	±–1+
		17	1+	0	1+	2+
		24	0	0	2+	0
482	12	19	1–2+	0	1–2+	±
		25	±	0	2+	0
216	10	10, 13, 16	0	0	0, 1+, ±	1+, 0, —
480	11	11, 13, 18	0	0	0, ±, 1+	2+, —, 3+
481	12	12, 17, 19	0	0	0	1+, ±, —
483	12	20, 27	0	0	2+, 1+	—
484	11	11, 13	0	0	±, 0	±, —

* The intensity and amount of fluorescence graded; 0, negative; ±, minimal; 1+, 2+ and 3+.

† Grading of the lesions by light microscopy was done without knowledge of the day of antigen elimination or the findings by fluorescent microscopy. Grade 0, negative; ±, minimal; 1+, 2+, and 3+; —, biopsy was not examined by light microscopy.

cedures were performed under light pentobarbital sodium (30 mg per kg) anesthesia using clean non-sterile technique. Renal cortical biopsy specimens, approximately 0.5 × 0.5 × 2 cm in size, were obtained using a sharp scalpel blade. Hemostasis was achieved by topical pressure, and penicillin (400,000 units) and streptomycin (0.5 gm) (Parke, Davis & Co., Detroit, Mich.) were injected intramuscularly to prevent infection.

Preparation of Tissue for Histologic Study. Part of each specimen was fixed in 10 per cent buffered formalin (pH 7.35) and paraffin embedded; sections were stained with hematoxylin and eosin and the periodic acid–Schiff (PAS) reagent. Another por-

TABLE III

RABBIT KIDNEY IN ACUTE SERUM SICKNESS NEPHRITIS INDUCED BY SECONDARY IMMUNE RESPONSE *

Rabbit number	Elimination BSA (day)	Kidney biopsy (day)	Fluorescent microscopy			Light microscopy
			IgG	BSA	$beta_{1C}$	
Group I						
861	5	5	1+	0	—	1+
		8	1+	0	—	1–2+
		11	2+	0	—	1+
		15	2+	0	—	±
863	5	6	1+	0	—	—
		9	±–1+	0	—	±
		11	1–2+	0	—	±
		15	±	0	—	—
869	6	6 †	1–2+ mes ‡	0	—	—
862	>15	7	0	0	—	1+
865	15	9, 17	0	0	—	1+, —
866	>15	7, 11	0	0	—	—, ±
868	>11	7, 11 †	0	0	—	—
Group II						
92	5	5	±–1+	0	±–1+	±
		7, 12	0, 0	0, 0	0, 0	1+, —
65	6	4	0	0	0	1–2+
		6	1+	0	±	±
		8	±	0	±	0
		14	0	0	0	0
81	5	5	2+	±–1+	1–2+	2+
		7	1–2+	0	±–1+	1+
		11	±	0	1–2+	1–2+
		18	0	0	0	—
91	5	5	1–2+ mes	0	1+ mes	1–2+
		7	±	0	0	0
		11, 18	0	0	0	—, ±
101	5	5 †	1–2+ mes	0	1+ mes	±
131	7	7	1+ mes	0	1+ mes	0
		12	0	0	±–1+	—
		15	0	0	± mes	0
76	7	6, 8, 13	0	0	0	1+, ±, —
95	8	8, 14	0	0	0	—, 0

* Group 1 861–869-secondary stimulation 5 weeks after primary. Group II 92–101-secondary stimulation 9 weeks after primary.

† Animal died after operation.

‡ Mesangial and stalk distribution of IgG and $beta_{1C}$.

tion of the specimen was immediately cut into cubes (0.5 mm^3), fixed in buffered 1 per cent osmium tetroxide (Merck & Co. Inc., Rahway, N.J.), dehydrated in 70 and 95 per cent alcohol, and embedded in Vestopal-W polyester resin (Martin Jaeger Co., Geneva, Switzerland). Sections cut at 1 μ were stained by the Wright–

Giemsa method [23] and examined by oil immersion light microscopy. Sections for electron microscopy were cut using an LKB Ultrotome and stained with uranyl acetate [24] and lead citrate.[25] These were examined with an RCA EMU-3D electron microscope at original magnifications of 3,000 to 15,000 and enlarged as desired.

Immunofluorescent Methods. Biopsy tissue obtained was rapidly frozen by immediate immersion into a 9.0 ml screwtop vial containing Isopentane (n-methylbutane) (Eastman Organic Chemicals, Rochester, N.Y.) which had been precooled in liquid nitrogen contained in a thermos bottle. The tissue was then stored at −65° C until sectioned. Before sectioning, the tissue was transferred to a −40° C freezer. A small cellulose sponge (Onkosponge no. 1, Histomed Inc., Paterson, N.J.) measuring 10 × 10 × 3 mm was placed on a cryotome chuck, precooled to −40° C and moistened with several drops of distilled water. The specimen was quickly and lightly placed on the sponge to which it became frozen while the chuck was maintained at a temperature of −40° C. After 30 minutes, the tissue and sponge were placed in a Lipshaw cryostat at −20° to −22° C. After equilibration at this temperature for 30 to 60 minutes, sections were cut at 4 μ. These were fixed to the frozen glass slide by the warmth of the finger and left at room temperature for 5 to 10 minutes to insure attachment to the glass. Slides were stored at −20° C until processed further. Sections were dried for 1 hour at 37° C in an incubator before fluorescent staining of unfixed tissue by the method of Ortega and Mellors.[26] Specificity of fluorescent staining was established by pretreating the section twice with untagged antiserum, or by absorbing the fluorescent antiserum with the specific antigen before staining.

The sections were viewed with a Zeiss microscope with an HB 200 light source. The ultraviolet activating filters were UG-1 or BG 12 with a KG-1 heat absorption filter. Barrier filters consisted of OG-4 and 2A Wratten (Kodak) filters. The intensity and amount of fluorescence were arbitrarily graded as 0, negative; ± minimal, 1+, 2+ and 3+. Photomicrographs were taken with an Exakta camera using Kodak Tri-X and Plus-X film.

Preparation of Antisera for Immunofluorescent Studies. Antiserum against BSA was prepared in rabbits using alum precipitated antigen; this was injected into footpads and multiple subcutaneous sites with BSA emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.). The antibody content of the pooled rabbit anti-BSA serum was 5.6 mg per ml. Antibody to rabbit IgG was prepared by immunizing goats with alum precipitated antigen (Cohn Fraction II, Nutritional Biochemicals Corporation, Cleveland, Ohio). Antibody to rabbit beta$_{1C}$ globulin was prepared according to the method of Mardiney and Müller-Eberhard.[27] Guinea pigs were immunized with beta$_{1C}$ globulin adsorbed on a suspension of activated Zymosan (Nutritional Biochemicals). All antisera used in this study showed one precipitin line with immunoelectrophoresis against the immunizing antigen and whole rabbit serum.

Globulins were precipitated from the antisera by the addition of 50 per cent saturated ammonium sulfate at 4° C. The precipitate was washed once with an equal volume of 50 per cent saturated ammonium sulfate, redissolved in 0.01M phosphate buffer (pH 7.35) in isotonic saline, and finally dialyzed until free of sulfate ions. The globulin preparation was conjugated with fluorescein-isothiocyanate [28,29] (Baltimore Biological Laboratory, Inc., Baltimore, Md.) using a ratio of .015 mg per mg of protein. Separation of free fluorescein from the labeled proteins was achieved by chromatography on Sephadex G-25 (fine) (Pharmacia Fine Chemicals, Inc., Newmarket, N.J.). Immediately before use, the gel-filtered, fluorescein-tagged antisera were absorbed twice with mouse liver powder (Baltimore Biological Laboratory).

Patient Study. To compare the morphologic similarity between acute serum sickness nephritis and acute poststreptococcal glomerulonephritis, a renal biopsy was made in an 8-year-old boy 6 days after the onset of acute proliferative glomerulonephritis; this was studied by the same methods. On admission to the hospital beta hemolytic streptococcus was cultured from a throat swab; an ASO titer was 50 Todd

units per cc. A low serum complement of 25 units (normal range of 45 ± 8) was found. Preparation of fluorescein-tagged antisera to human IgG and $beta_{1C}$ globulin and complement assay methods have been previously described.[5]

Results

Fluorescent Microscopy

Serum Sickness Nephritis (Primary Immune Response) (Tables I and II). Glomerular deposition of IgG and BSA was found at the time of complete clearance of the antigen from the circulation. Characteristically, the fluorescence was discrete and nodular with a fine, granular quality (Fig. 1). Some glomeruli showed focal fine linear areas of staining within the basement membrane. In approximately one third of the animals, glomerular involvement with deposits of IgG was diffuse whereas in the remaining rabbits localization was more focal and local. The designations of 'focal' and 'local' are those used by Meuhrcke, Kark, Pirani and Pollack.[30] 'Local' refers to involvement of a portion of one glomerulus; 'focal' indicates lesions of some glomeruli and not others. Early in the course of the disease, glomerular localization of BSA as fine discrete deposits (Fig. 2) was detected in only 3 rabbits (rabbits 62, 69 and 74) (Table I). In group II (Table II), where a more intensified effort to detect the BSA was made by regularly performing biopsies at the time of antigen elimination, BSA was found within the glomeruli in 6 of 7 rabbits demonstrating IgG deposition. In each case, although the antigen deposits were morphologically similar to IgG, BSA localization was much less extensive, being more focal and local in distribution. BSA was usually detected in the glomeruli for periods of 1 to 4 days; antigen was however found as late as 5 days (rabbit 69) and 8 days (rabbit 218) after BSA was eliminated from the circulation.

As the course of the nephritis progressed the deposits of IgG became larger and more discrete (Fig. 4). Nodular deposits were more clearly orientated along the glomerular capillary wall. At higher magnifications it appeared as though immune deposits were located on the epithelial side of the glomerular basement membrane (Fig. 8). There was no evidence that the fluorescence represented nuclear, cytoplasmic or cellular staining. During the first week of serum sickness, glomerular localization of IgG and $beta_{1C}$ globulin appeared (Table III) in identical distribution, with staining of similar intensity and extent. Two weeks after the onset of nephritis IgG staining was no longer demonstrable, or was considerably diminished, whereas strongly positive staining for $beta_{1C}$ globulin was commonly observed. $Beta_{1C}$ globulin was also detected in 4 rabbits (rabbits 216, 480, 483 and 484) where IgG could never be demonstrated.

Serum Sickness Nephritis (Secondary Immune Response) (Table

III). At the time of antigen elimination, the deposits of IgG and $beta_{1C}$ globulin were found as fine discrete granular deposits in the mesangium and supporting structures of the glomerular capillary bed (Fig. 6). Within 48 hours this distribution changed into one of very large coarse deposits found focally and locally within the glomeruli, occasionally intimately related to the glomerular capillary wall (Fig. 7).

In 9 of 10 animals in the 2 experimental groups (Table III), antigen clearance occurred between days 5 and 7, and deposits of IgG were found. Among these only rabbit 81 had glomerular deposits of BSA. Rabbits in group II were tested for $beta_{1C}$ globulin localization which was present as extensively and with the same distribution as IgG. Glomerular localization of immunoglobulin was evident for 10 days in rabbits 861 and 863; in the remaining animals with nephritis the immune deposits were evident between 2 and 6 days after antigen elimination. In group II where antigen was injected secondarily 9 weeks following primary immunization, there was a higher incidence of rapid antigen clearance (days 5 to 7) from the circulation and glomerular localization of IgG as compared with group I. The extent and ultimate duration of immune globulin deposition in group II, however, was not nearly as pronounced as in group I in which the second injection of antigen was provided 5 weeks after primary immunization.

Light Microscopy

The main abnormality detected in rabbits developing serum sickness nephritis by a primary immune response consisted of focal areas of cellular proliferation associated with narrowing of the glomerular capillary loops (Fig. 3). Moderate infiltration with polymorphonuclear leukocytes was also present. Stalk thickening and increased cellularity of the mesangium were occasionally observed. Focal adhesions of Bowman's capsule appeared in instances of severe nephritis; crescent formation, however, was not noted. All rabbits exhibiting IgG and $beta_{1C}$ globulin deposits in glomeruli were found to have marked abnormalities by light microscopy. Although rabbits 59, 65, 71, 216, 480 and 481 showed mild and moderate signs of glomerulonephritis, depositions of IgG or $beta_{1C}$ globulin were not detected within the glomeruli.

When nephritis was induced using the secondary immune response, the abnormalities again consisted of focal areas of increased cellularity, endothelial proliferation and narrowing of glomerular capillary loops. These findings were present to a varying degree in all the rabbits having immune deposits within the glomeruli by fluorescent microscopy. Here again comparable findings by light microscopy were present in rabbits 862, 865 and 76 which lacked IgG or $beta_{1C}$ globulin deposits.

Glomerular Ultrastructural Changes in Acute Serum Sickness Nephritis

Seventeen serial biopsy specimens obtained during the course of experimental serum sickness nephritis in 7 rabbits were examined by electron microscopy (Table IV). These animals were those shown to

TABLE IV

GLOMERULAR ULTRASTRUCTURE IN RABBIT KIDNEY IN ACUTE SERUM SICKNESS NEPHRITIS

	Rabbit number	Elimination BSA (day)	Kidney biopsy (day)	Increased cellularity	Basement membrane * deposits	
Immunofluorescent-positive						
	62	13	14	1+ focal	none	
			16	1–2+ diffuse	many	small
			21	1–2+ diffuse	many	large
			28	2–3+ focal	few	large
	63	14	14	3+ diffuse	none	
			16	2–3+ diffuse	occas.	medium
			23	3+ diffuse	many	small
	68	12	13	3+ diffuse	many	small
			24	3+ diffuse	many	large
			31 ‡	3+ diffuse	many	large
	69	12	17	2+ focal	few	medium
			28 ‡	2+ diffuse	few	large
	73	16	17	3+ diffuse	none	
			22	2+ diffuse	none	
	218	10	13 ‡	1–2+ focal	many	small & large
	861 †	5	11	2+ focal	none	
			15	2–3+ focal	many	medium
Immunofluorescent-negative						
	59	17	15	2–3+ diffuse	none	
	61	13	20	0	none	
	64	16	20	2+ focal	none	
	65	15	13	1+ focal	none	
	71	17	27	1+ focal	none	

* Basement membrane masses graded by size as follows: small, 0.25 to 1.0 μ; medium, 1.0 to 2.0 μ; large, over 2.0 μ.

† Nephritis induced by secondary immune response.

‡ Oil immersion light microscopy revealed discrete basement membrance masses (Fig. 5) (1-μ sections, Giemsa stain).

have glomerular deposition of IgG and complement (Tables I to III). Five additional rabbits which did not have deposition of IgG and complement by immunofluorescence were also studied.

The earliest abnormality detected in the glomeruli consisted of focal and diffuse narrowing of the capillary lumens. This change, evident at

the time of complete antigen clearance from the circulation, resulted from an increase in the cytoplasmic volume of endothelial cells, and an increased number of both endothelial and centrilobular (mesangial) cells. Occasionally polymorphonuclear leukocytes or circulating macrophages were observed. The latter displayed fine cytoplasmic projections which extended to, but did not penetrate the endothelial cell membrane. The leukocytes contained numerous cytoplasmic lysosomes in varying stages of degranulation.

Two to 3 days after antigen clearance an ultrastructural change which we have found to be characteristic of this lesion was observed in 6 of 7 rabbits. Numerous discrete electron-dense masses approximately 0.25 to 1.00 μ in diameter were found within the glomerular basement membrane (Fig. 8). The masses were homogeneous and spherical, projecting from the epithelial side of the lamina densa of the glomerular basement membrane. In these areas the epithelial plasma membrane appeared to be deflected around the electron-dense masses and away from the basement membrane. Where the dense basement membrane deposits were found, the foot processes of the overlying epithelium were absent (Figs. 8 and 9). Vacuoles containing a granular material were observed within the epithelial cytoplasm. A rim or halo of dense, finely granular, epithelial cytoplasmic material was always observed surrounding the dense masses which projected from the basement membrane. The halo of dense material was separated from the basement membrane masses by the epithelial cell plasma membrane.

With progression of serum sickness nephritis serial biopsy specimens demonstrated that the electron-dense masses became fewer but much larger, their size sometimes reaching 2 to 3 μ in diameter. (Fig. 10) The masses also tended with time to become more pedunculated and to extend further into the epithelial cell. Occasionally they appeared to be separated from the basement membrane (Fig. 9); serial sections, however, revealed that the masses were always in contact with basement membrane. Rabbit 73, which showed clear evidence of proliferation and swelling of endothelial cells, exhibited no electron-dense deposits.

Although all glomeruli in Vestopal-embedded tissue cut at 1 μ revealed endothelial swelling and proliferation when examined by oil immersion microscopy, discrete nodular deposits were detected in glomeruli in only 3 rabbits by this technique. These were clearly attached to or contained within the basement membrane and stained intensely with Wright–Giemsa (Fig. 5).

Biopsy tissues from 5 rabbits which did not develop deposits of IgG within glomeruli exhibited varying degrees of hypercellularity and cellular proliferation. No basement membrane lesions or electron-dense deposits were found.

Tissue from rabbit 861 which developed nephritis after secondary antigenic stimulation with BSA was examined by electron microscopy. The glomerular basement membrane in this animal showed nodular lesions similar to those observed in acute serum sickness.

Correlation of Acute Serum Sickness Nephritis with the Lesions of Acute Poststreptococcal Nephritis in Man

The findings in the patient were similar to those revealed in previous studies in children with acute poststreptococcal glomerulonephritis.[5] IgG, and $beta_{1C}$ globulin deposition was nodular and discrete (Fig. 11) with localization on the epithelial side of the basement membrane (Fig. 12). Ultrastructural lesions which were strikingly similar to serum sickness nephritis in the rabbit featured electron-dense basement membrane masses which projected from the epithelial side of the lamina densa (Fig. 13).

Correlation of Antigen Clearance and Formation of Complexes with Development of Serum Sickness Nephritis

In the first group of animals developing serum sickness during a primary immune response (Table I), clearance of antigen from the circulation occurred between the twelfth and 17th day. With the exception of rabbit 61, all animals eliminating the antigen by day 14 (rabbits 62, 63, 68 and 69) developed nephritis as evidenced by either glomerular deposition of antigen and IgG, abnormalities by light microscopy or both. Rabbit 73 did not clear the antigen until day 16 but still developed nephritis. Among the second group of rabbits similarly studied (Table II) all the rabbits cleared the BSA from the circulation between days 10 and 13. In this group, however, only 7 of 12 animals developed immunofluorescent evidence of nephritis by antigen and IgG deposition. Animals more consistently showed $beta_{1C}$ globulin deposition and evidence of nephritis by light microscopy.

During antigen elimination by the secondary immune response 3 animals (rabbits 861, 863 and 869) in group 1 cleared the BSA ^{131}I from the circulation on the fifth and sixth day; the remaining 4 rabbits in this group (rabbits 862, 865, 866 and 868) did not eliminate the antigen for periods of 11 to over 15 days. In rabbits 861, 863 and 869, which eliminated the antigen on days 5 and 6 and developed nephritis, the amount of circulating BSA ^{131}I complexed to antibody rose to a maximum of 1.2, 1.5 and 1.7 per cent of administered antigen respectively. The 4 remaining animals did not develop nephritis, and antigen was eliminated more slowly; 2 of these rabbits (866 and 868), however, bound 1.2 and 2.2 per cent of the BSA ^{131}I with IgG, but rabbits 862

and 865 only developed maximum complexing of antigen to levels of 0.4 and 0.3 per cent respectively.

In group II (Table III) antigen elimination occurred between 5 and 8 days in all animals and nephritis was a concomitant in 7 of 8 rabbits. Six rabbits exhibited deposits of IgG and $beta_{1C}$ globulin and the seventh animal showed abnormalities only by light microscopy.

Discussion

Correlation of light, fluorescent and electron microscopic findings in the rabbit kidney in acute experimental serum sickness nephritis has extended prior descriptions of the immunopathologic features in this experimental model. We have shown that following antigen clearance from the circulation, BSA, IgG and $beta_{1C}$ globulin were deposited in a finely granular form with distribution along the glomerular capillary wall. At this stage light and electron microscopy revealed increased cellularity and proliferation of glomerular capillary endothelial cells. With increasing time characteristic discrete nodular deposits of IgG and $beta_{1C}$ globulin were found within the glomerular basement membrane. Ultrastructural examination at this time revealed discrete electron-dense 'humps' projecting from the epithelial side of the glomerular basement membrane. In some instances the nodular basement membrane masses were detected by oil immersion microscopy in thin sections of osmium fixed tissue.

In general the localization of $beta_{1C}$ globulin within the glomerulus in acute serum sickness was found to parallel that of IgG. In 4 rabbits nodular deposits of $beta_{1C}$ globulin were found when IgG could not be demonstrated. Relatively late in the development of this experimental lesion deposits of $beta_{1C}$ globulin appeared in most animals but IgG was not found. When serum sickness nephritis was induced by a secondary immune response fine granular deposits of IgG occurred initially in the mesangium of the glomerulus. Later well defined nodules of IgG seemed to have a random focal and local distribution. Whereas antigen (BSA) could be detected in most animals developing nephritis by a primary immune response, this was found in only 1 instance among the 9 animals developing nephritis as a consequence of the secondary immune response.

Mellors, Arias-Stella, Siegal and Pressman[10] showed that in acute serum sickness nephritis, IgG was deposited within the glomerulus, and studies by Dixon, Vazquez, Weigle and Cochrane[2] revealed that antigen and host IgG were present together in the glomerulus at the onset of this experimental nephritis. Prior to our studies, however, no detailed investigation describing the immunopathologic alterations late in the

development of the glomerular lesions in this experimental disorder were reported. We have shown that nodular accumulations, demonstrated by immunofluorescence and electron microscopy constituted a characteristic feature.

Kniker and Cochrane[31] have recently reported the localization of beta$_{1C}$ globulin as granular deposits on the glomerular basement membrane. Their studies were made early in the course of acute serum sickness; they did not observe deposition of beta$_{1C}$ globulin in the absence of IgG. In our studies beta$_{1C}$ globulin deposits were often noted in the absence of IgG. Although Feldman[11] found rare electron microscopic basement lesions at the time of antigen elimination from the circulation, he emphasized endothelial proliferation. The later lesions of serum sickness, however, bear a strong resemblance to the changes we have observed in acute glomerulonephritis and seem to us similar to some of the changes observed by Dixon and co-workers[3] in their model of chronic glomerulonephritis produced by daily antigen administration over a period of several months.

Perhaps even more striking is the similarity of the experimental serum sickness lesions described herein to those which are a feature of acute poststreptococcal glomerulonephritis in man.[14,20] Our electron microscopic studies further extend the similarities between the two renal disorders since in both situations electron-dense masses on the epithelial side of glomerular basement membrane have now been shown to be a characteristic feature. The lesions in Dixon's chronic glomerulonephritis model, however, show significant differences from the lesions of late serum sickness described in this paper. Many of the lumpy deposits in the chronic glomerular lesion are smaller, more confined to the substance of the basement membrane and antigen can be easily demonstrated by immunofluorescence. Our nodular lesions of late serum sickness are more uniform and discrete, in which IgG and complement are readily demonstrable but antigen cannot be found.

Germuth and associates,[32,33] and Dixon and co-workers[2] defined the pathogenetic significance of soluble antigen–antibody complexes. It has been shown that the formation, and subsequent elimination of complexes from the circulation is associated with both deposition of antigen and antibody in the glomeruli and with the subsequent development of glomerulonephritis. In our experiments as well, a general correlation was noted between "immune" elimination of antigen and immunoglobulin and complement localization in glomeruli. The correlations however were not absolute. In an initial experiment (Table I) animals showing "immune" elimination were observed with minimal or no antigen or IgG deposition within glomeruli. Similarly in a second group of rabbits

(Table II) in which "immune" elimination was more uniform, IgG and antigen were not demonstrated in some, although complement deposition and abnormalities were evident by light microscopy.

Where antigen elimination was produced by a secondary immune response (Table III) the glomerular localization of immune globulins occurred on days 5 to 7 in 9 of 16 rabbits. In this group maximum demonstrable complexing of antigen with IgG varied between 1.2 and 2.2 per cent. Surprisingly these values were similar to the amount of complexing reported previously in the primary response.[2] This occurred despite the fact that much more antigen was available for complex formation at the time of "immune" elimination in the secondary than in the primary response. The relatively low levels of complexes in the circulation in the secondary response might reflect the formation of complexes more rapidly removed by the reticuloendothelial system.

From the foregoing it is apparent that rapid "immune" elimination of antigen, and the formation of antigen–antibody complexes are not the only factors important in the pathogenesis of serum sickness nephritis. Additional important parameters such as the kinetics of antibody production, the specific nature of the complexes formed, factors responsible for glomerular localization of macromolecules,[34] and the individual vulnerability of different animals to injury by immune complexes must be considered.

Localization of beta$_{1C}$ globulin within the glomerulus without demonstrable IgG has been observed. This discrepancy is hard to explain on the basis of the sensitivity of the tests involved. This feature, however, is similar to that which we have previously reported in patients with acute poststreptococcal glomerulonephritis.[5] A parallel may have been encountered by Leddy, Hill, Swisher and Vaughn[35] who found complement on red cells in patients with hemolytic anemia where IgG could not be demonstrated. Perhaps as proposed by Leddy this phenomenon can be explained by the assumption that IgG is present but sterically hindered from reacting with its homologous antiserum by the molecules of complement covering the antigen–antibody combination.

As described by other workers, localization of antigen could only be detected for very short periods of time, usually 2 to 3 days after the onset of nephritis. The most likely explanation of this phenomenon is the combination of specific antibody with binding sites still available on the deposited antigen. Of note, only 1 rabbit among 9 developing nephritis with the secondary immune response showed deposits of BSA, a finding which may result from the rapid rate of antibody synthesis and the consequent rapid saturation of antigenic sites.

Summary

A correlated study of the immunopathologic alterations in the rabbit kidney during serum sickness nephritis using light, fluorescent and electron microscopy has emphasized the later stages in the development of the renal lesions.

Immunofluorescent studies showed that IgG, antigen and $beta_{1C}$ globulin were deposited within the glomerular capillary wall as discrete nodular deposits. In certain instances $beta_{1C}$ globulin alone was found in the deposits.

By electron microscopy, nodular electron-dense deposits within the glomerular basement membrane were regularly encountered. These resembled the nodules observed in acute poststreptococcal glomerulonephritis in man, and in certain aspects of experimentally induced chronic glomerulonephritis following repeated injections of antigen as described by Dixon.

Serum sickness nephritis produced by a secondary immune response was studied to compare the formation of antigen–antibody complexes and the immunopathologic features in classical serum sickness nephritis.

Additional evidence of a similarity between experimental serum sickness nephritis and acute poststreptococcal glomerulonephritis in man has been provided.

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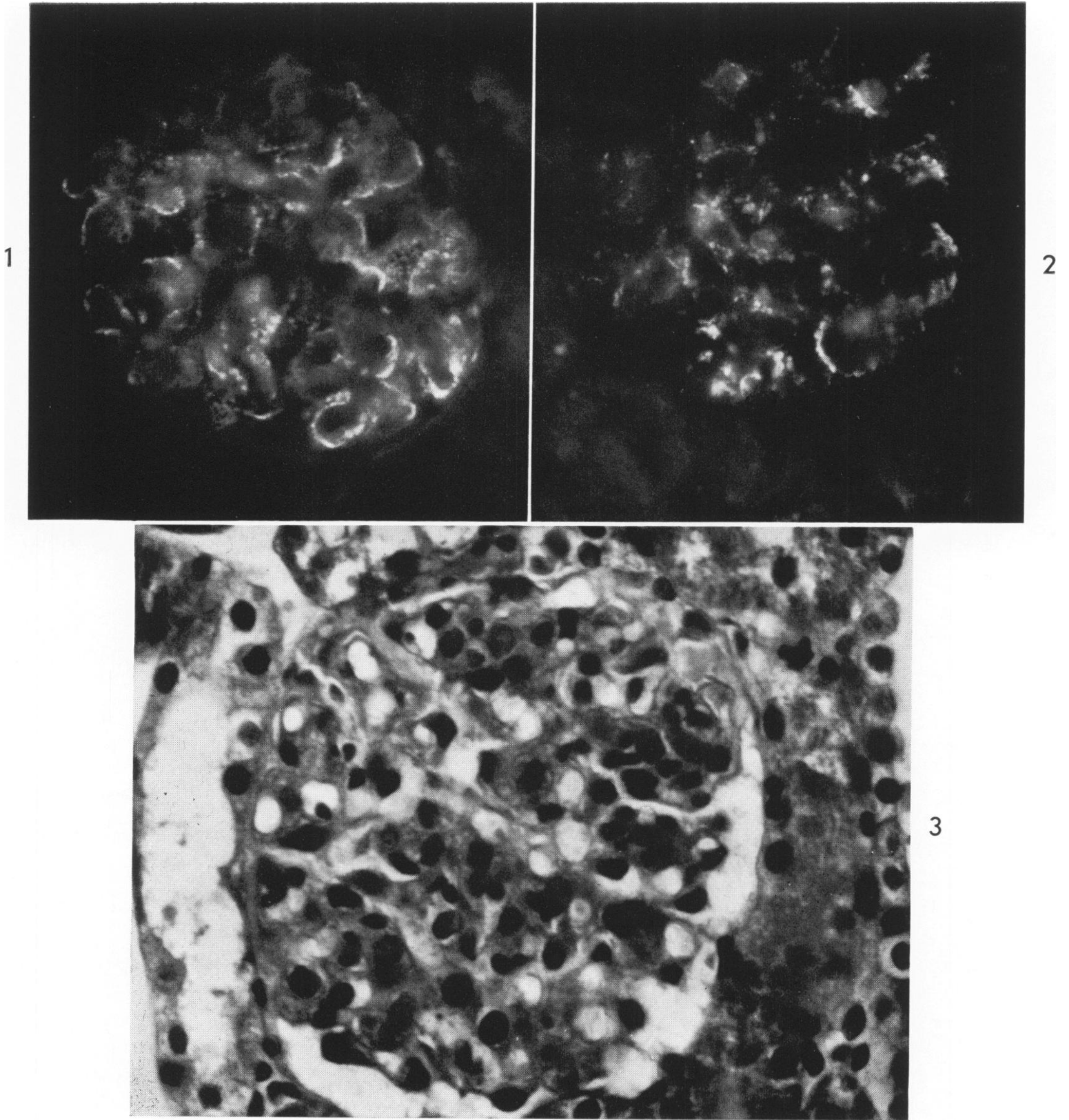
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. A rabbit glomerulus at the onset of serum sickness nephritis. Fine granular deposits of host IgG and focal areas of linear basement membrane staining are present. A morphologically similar distribution is seen when the tissue is stained for rabbit $beta_{1C}$ globulin. Fluorescent microscopy. × 350.

FIG. 2. Glomerular localization of BSA at the time of antigen elimination is less extensive but the fine granular deposits of antigen are morphologically similar to the distribution of IgG. Fluorescent microscopy. × 350.

FIG. 3. Glomerulus in a rabbit 2 days after antigen elimination in serum sickness nephritis. Moderate glomerulitis (1 to 2+) is characterized by focal areas of increased cellularity and narrowing of the glomerular capillary loops. Hematoxylin and eosin stain. × 400.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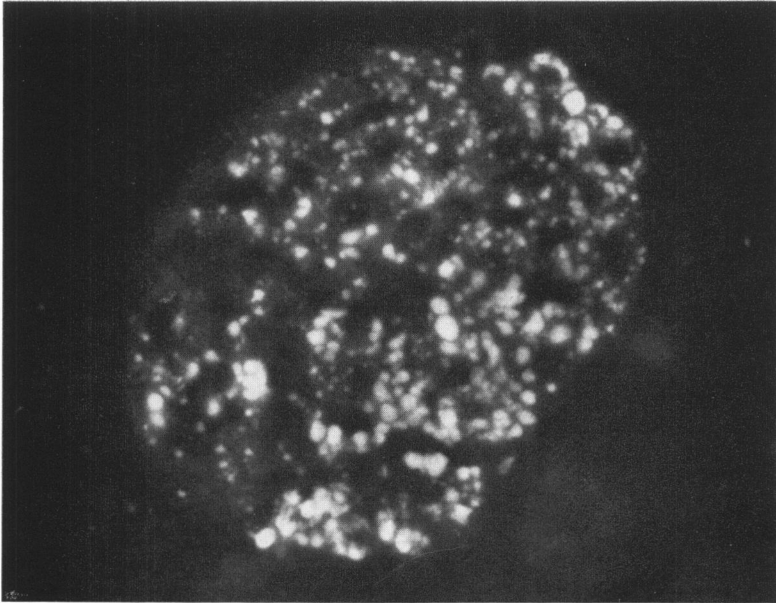

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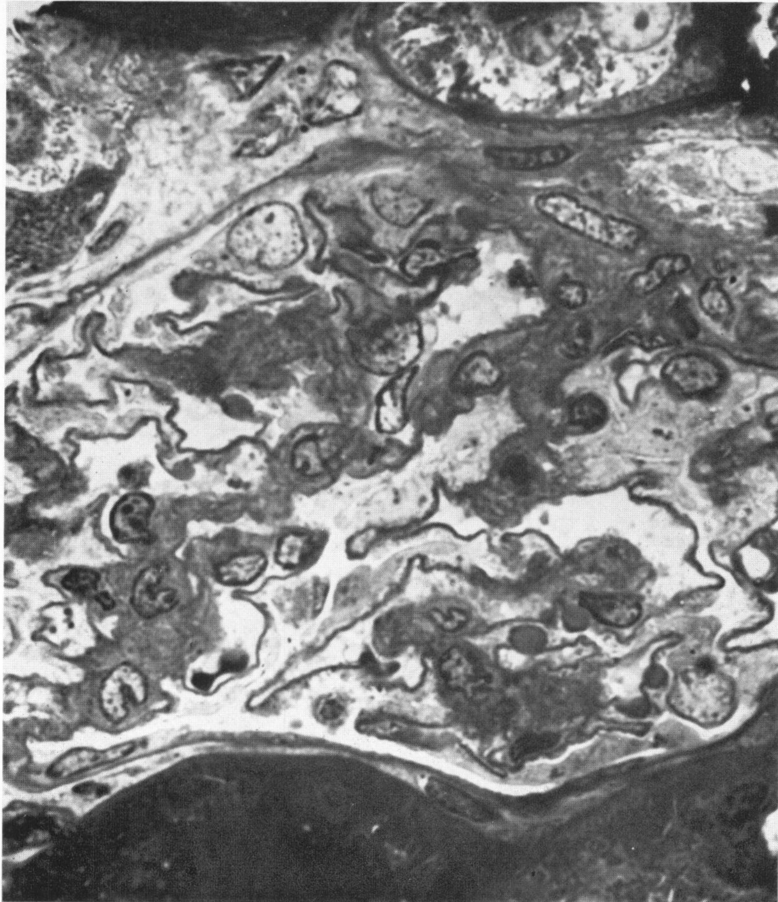

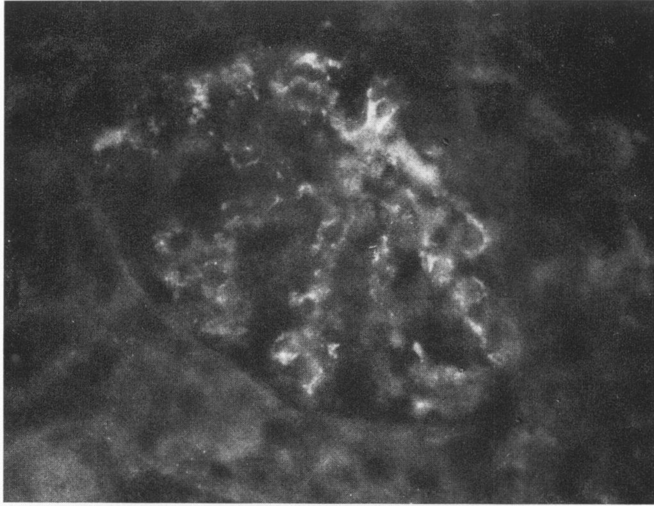

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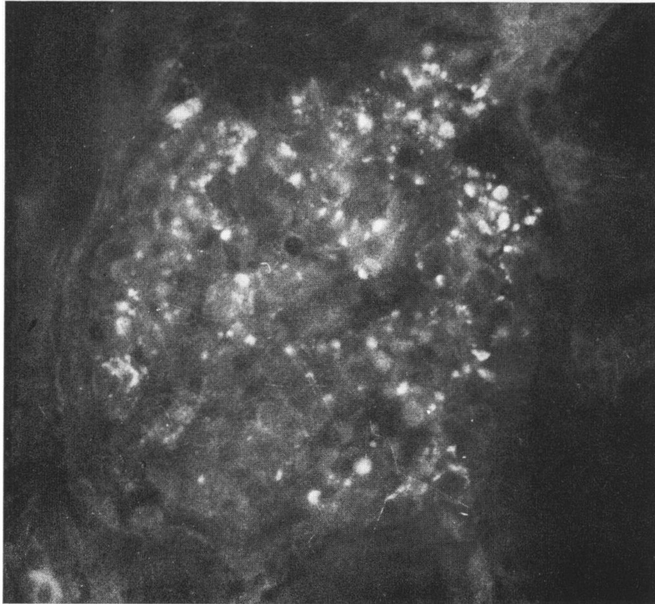

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FIG. 4. Twelve days after the onset of serum sickness nephritis. Large discrete nodular deposits of $beta_{1C}$ globulin are evident. In peripheral capillary loops the deposits follow the course of the glomerular capillary wall. A similar distribution, with less intense fluorescence is seen when the tissue is stained for rabbit IgG. Fluorescent microscopy. × 400.

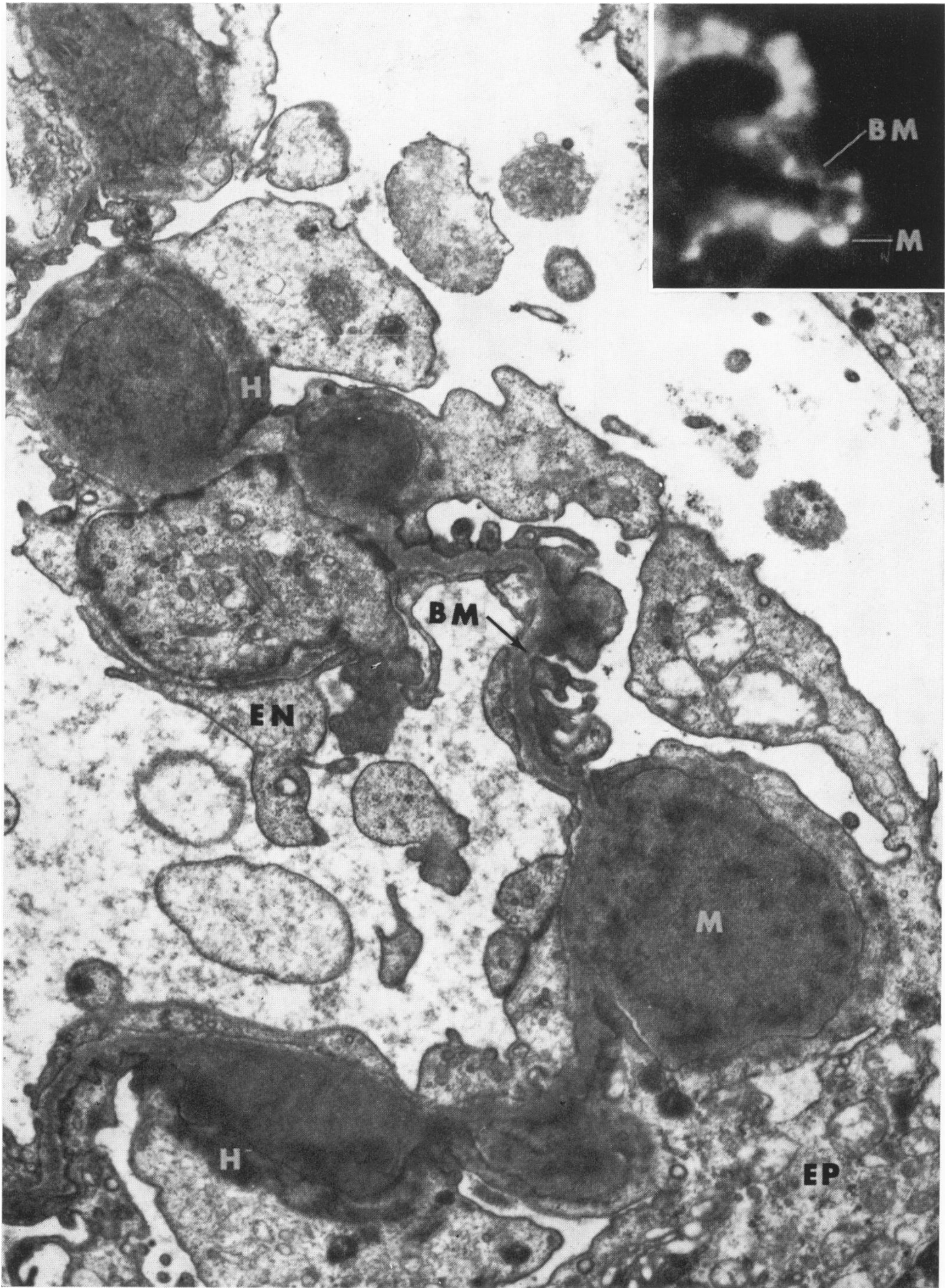
FIG. 5. Renal tissue from a rabbit 2 weeks after the onset of nephritis. Discrete deposits (arrows) are associated with the basement membrane. Section cut at 1 μ; Wright–Giemsa stain. × 1,100.

FIG. 6. Serum sickness nephritis induced by a secondary immune response. A distinct mesangial distribution of IgG is evident at the time of antigen clearance from the circulation. Focal circular areas of fine granular fluorescence appear to be located within the cytoplasm of cells having a stalk distribution characteristic of mesangial cells. Fluorescent microscopy. × 350.

FIG. 7. Large coarse nodules of IgG appear with a random distribution throughout a glomerulus 1 week following antigen clearance by a secondary immune response. Fluorescent microscopy. × 350.

FIG. 8. A portion of the glomerulus in a rabbit 4 days after the onset of serum sickness nephritis. Seven discrete electron-dense masses are present. These project from the epithelial side of the basement membrane (BM). Adjacent epithelial cells (EP) exhibit a loss of foot processes and intracytoplasmic accumulation of a finely granular dense material (H). The largest electron-dense mass (M) measure 2.6 μ. $\times$ 17,000.

Inset. A peripheral capillary loop in the same animal shows nodular deposits of $beta_{1C}$ globulin on the epithelial side of the basement membrane. Fluorescent microscopy. $\times$ 700.



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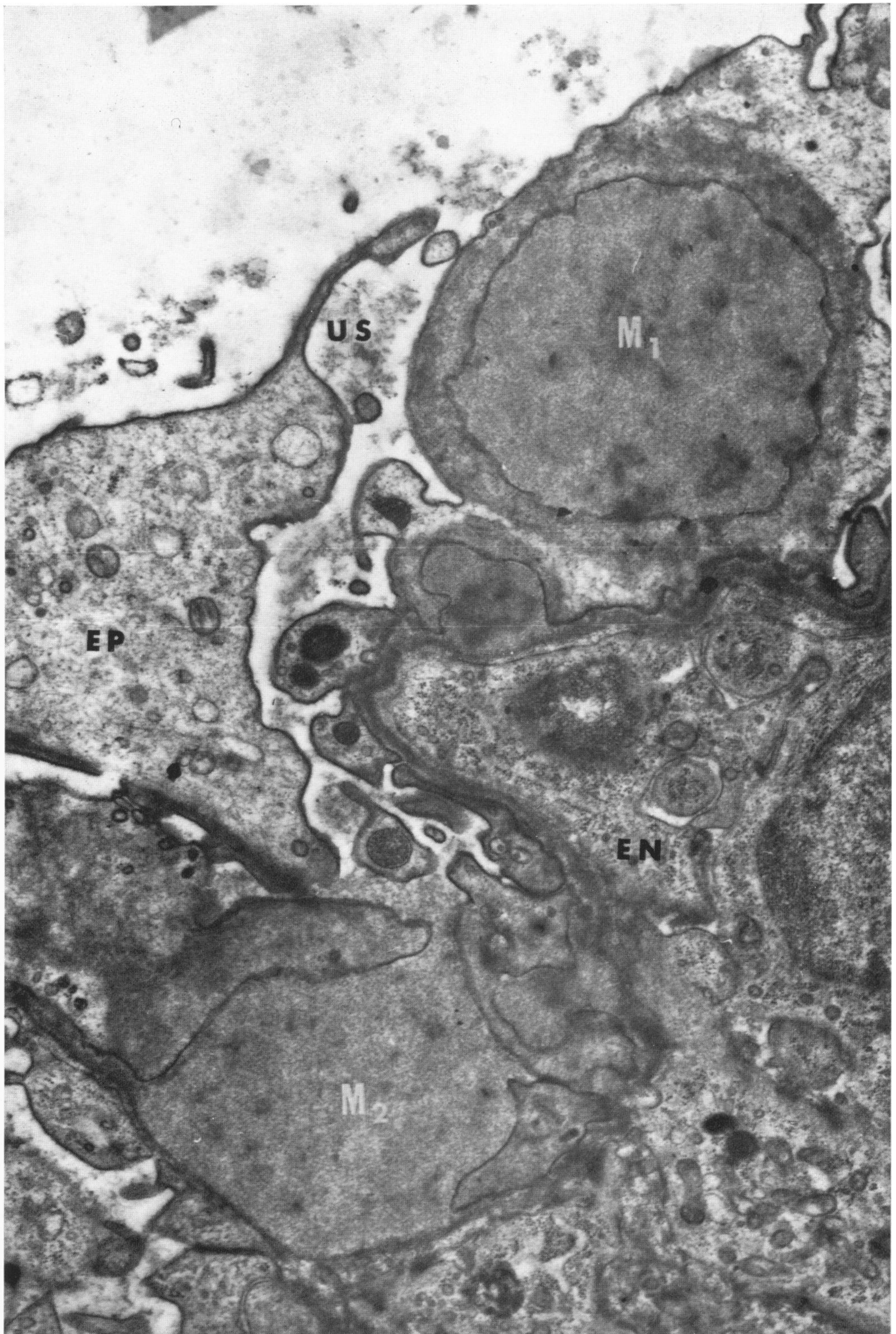


FIG. 9. Serum sickness, 3 weeks after onset. Four electron-dense basement membrane lesions are shown. One mass (M_2) appears to be communicating with the urinary space (US). × 19,700.

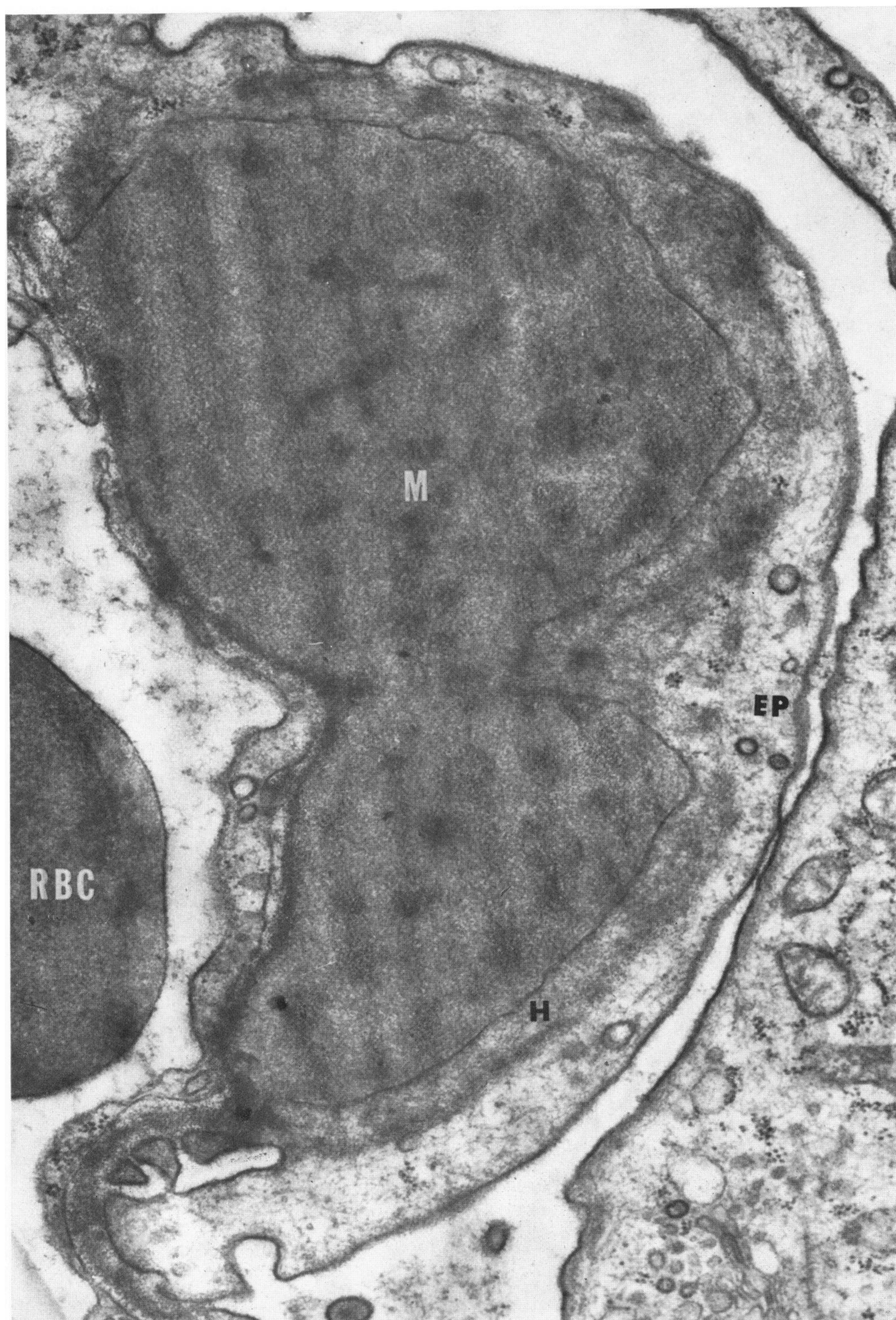
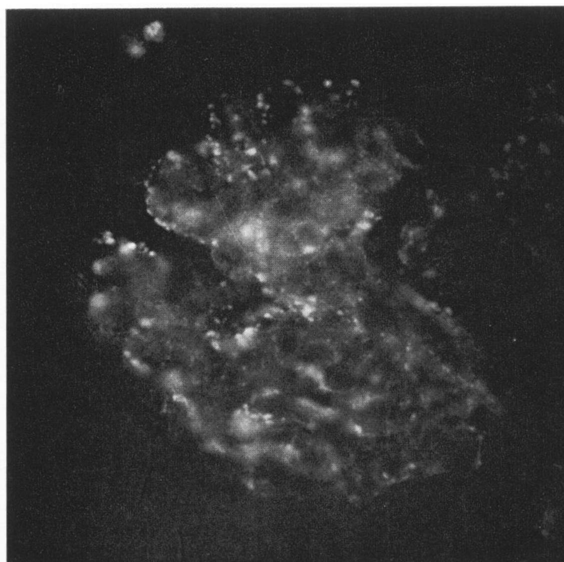


FIG. 10. A large electron-dense basement membrane lesion ($3.4 \times 2.1\ \mu$). This mass is bilobed and may possibly represent the fusion of two previously smaller masses. $\times$ 44,600.



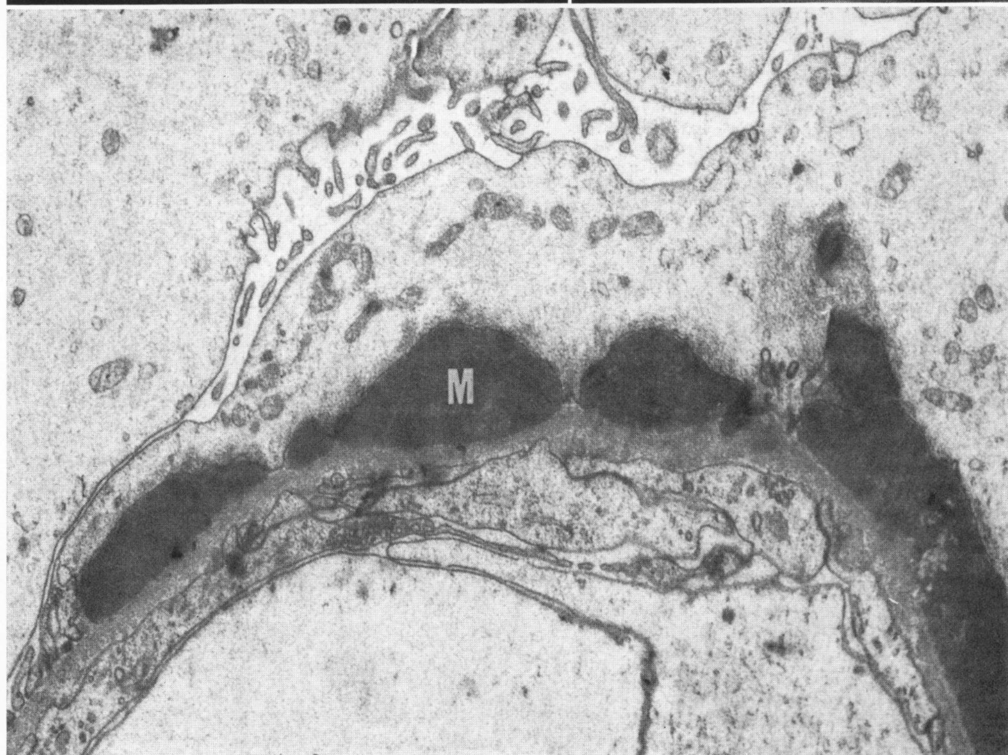


FIG. 11. Renal biopsy, patient with acute poststreptococcal glomerulonephritis. Discrete deposits of IgG are shown. Fluorescent microscopy. × 350.

FIG. 12. A segment of a glomerular capillary loop in acute glomerulonephritis. Immune deposits of IgG appear within the glomerular capillary wall. Fluorescent microscopy. × 1,800.

FIG. 13. Acute poststreptococcal glomerulonephritis. In a portion of a glomerulus prominent electron-dense 'humps' (M) appear on the epithelial side of the basement membrane. × 13,300.