PATHOGENIC FACTORS IN VASCULAR LESIONS OF EXPERIMENTAL SERUM SICKNESS*

By WILLIAM T. KNIKER,‡ M.D., and CHARLES G. COCHRANE,§ M.D.

(From the Division of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, California)

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Abundant evidence indicates that localized antigen-antibody (Ag-Ab) complexes induce inflammatory responses of increased vascular permeability, endothelial proliferation, leukocytic infiltration, and necrosis (1). Typical examples of the inflammatory reaction associated with localized immune complexes may be found in serum sickness. These include proliferative and necrotic lesions in the cardiovascular system and an acute glomerulonephritis. The observed tissue lesions have been shown (2) to follow the deposition of soluble Ag-Ab complexes. The actual damage undoubtedly results from the interaction of these complexes with many host humoral, cellular, and tissue factors. One such possible factor, the polymorphonuclear leukocyte (PMN), plays an important role in several other immunological inflammatory processes. Besides being essential for the development of vasculitis and necrosis in Arthus lesions (3–5), PMN's also are instrumental in removing Ag-Ab complexes from the damaged vessels (6). Present work indicates that the PMN plays an important pathogenic role in the development of glomerulitis and proteinuria subsequent to intravenous injection of heterologous nephrotoxic serum in rats and rabbits (7). It is also apparent that much of the vasculitis in skin following intradermal injection of heterologous basement membrane antiserum is dependent upon the presence of PMN's (8).

The serum complement (C′) system is another host factor that is involved in immunologically induced tissue damage. Both in Arthus lesions (9) and in early nephrotoxic nephritis (7), participation of at least part of the C′ system is apparently essential in bringing about accumulation of PMN's in the site of reaction.

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§ Established Investigator, the Helen Hay Whitney Foundation.

The present studies were undertaken to evaluate the role played by PMN's and C′ in the pathogenesis of the lesions seen in serum sickness.

Materials and Methods

Induction of Serum Sickness.—Male albino New Zealand rabbits weighing 1.7 to 2.7 kg were used. To produce serum sickness, bovine serum albumin (BSA) (Armour Pharmaceutical Company, Kankakee, Illinois) was injected in a single intravenous dose of 250 mg/kg body weight. The BSA was labeled with I^{131} (I* BSA) in order to follow its elimination from the circulation (10). In some animals the amount of I*BSA in the plasma that was bound to host gamma globulin (during immune elimination) was determined by the ammonium sulfate fractionation technique (11).

To enhance the immune response to I*BSA and possibly thereby to increase the number of lesions, two procedures were employed. The first was the intravenous administration of pooled rabbit anti-BSA serum in a dose of 5 mg antibody nitrogen/kg body weight given 18 hours before the I*BSA. Precipitin analysis was carried out according to the method of Heidelberger and Kendall (cited in reference 12). The second means of enhancing the immune response was the incorporation of *Escherichia coli* endotoxin with the I*BSA, using 10 μg per rabbit. With these means of enhancement, an average of 90 per cent of prepared rabbits eliminated over 99 per cent of the I*BSA within 9 to 11 days after injection. Only these were included in the study. Animals eliminating 99 per cent of injected I*BSA earlier than 9 days could not be treated long enough in the experimental regimens. Those rabbits reaching the same point in immune elimination after 11 days were not used, since they generally manifested fewer lesions and showed a diminution in the rate of elimination of I*BSA when treated.

Sacrifice and Morphological Study of Tissue.—Animals were sacrificed at the time they had eliminated over 99 per cent of the I*BSA unless noted. Specimens of cutaneous Arthus test sites, lung, kidney, spleen, and mesenteric lymph node were fixed in Bouin's solution or quickly frozen for fluorescent antibody studies. The heart was kept in the fixative until hardened, at which time 3 to 4 cross-sections were made through its base to include the coronary outflow tracts. If petechiae were found on a kidney, they were rated as few (less than a dozen), or many (over a dozen). Histologic changes in kidneys were scored by the system of Germuth (13).

Immunofluorescent Method.—The fluorescent antibody technique as described by Coons and Kaplan (14) was employed with minor modifications (6). One important modification was an initial washing of the tissue sections 30 seconds in buffered saline before fixation. Tissues were stained with the fluorescent antisera listed below. In addition, appropriate control staining of representative tissue sections was carried out (15).

Production of Antisera for Immunofluorescent Studies.—Techniques for the preparation of pooled rabbit anti-BSA serum (6), pooled sheep anti-rabbit gamma globulin serum (6), and pooled guinea pig antiserum to rabbit β1C-globulin (15) have already been described. Rabbit antiserum to sheep gamma globulin was obtained from Antibodies Incorporated, Davis, California. To obtain guinea pig antiserum to rabbit fibrin, 300 N.I.H. units of bovine thrombin (Parke, Davis and Co., Detroit) were added to 65 ml of pooled fresh rabbit plasma. After incubation at room temperature 2 hours and overnight at 4°C, the fibrin clot was teased apart and washed three times in saline, then incorporated with incomplete Freund's adjuvant (60 ml final volume). Guinea pigs received two subcutaneous injections of 2 ml, 13 days apart, and were exsanguinated 2 weeks after the second injection. The antisera were absorbed with defibrinated rabbit serum, and showed one precipitin arc in the β-1 region with rabbit plasma on immunoelectrophoresis, and no arcs with rabbit serum.

Production of Sheep Antisera to Rabbit Leukocytes.—Antisera to rabbit PMN's and lymphoid cells were prepared in sheep. The method of Cohn and Hirsch was employed to obtain PMN's for immunization (16). The final suspension of PMN's was washed, adjusted to 20,000/mm^3

in saline, and incorporated into incomplete Freund's adjuvant for immunizing sheep. Five ml were injected subcutaneously at monthly intervals for several months before serum was procured. Lymphoid cells were obtained from mesenteric lymph nodes of normal rabbits. The cells were teased from the nodes in saline, washed, and adjusted to contain 20,000 cells/mm^3; 99 to 100 per cent of these were mononuclear cells. They were injected into sheep in the same manner as that used for the PMN's. Sera were obtained after several months of immunization and were stored frozen until used.

To determine the presence of antibodies in the sheep antisera to the various rabbit blood cells, agglutination tests were performed. PMN and lymphocyte suspensions as prepared for immunization of sheep were used in the tests. Red blood cells (RBC's) and platelets were obtained by bleeding normal rabbits into siliconized tubes containing 2 per cent disodium EDTA (9 parts blood to 1 part EDTA). Platelets obtained by differential centrifugation (17) were suspended in saline-EDTA (9:1) at a concentration of 100,000/mm^3. A 0.5 per cent suspension of washed RBC's in saline-EDTA was prepared. Sheep antisera were tested for agglutinins against the various rabbit blood cells using the method of Dausset and Nenna (cited in reference 18). The antisera had high titers to all cells tested.

Purification of Sheep Antisera.—To remove cross-reacting antibodies, the sheep antisera were repeatedly absorbed with rabbit plasma, platelets, and RBC's. Anti-PMN sera also were absorbed with lymph node cells and antilymph node sera were absorbed with PMN's. Although the agglutinating titer to the homologous cell species dropped slightly, titers to the other cells were markedly reduced or abolished. The absorbed pooled antisera were fractionated in 40 per cent ammonium sulfate; after dialysis against buffered saline and addition of merthiolate 1:10,000, each globulin solution was stored at −20°C. Just before experimental use the globulin solution was ultracentrifuged in a Spinco model L centrifuge using a No. 30 head at 78,000 *g* for 2 hours; the sediment was discarded. Protein concentration, determined by the micro-Kjeldahl method (19), was in the range of 4.1 to 6.1 gm per cent. Pooled normal sheep serum (Colorado Serum Company Denver, Colo.), was fractionated and centrifuged in the same manner.

Experimental Groups.—Rabbits were divided into four groups. The first group, consisting of untreated controls, received only the serum sickness–inducing regimen. The second group, in addition, received anti-PMN globulin in a dose sufficient to depress circulating PMN's below 300/mm^3 during the final 1.5 to 3 days before sacrifice. The first dose, 6 ml intravenously and 6 ml intraperitoneally, was given within 24 hours of the onset of immune elimination of antigen. All subsequent doses were given intravenously; by the time of sacrifice a total of 25 to 40 ml of anti-PMN globulin had been administered. In some animals, mechlorethamine HCl (nitrogen mustard, HN_2), 1.75 mg/kg intravenous, was given with the initial dose of globulin. To prevent overwhelming infection, penicillin 3000 μ and streptomycin 50 mg were administered intramuscularly daily during the period of immune elimination. These antibiotics were given to animals in other experimental groups at the same time. The two other groups served as treatment controls; the third received antilymph globulin and the fourth received normal sheep globulin in doses and at intervals equivalent to the animals in the anti-PMN group. Several of the animals in groups three and four were given nitrogen mustard with their first globulin injection; those rabbits showing PMN depletion before sacrifice were discarded. No difference otherwise was noted between those receiving or not receiving HN_2 and hence, no differentiation between the two is noted. Other animals, whose immune response to I*BSA was abrogated so that they could not develop serum sickness, were placed into each of the experimental groups. The immune response in these was prevented by injections of BSA in the neonatal period (20) or by x-irradiating animals with 450 roentgen one day before injecting I*BSA (21).

Hematologic Parameters of Experimental Animals.—During the treatment period, frequent blood total leukocyte counts, differential counts, and platelet counts were obtained. Hematocrit

measurements were made before treatment was started and at sacrifice. Reversed passive Arthus tests, using 100 μg rabbit anti-BSA nitrogen in 0.1 ml, were performed on animals at various times during immune elimination. Besides providing tissue for morphologic analysis, these served as sensitive indicators of the degree of PMN suppression, the test sites of treated animals remaining negative in the gross if the PMN levels were maintained below 300/mm^3. The degree of inflammation in the Arthus sites was scored macroscopically (22) and microscopically (9) according to systems already described.

Hemolytic Complement (C′) Activity Determination.—Samples of blood were obtained in neutralized EDTA (0.01 M final concentration). EDTA was employed since circulating Ag-Ab complexes might be capable of depleting C′ activity *in vitro*. After centrifugation at 4°C, the separated plasma was stored in an acid-cleaned tube at −70°C. Specimens were assayed for hemolytic C′ activity using a micromodification of the $C'H_{50}$ test described by Osler *et al.* (23). To assay a single specimen three tubes were used. Each contained 1.5 ml of reactants of which 0.5 ml was 5×10^7 sensitized sheep cells (EA) suspended in veronal buffer with 0.1 u. heparin/ml (VB-hep). EA were prepared by incubating sheep cells (1×10^9 ml) with an equal volume of hemolysin (1:500) (Colorado Serum Company) for 30 minutes at 37°C. To the three tubes at 0°C were added 0.1 ml, 0.5 ml, and 1.0 ml of the diluted rabbit plasma being tested, with enough of the VB-hep buffer (added to the EA before the rabbit plasma) to make a final volume of 1.5 ml. Immediately before testing, each plasma sample had been diluted 1:10 in VB-hep buffer containing equal amounts of $CaCl_2$ and $MgCl_2$, which together totaled the molar concentration of EDTA in the plasma sample. A fourth tube, containing 1 ml of the diluted plasma, served as a color control. After incubation for 1 hour at 37°C, VB-hep buffer was added to bring the total volume of all tubes to 5.0 ml. After centrifugation, supernatants were read spectrophotometrically at 412 mμ. The 100 per cent lytic standard consisted of 0.5 ml EA lysed by 4.5 ml of 0.1 per cent Na_2CO_3. Under the test conditions, normal rabbit plasma yielded 100 to 150 $C'H_{50}$ units/ml.

Measurement of Proteinuria.—During the final 3 days of immune elimination, 24-hour urine samples were collected and tested for protein using the sulfosalicylic acid precipitation method (24). Significant proteinuria was defined as a loss in excess of 15 mg in one 24 hour period.

RESULTS

The Effect of PMN Depletion on Cardiovascular Lesions.—

Pathologic changes in the hearts in untreated rabbits: The cardiovascular lesions developing in the normal rabbits with serum sickness were analyzed by the histologic criteria outlined in the first column of Table I. Coronary artery lesions were concentrated at the aortic valve outflow areas and major branches, particularly at bifurcations. Endothelial proliferation, seen in two-thirds of the animals, was characterized by proliferation and swelling of endothelial cells and was accompanied by an influx of mononuclear cells and PMN's (Fig. 1). Subendothelial deposits of amorphous pink-staining material, often associated with RBC's, appeared in half of the cases. Medial necrosis, occurring in one-half of the control rabbits, included distortion and disruption of the media with aggregation of intact and disrupted PMN's (Fig. 2). These changes in the media were at all times associated with dissolution of portions of the internal elastic lamina beneath areas of endothelial proliferation (Figs. 2 and 3). This latter finding only occurred in the presence of accumulated PMN's. Most of the rabbits with

medial necrosis of arteries exhibited zones of fibrinoid deposits in markedly inflamed vessels (Fig. 2). In such cases, leukocytic and histiocytic infiltrates, edema, and fibrinoid deposits extended into adjacent tissue (Fig. 4). Endocardial proliferation was found in all animals, most abundantly in the aortic valve. Histologically, it resembled the proliferation of the arterial endothelium, and was associated with pink amorphous deposits half the time. The myocarditis seen in serum sickness was easily distinguished from the so called sporadic round cell myocarditis of rabbits, found in about a fourth of all animals. Focal lesions were found in all layers of the heart in two-thirds of the animals. The lesions adjacent to blood vessels in the myocardium were composed of aggregates of

TABLE I

Cardiovascular Histologic Lesions in Serum Sickness in Normal and Treated Rabbits

	Untreated control	Anti-PMN	Normal globulin	Antilymph
	per cent	*per cent*	*per cent*	*per cent*
Heart	(29 animals)	(23 animals)	(20 animals)	(12 animals)
Arterial endothelial proliferation	65	17	68	67
Arterial subendothelial deposits	48	17	47	42
Arterial medial necrosis	52	0	58	42
Arterial fibrinoid deposits	38	0	53	33
Endocardial proliferation	100	83	100	75
Endocardial deposits	48	39	25	50
Myocarditis	65	57	85	92
Lung (arteries)	(32 animals)	(26 animals)	(17 animals)	(8 animals)
Endothelial proliferation	78	54	88	42
Subendothelial deposits	28	35	24	25

histiocytes, lymphocytes, elongated cells resembling Antischkow's myocytes, and giant cells (Fig. 5).

Heart lesions in PMN-depleted rabbits: When serum sickness was induced in rabbits depleted of polymorphs, arterial endothelial lessions were largely inhibited, and medial necrosis and fibrinoid deposits were completely abolished, as noted in Table 1. The internal elastic lamina was always intact and clearly demarcated the endothelium from the media. However, the endocardial lesions and myocarditis occurred almost as frequently as in untreated controls. The levels of formed blood elements in these animals are presented below.

Heart lesions in control rabbits treated with normal sheep globulin and antilymph globulin: Arterial lesions were about as numerous as those found in untreated controls. Both groups manifested a higher incidence of myocarditis than controls. The antilymph animals, like the anti-PMN animals, had

a slightly but probably significantly diminished number of endocardial proliferative lesions.

Pathologic changes in pulmonary arteries of normal and treated rabbits: Lesions were found in pulmonary and bronchial arteries, ranging in size from arterioles to large muscular vessels (Fig. 6). Endothelial proliferation was more commonly found and subendothelial deposits less commonly found in pulmonary arteries than in coronary arteries of untreated controls and normal globulin treated animals. Medial necrosis was infrequently seen in those groups, and fibrinoid deposits were rare. Over half of the PMN-depleted animals exhibited endothelial proliferation, the majority of these also showing subendothelial deposits.

Immunofluorescent analysis of cardiovascular lesions: Although the number of rabbits tested was small, fluorescent antisera to BSA, RGG, and C′ revealed positive particulate granules or small clumps in severely inflamed coronary and pulmonary arteries. These deposits were limited to areas of vasculitis, where distortion and fragmentation of the autofluorescent internal elastic lamina was quite evident (Fig. 3). In severe arterial lesions of rabbits sacrificed near the time of complete antigen elimination, the amount of immune reactants was quite small. Such a diminution of observable immunologic deposits was also noted in Arthus reactions of 24 to 48 hours' duration.

Lesions in Kidneys of Normal and Treated Rabbits.—

Lesions in normal rabbits: Results are listed in Table II. A striking feature of serum sickness produced under the conditions of these experiments was the severity of the renal disease. Macroscopically the kidneys were swollen and pale; their surface was diffusely spotted with petechiae (Fig. 7). Petechiae occurred in most of the control animals. Up to two or three PMN's were often found per section of affected glomeruli, but even in those showing severe inflammation it was rare to find more than ten (Fig. 8). Most animals had tubular casts and proteinuria. The presence of casts correlated well with the degree of proteinuria observed on the final day; animals with less than 50 mg of proteinuria rarely showed casts, while those with losses over 100 mg nearly always had casts.

Lesions in PMN-depleted animals and globulin-treated controls: As compared to untreated controls (Table II), PMN-depleted rabbits exhibited fewer petechiae on the surface of kidneys. PMN's could be found in occasional glomeruli of a few animals in this group. Proteinuria was not significantly different from that in the untreated controls. Average urine volumes were significantly lower in the PMN-depleted group. The two treatment control groups that received a sheep globulin were similar to the anti-PMN group in degree of glomerular proliferation and in urine volume.

Immunofluorescent findings in the kidney: In all experimental groups BSA, RGG, and C′ were found in glomeruli of animals with more than minimal glomerular proliferation. The fluorescence, noted in a basement membrane dis-

tribution outlining the capillary loops, exhibited a finely granular pattern (Fig. 9). Fluorescent antifibrin revealed the presence of fibrin (or fibrinogen) deposited in small foci without regard for anatomical structures. This fluorescence was found as well in control groups in which the rabbits were made unresponsive to the BSA injection and in which serum sickness did not develop. In animals receiving either of the sheep antileukocyte globulins, a diffuse outlining of

TABLE II

Renal Lesions in Serum Sickness in Normal and Treated Rabbits

	Untreated controls (35 animals)	Anti-PMN (27 animals)	Normal globulin (20 animals)	Antilymph (12 animals)
Surface Petechiae				
None, *per cent*	15	48	5	42
Few, *per cent*	32	33	25	16
Many, *per cent*	53	19	70	42
Histology				
Glomerular proliferation	++-+++	+-++	+-++	+-++
Glomerular PMN's	+++	0-+	+++	++
Tubular casts, *per cent*	83	67	80	58
Urinary Findings				
Significant proteinuria, *per cent*	93	95	100	75
Average daily protein loss, *mg*	414 (17–2500)	356 (53–1289)	268 (11–1135)	194 (19–527)
Average daily volume, *ml*	89 (30–173)	65 (40–106)	73 (26–117)	64 (36–106)

all glomerular capillary basement membranes was obtained in some animals on staining with fluorescent anti-sheep globulin.

Effect of antileukocyte globulin treatment on lymphoid tissue: The anti-PMN treatment might have prevented the development of cardiovascular lesions by releasing products of cellular destruction whose inhibitory effect was not caused by PMN depletion. In both the anti-PMN and antilymph rabbits, there was marked cellular destruction in the spleen and slight diminution of lymphocytes in mesenteric nodes. Despite these alterations, the rate of I*BSA elimination was not diminished in either group. Since antilymph animals had no appreciable curtailment of cardiovascular lesions, the observed lymphoid cellular damage

in both antileukocyte groups could not have influenced the serum sickness lesions.

*Lack of serum sickness lesions in rabbits incapable of responding to I*BSA:* It was necessary to find whether the two methods employed to accentuate the immune response to I*BSA, *i.e.*, antiserum to BSA and endotoxin, and the administration of sheep antileukocyte globulin could in themselves cause tissue lesions. To determine this, rabbits unresponsive to BSA were placed in each of the experimental groups, excluding the antilymph group (Table III). Such animals did not exhibit immune elimination of I*BSA, and showed no significant

TABLE III

Studies in Animals Rendered Unresponsive to BSA

	Kidney			Heart				Lung
	Glomerular proliferation	Tubular casts	Proteinuria	Endocardial proliferation	Arterial Endothelial Proliferation	Arterial medial necrosis	Myocarditis	Arterial lesions
X-irradiated. No sheep globulins (4 animals)	0	0	0	0	0	0	0	0
Tolerant to BSA. Treated with normal sheep globulin (5 animals)	0	0	0	1*	0	0	1*	0
Tolerant to BSA. Treated with anti-PMN globulin (10 animals)	1(1+)	0	2‡	0	0	0	0	0

* One small focus of valve endocardial proliferation and rare "typical" myocarditic lesions.
‡ Single 24-hour specimens of 16 mg and 43 mg protein.

pathologic alterations of the cardiovascular and renal systems. Using fluorescent techniques, no BSA, RGG, or C′ was found in glomeruli. However, fibrin (fibrinogen) and sheep gamma globulin were found in the same patterns as noted in the experimental groups that manifested lesions of serum sickness.

Hematologic parameters and Arthus reactions in the study groups: Effects of the treatment regimens on blood elements at the time of sacrifice are presented in Table IV. In the anti-PMN animals, circulation of PMN's virtually ceased within 12 hours of institution of globulin treatment. In the other three groups, PMN levels remained above 300/mm^3 during immune elimination. Lower average total leukocyte and PMN counts, in the globulin treated groups, as compared to untreated controls, reflect the response to nitrogen mustard, given to some animals. In the antilymph group, numbers of circulating lymphocytes were

quite low. Not recorded in the table are the platelet counts which ranged from 1×10^6/mm³ down to 75,000/mm³ in each group without appreciable differences being noted. In the three treated groups, hematocrit levels fell about 25 per cent on the average.

TABLE IV

Hematologic Parameters at Time of Sacrifice

	Untreated control	Anti-PMN	Normal globulin	Antilymph
		Blood count /mm³		
Total leukocytes	9216 (3300–26,620)	2030 (110–9790)	6670 (990–21,780)	3100 (550–25,080)
PMN's	4470 (655–11,200)	112 (0–317)	2535 (292–11,700)	1769 (286–11,030)
Lymphocytes	4286 (1720–14,920)	1745 (110–8400)	3934 (850–14,700)	1160 (297–14,070)
Monocytes	276 (0–1125)	122 (0–979)	134 (0–408)	62 (0–290)
Eosinophiles	46 (0–532)	None	None	31 (0–119)
Basophiles	138 (0–745)	51 (0–549)	67 (0–218)	78 (0–114)
		Average hematocrit		
Hematocrit value	40 (28–49)	28 (13–43)	29 (21–35)	30 (11–37)
		Reverse passive Arthus reactions		
Gross edema, hemorrhage	3 − 2+	1+ − 0	3 − 1+	3 − 1+
Histologic edema, vasculitis	3 − 2+	1+ − 0	3 − 1+	3 − 1+
Local BSA, RGG, C′	+	+	+	+

Arthus sites were placed on the rabbits each day during treatment to reflect the effectiveness of PMN depletion. Their macroscopic appearance on the day of sacrifice is shown on Table IV. When the PMN count remained below 300/mm³, no macroscopic Arthus lesions developed. Variability of inflammation found in the non-PMN–depleted groups terminally was due to differences in levels of plasma I*BSA that were able to combine with intradermally injected

anti-BSA, and in the number of circulating PMN's. By immunofluorescence BSA, RGG, and of special note, C′ were localized in Arthus sites of animals in all study groups.

Serum complement activity: In untreated controls and normal globulin treated animals there was a wide variation in serum hemolytic C′ activity at the time of sacrifice as compared to activity present before the onset of immune elimination. Per cent loss of $C'H_{50}$ units ranged from 0 to 96, the average being 57 per cent. Animals in the anti-PMN and antilymph groups had greater losses than

TABLE V

Incidence of Lesions in Rabbits Sacrificed at Different Times During Immune Elimination of I BSA*

	3.7 to 1.2 per cent remaining 1* BSA (15 animals)	Less than 1 per cent remaining 1* BSA (49 animals)
Cardiac findings		
Arterial endothelial proliferation, *per cent*	100	67
Arterial subendothelial deposits, *per cent*	71	48
Arterial medial necrosis, *per cent*	93	54
Arterial fibrinoid deposits, *per cent*	73	45
Endocardial proliferation, *per cent*	100	100
Endocardial deposits, *per cent*	69	38
Myocarditis, *per cent*	76	73
Renal findings		
Surface petechiae, *per cent*	33	90
Glomerular proliferation	+-++	++-+++
Significant proteinuria, *per cent*	43	96
Average daily proteinuria, *mg*	41	354

the untreated rabbits developing serum sickness, the average loss being 85 per cent with a range of 50 to 94 per cent. The range was comparable in these two groups, and overlap of $C'H_{50}$ values between these rabbits and the untreated or normal globulin treated rabbits was frequent.

Incidence of lesions at various times during immune elimination: Data presented in Tables I and II concerned animals that had eliminated I*BSA within 9 to 11 days. Untreated animals whose rate of immune elimination suggested that they would completely eliminate I*BSA within the same period were sacrificed earlier in immune elimination. Data comparing the incidence of certain lesions at two stages of elimination is presented in Table V. Animals with over 1 per cent I*BSA still circulating, sacrificed 7 to 9 days after antigen injection, had significantly greater numbers of arterial lesions. Indeed, in results not tabulated, florid cardiovascular lesions could be found very early in immune elimi-

nation, 6 days after I*BSA administration. The maximal incidence of cardiovascular lesions coincided with peak levels of circulating BSA–anti-BSA complexes (1.5 to 4.0 per cent of injected I*BSA), found on the 6th and 7th day after I*BSA injection. Conversely, renal changes were minimal early in immune elimination, becoming maximal at the time of complete elimination of antigen.

DISCUSSION

An essential role played by the polymorphonuclear leukocyte (PMN) in the development of vascular inflammation in serum sickness is strongly suggested by these studies. Fifty-two per cent of control rabbits developed necrotic arterial lesions, and in 38 per cent, there were associated fibrinoid deposits. Animals devoid of circulating PMN's did not show necrosis or fibrinoid changes in arterial and venous walls. Arterial endothelial proliferation was inhibited also, but to a lesser degree, suggesting that some reaction to the localized antigen-antibody complexes occurred despite the absence of PMN's.

The data also suggest that one of the key structures of the vessel wall attacked by the PMN's was the internal elastic lamina. Necrosis of the media of arteries in control rabbits was almost invariably associated with a fragmentation of the internal elastic lamina (Fig. 2) and an accumulation of PMN's. Indeed, in zones of arterial endothelial proliferation in which few or no PMN's were present, rupture of the internal elastic lamina did not occur and inflammation did not extend into the medial layer of the artery. Furthermore, in PMN depleted rabbits, the internal elastic lamina beneath areas of arterial endothelial proliferation never showed evidence of disruption. In results to be reported at a later date, it was found that carbon injected intravenously shortly before sacrifice into rabbits with serum sickness localized in the arterial lesions. The carbon passed into the intimal layer and apparently stopped at the lamina elastica. If PMN-associated areas of disruption were present in the lamina, the carbon passed through these into the medial layer. These data, then, suggest a progressive sequence initiated by the deposition of antigen-antibody complexes in the intima of arteries. Complement is activated and PMN's enter the reaction. These cells, in a manner not yet clear, bring about focal destruction of the lamina elastica and the inflammation then progresses into the medial layer of the artery. In the Arthus reaction and early nephrotoxic nephritis, complement binds to localized immunologic reactants, and liberates an activated C′5 to C′ 6 complex which is strongly chemotactic for PMN's (25). Damage to basement membrane structures then ensues (7, 8).

Control studies in which rabbits were treated with normal sheep globulin or anti-rabbit lymph node globulin indicated that the inhibition of cardiovascular lesions in the PMN-depleted rabbits was a specific effect. That the treatment regimens did not in themselves induce pathologic alterations was demonstrated

in rabbits rendered incapable of responding immunologically to BSA by inducing tolerance to BSA at birth or by X-irradiation. Such animals receiving the normal globulin or anti-PMN treatment were free of significant vascular and renal lesions.

The endocardial, myocardial, and pulmonary arterial lesions were not affected by PMN depletion. In untreated or treated animals, necrosis in these lesions is rarely seen and PMN accumulation is sparse. While the pathogenic mechanisms in these three lesions may differ from those of the cardiac arterial lesions, definitive evidence is not at hand. In addition, no significant effect of PMN depletion was noted on the renal alterations with the exception of the petechiae noted macroscopically. Proteinuria was not inhibited. However, these results are not conclusive, since it was not possible to effect PMN depletion before the onset of immune elimination without diminishing the immune response to I*BSA. Hence, any PMN activity in the glomeruli in the earliest stages of serum sickness could not be prevented. It has been shown that the action of PMN's in glomeruli during the first hours of nephrotoxic nephritis was sufficient to cause persistent damage (7).

By immunofluorescent techniques, antigen (BSA) and host antibody (RGG) were found localized in affected glomeruli in the form of discrete granules or clumps, as noted previously (2). Using a modification of the fluorescence method, these immune reactants were seen distributed along the basement membrane. By electron microscopy, alterations in the glomerular basement membrane and occasional small deposits within it have been described in serum sickness (26). Whether these changes are related to the presence of immune reactants has not been determined. Host β1C-globulin or complement (C′) was localized with the immune reactants both in glomerular and arterial lesions. This finding suggests the participation of C′ in the pathogenesis of vascular lesions. Recent studies indicate that C′ attracts PMN's to Ag-Ab complexes both *in vivo* (7, 9) and *in vitro* (25). Whether such a mechanism exists in the PMN-dependent lesions of serum sickness is not known. Attempts to determine this role of C′ would be difficult, although efforts have been exerted in this direction (27).

Fibrin (or fibrinogen) is not deposited in the pattern typical for the immune complexes, but rather in an amorphous fashion in various glomerular, tubular, and vascular renal structures. Since these deposits also appear in kidneys of animals without serum sickness, a significant role for fibrin in the pathogenesis of the glomerulitis cannot be evinced. Administration of sheep antiserum to rabbit leukocytes is associated with deposition of sheep gamma globulin diffusely on glomerular basement membranes in a smooth, linear fashion. This could represent an antibody to leukocytic antigen(s) which cross reacts with renal basement membrane. This phenomenon does not in itself induce significant glomerular changes or proteinuria.

When serum sickness was induced by a single injection of I*BSA, only 30 to 50 per cent of rabbits eliminated the antigen before 12 days and developed typical lesions. To augment the immune response to I*BSA, endotoxin and pooled rabbit antiserum to BSA proved effective individually. The combined use of both enhancing methods increased the number of vigorously responding animals to 80 to 100 per cent. Although the character of the lesions was not changed, augmentation in the numbers of lesions and their severity was pronounced. Particularly enhanced were the lung arterial lesions, pancarditis, and renal petechiae.

It is evident the cardiovascular lesions in serum sickness appear early in immune elimination and are maximal at a time when peak levels of Ag-Ab complexes circulate. Yet the renal pathologic changes are most pronounced days later at the completion of elimination. An explanation of this dichotomy would be illuminating as regards differences in pathogenesis of vascular *versus* renal lesions. Possible reasons for the dichotomy might relate to size of circulating complexes, quality of antibody in the complexes, variations in kinds and amounts of local mediators, etc.

SUMMARY

The present studies suggest that polymorphonuclear leukocytes (PMN's) are essential for the development of cardiovascular lesions in serum sickness. In the absence of PMN's, necrotic vascular lesions were never seen and endothelial proliferation in arteries was inhibited. Zones of fibrinoid deposits did not occur. By contrast, at least two-thirds of the control animals exhibited endothelial proliferation, and half had necrosis of arterial walls, usually with fibrinoid deposits. In arterial lesions that involved the intima and media, the internal elastic lamina was disrupted. This was associated with accumulations of PMN's and was prevented when PMN's were depleted. The observations suggested that the elastic lamina acts as a barrier to the outward spread of inflammation in arteries and that it is an important substrate of PMN action. Although glomerulitis and proteinuria developed in PMN-depleted animals, no conclusions could be drawn concerning the pathogenic role of PMN's in renal lesions, since PMN depletion could not be effected before the onset of immune elimination without influencing the immune response itself.

Host complement (β1C-globulin) was localized along with the antigen and rabbit gamma globulin in glomeruli and arteries showing lesions. In the glomeruli these deposits formed a granular lining along the area of the basement membrane. In arteries the fluorescent amorphous particles were in the intima and media of inflamed vessels.

The immune response to BSA and the incidence and severity of cardiovascular and renal lesions were enhanced by the intravenous administration of pooled rabbit antiserum to BSA given 18 hours before BSA antigen and by injecting

endotoxin along with the BSA. These additions to the usual procedure of inducing serum sickness did not appear to change the quality of the disease.

In normal rabbits, the peak incidence of cardiovascular lesions was early in immune elimination of antigen, at a time when levels of circulating complexes was maximal. Conversely, the severest renal injury was noted several days later, at the completion of immune elimination.

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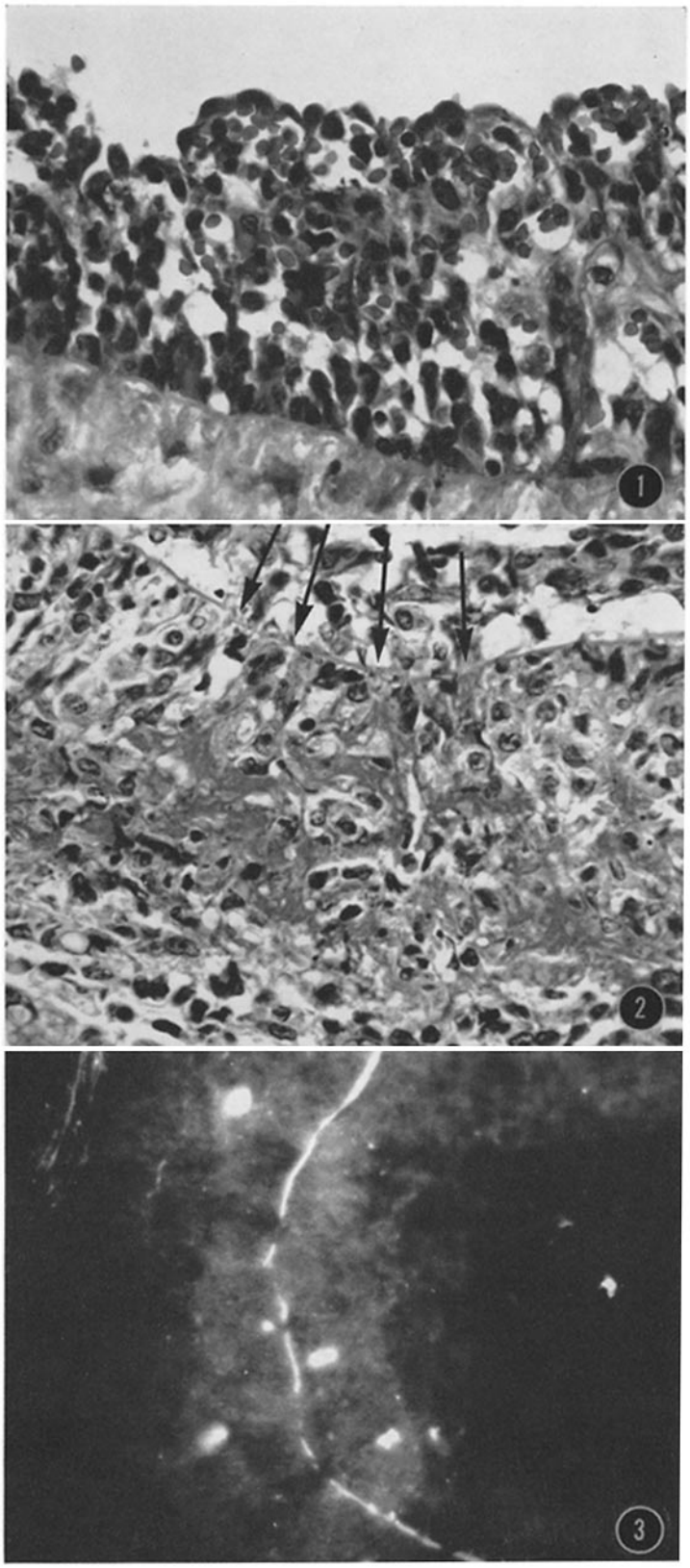
EXPLANATION OF PLATES

PLATE 12

FIG. 1. Arterial endothelial proliferation with aggregates of histiocytes, PMN's, RBC's, and deposits of amorphous pink-staining material. The internal elastic lamina is intact and the media is normal. Coronary artery, hematoxylin-eosin × 500.

FIG. 2. Arterial inflammation with endothelial proliferation (top) and medial necrosis, associated with aggregates of intact and disrupted PMN's, and fibrinoid deposits (bottom). Several breaks in the internal elastic lamina are marked by arrows. Fibrinoid deposits and disruption of the internal elastic lamina are always associated with infiltrates of PMN's. Coronary artery, hematoxylin-eosin × 400.

FIG. 3. Severely inflamed artery, section stained with fluorescent anti-BSA. The bright linear structure is the autofluorescent internal elastic lamina, which is fragmented at many points. (Compare with Fig. 2, showing a similarly involved vessel.) The faint granular fluorescence in the intima (right) and the media (left) is due to the presence of residual BSA–anti-BSA complexes. The globular spots of bright fluorescence in the vessel wall are due to autofluorescence in eosinophiles. Coronary artery, × 400.



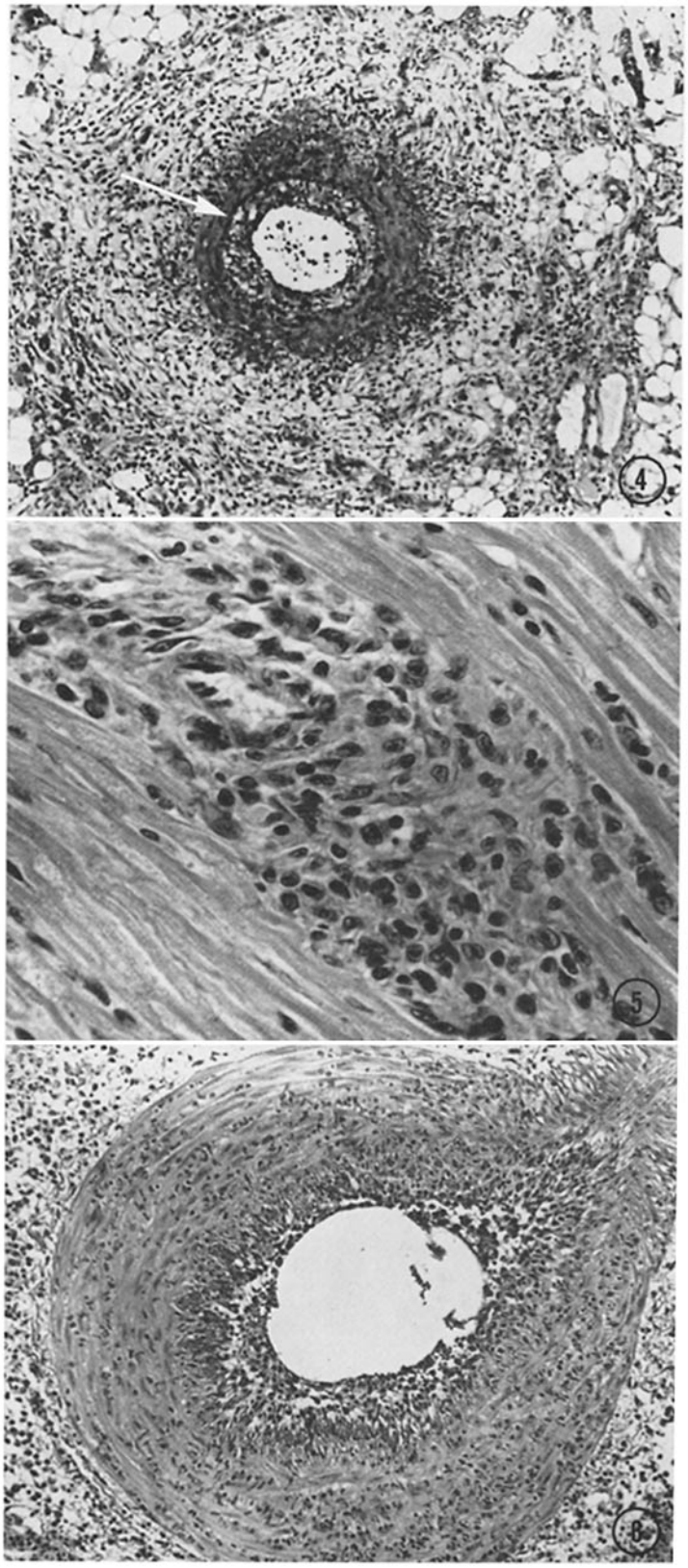
(Kniker and Cochrane: Vascular lesions of experimental serum sickness)

PLATE 13

FIG. 4. Severe inflammatory involvement of artery, involving all layers. Arrow marks internal elastic lamina which is diffusely fragmented. Leukocyte infiltration, edema and fibrinoid deposits extend far into epicardial tissue. Coronary artery, hematoxylin-eosin × 85.

FIG. 5. Focal myocarditis lesion surrounding venule. Mononuclear cells and spindle cells with elongated nuclei, similar to Anitschkow's myocytes, predominate in this example. Myocardium, hematoxylin-eosin × 350.

FIG. 6. Endothelial proliferation with intimal amorphous deposits and early medial necrosis without fibrinoid deposits in medium-sized pulmonary artery. hematoxylin-eosin × 150.



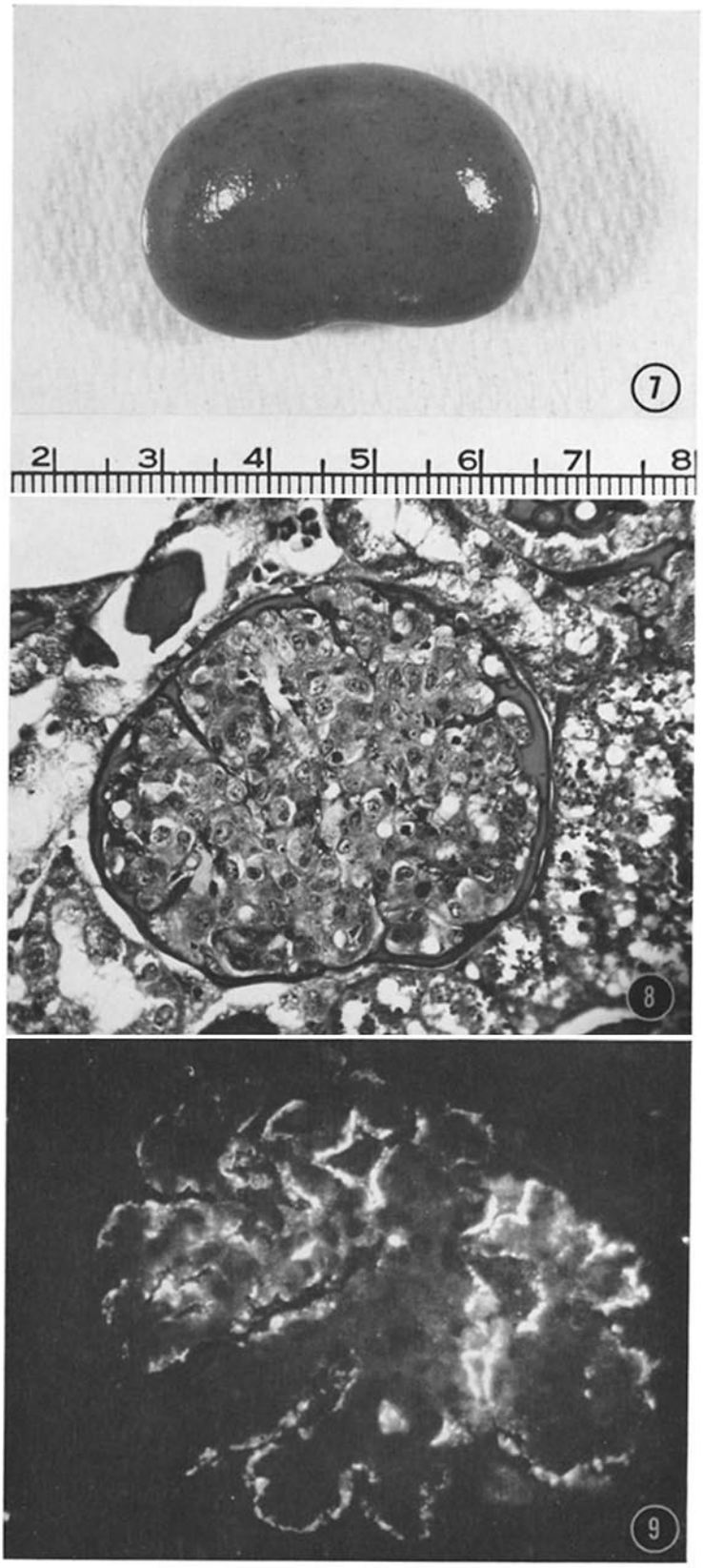
(Kniker and Cochrane: Vascular lesions of experimental serum sickness)

PLATE 14

FIG. 7. A kidney whose surface is diffusely covered with petechiae, from a rabbit with serum sickness. Centimeter scale on figure.

FIG. 8. Severe glomerulitis (3+) in a rabbit with serum sickness. Intense endothelial cell proliferation has closed the capillary lumens of the tufts and led to marked swelling of the glomerulus. Proteinaceous fluid fills Bowman's space and a tubular cast is present (top). A few PMN's can be recognized in the glomeruli. Hematoxylin-eosin × 450.

FIG. 9. A glomerulus of a rabbit with serum sickness; the glomerulitis was graded severe (3+) histologically. Stained with fluorescent anti-RGG bright granular deposits outline the capillary tufts in a basement membrane pattern. By fluorescence, BSA and rabbit C′ also can be found deposited in an identical fashion. × 500.



(Kniker and Cochrane: Vascular lesions of experimental serum sickness)