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Relationship of Circulating Antigen-Antibody Complexes, Antigen Elimination, and Complement Fixation in Serum Sickness.*† (24304)

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The symptoms and lesions of both clinical and experimental serum sickness usually develop during rapid immune elimination of antigen(1-5). In rabbits, this immune elimination of antigen is associated with circulating antigen-antibody complexes(6,7) and with a fall in serum complement(8,9). The present serologic and histologic studies were undertaken to find whether the level and persistence of the complexes, in the circulation, and/or magnitude of the fall in complement could be correlated with extent of the associated morphologic manifestations of serum sickness.

Materials and methods. Bovine serum albumin (BSA), Armour and Co., Lot No. P67908 was trace-labelled with I^{131} (I*) according to the method described by Talmage *et al.*(10). Twenty-six albino rabbits, weighing approximately 3 kg, were injected intravenously with 250 mg of I*BSA/kg and bled (2.5-3.0 ml sera) every 1 to 2 days starting on either the first or third day following the injection. The sera were analyzed for total I*BSA, I*BSA bound to globulin, and complement. Total I*BSA activity was determined by counting the protein-bound I* in aliquots of sera, following trichloroacetic acid precipitation. These counts were converted to % of injected I*BSA remaining in the total blood volume(10). All samples contained sufficient counts to insure a maximum counting error of less than 5%. I*BSA present as a circulating I*BSA-anti BSA complex was determined by the ammonium sulfate technic of Farr(11). Aliquots of sera were diluted with equal volumes of 0.15 M NaCl and the globulin of these mixtures was precipitated at 37°C with equal volumes of saturated ammonium sulfate. The precipitates were washed twice with 50% saturated ammonium sulfate and were counted for I*BSA activity. The counts were converted to % of injected BSA which was bound to the total serum globulin. The hemolytic activity of the sera (CH_{50}units) was determined by the method of Osler *et al.*(12). All animals which showed an immune elimination of the I* antigen before the sixteenth day following injection were sacrificed 1 day after antigen was eliminated. With 2 exceptions, all other animals were sacrificed on the 16th or 17th day. One rabbit which showed no immune elimination of antigen was sacrificed on the 14th day following injection of the antigen. Another rabbit which did not show an immune elimination until the 18th day was sacrificed one day later. Tissues obtained at autopsy from all animals were fixed in formalin, embedded in paraffin, cut at 5 μ and stained with hematoxylin and eosin for microscopic examination. From every animal the tissues taken for section in-

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cluded one or more blocks from each kidney and at least 2 blocks transecting the base of the heart, including major coronary vessels, cardiac valves, and atrial and ventricular en-

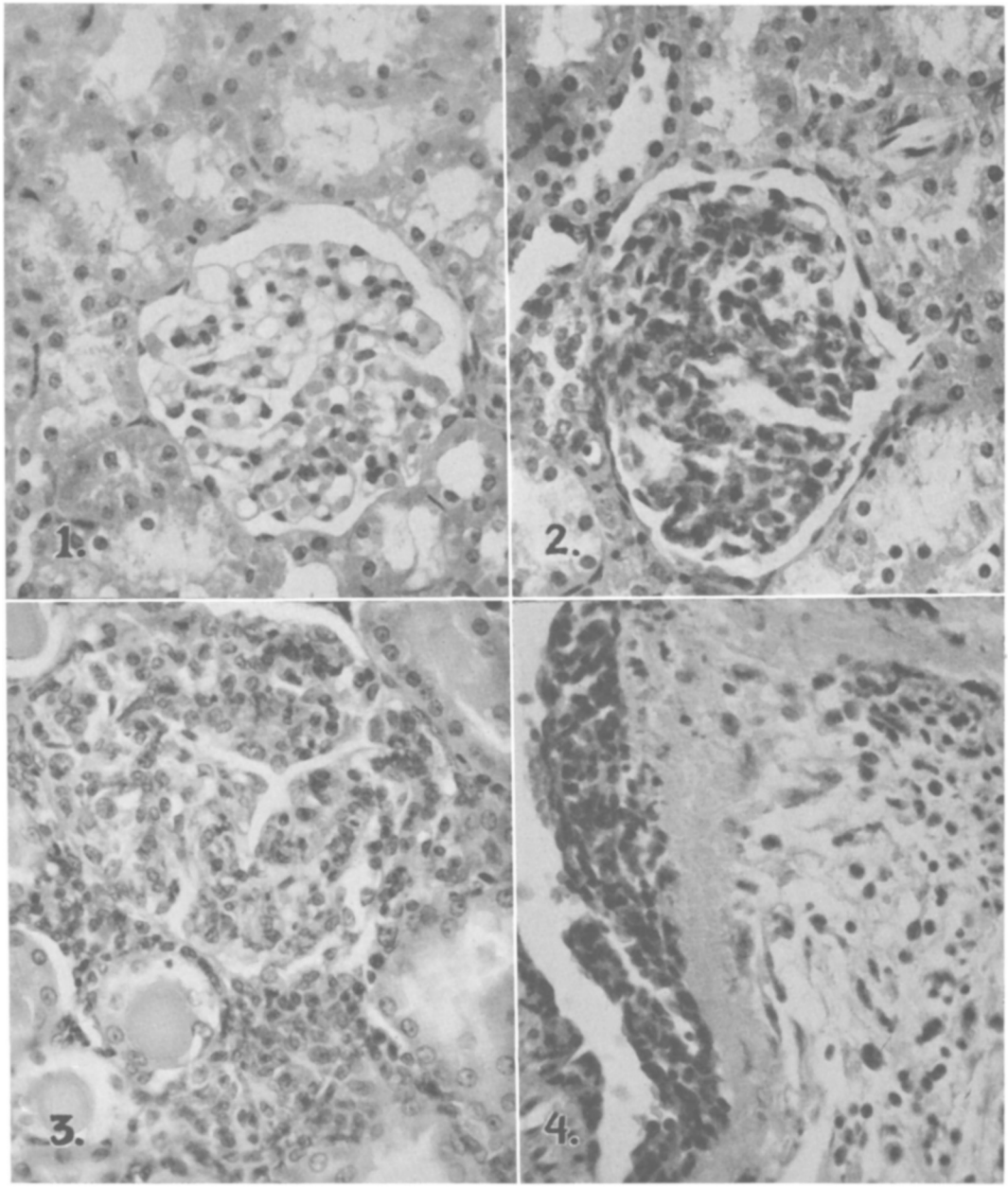

FIG. 1. Normal rabbit glomerulus.

FIG. 2. Glomerulus from rabbit 5151 which had circulating complexes without accelerated elimination of antigen. This is an example of moderate glomerulitis with an increased number of endothelial and epithelial cell nuclei and scarcity of patent glomerular capillaries. Same magnification as Fig. 1.

FIG. 3. Glomerulus from rabbit 5270 showing 3+ glomerulitis. Marked proliferative changes making the glomerulus virtually bloodless. Same magnification as Fig. 1.

FIG. 4. Base of mitral valve leaflet from rabbit 5151. Note proliferation of endocardium and inflammatory infiltrate in substance of valve.

docardium. Sections of these tissues were studied to determine incidence and degree of glomerulonephritis, arteritis, and endocarditis. In accompanying Table: + glomerulonephritis indicates a mild hypertrophy and hyperplasia of endothelial and epithelial cells; ++ glomerulonephritis involves most glomeruli and consists of moderate hypertrophy and hyperplasia of glomerular cells with occlusion of many of the capillaries (Fig. 2); +++ glomerulonephritis involves virtually all glomeruli with marked hypertrophy and hyperplasia of endothelial and epithelial cells leading to overall glomerular enlargement, avascularity and obliteration of the capsular space with or without evidence of glomerular hemorrhage (Fig. 3); and ++++ includes the previous changes plus evidence of focal glomerular necrosis. Arterial changes are graded from + to ++++, depending upon amount of intimal reaction, vascular necrosis, and inflammatory exudate, with + lesions showing only swelling of intima and slight focal inflammatory exudate and ++++ lesions showing almost complete fibrinoid necrosis of vessel walls and extensive inflammatory reaction. Endocarditis usually consisted of mononuclear infiltration of mural or valvular endocardium, proliferation of endocardial lining cells and occasional foci of subendocardial fibrinoid necrosis (Fig. 4). Glomerular alterations have been used as a basis for correlation of morphologic and serologic developments in these rabbits. The characteristic proliferative change in glomeruli is the most frequent morphologic manifestation of serum sickness. Since serum sickness affects the kidneys more or less generally, the study of at least 1 complete cross section of each kidney would seem to afford an ade-

TABLE I. Histologic and Serologic Changes in Rabbits Injected with 250 mg BSA per kg.

Rabbit No.	Day of antigen elimination	Lesions of serum sickness: Glomerulonephritis	Arteritis	Endocarditis and valvulitis	Decrease in serum complement (% CH_{50} units)	Maximum BSA in complex form*
5271	11	++	++++	+	67	1.4
5276	"	++++	++++	+	65	1.7
5150	12	+++	+	++		1.3
5261	"	++	++++	++		1.9
5270	"	++++	++++	++	78	1.0
5277	"	++++	0	+		.9
5153	13	++	0	++		1.5
5258	"	++	++	+	78	1.4
5263	"	++++	+	++	71	2.1
5264	14	0	+	0	66	1.5
5152	15	0	0	0		1.7
5259	"	++	0	0	72	1.5
5262	"	++++	++	++	83	1.9
5265	"	0	0	0	66	1.6
5272	"	+++	0	0	61	1.6
5273	"	++	0	0		.4
5274	"	+++	0	0	71	1.2
5154	17	+	0	0		.5
5149	18	0	0	0		1.7
5268	†	0	0	0		.0
5269	†	0	0	0		1.1
5275	†	0	0	0		.7
5151	‡	++	0	++	0	3.5
5260	§	+	0	0	0	.5
5266	§	++	0	0	0	.7
5267	§	0	0	0	0	1.7

* % of total BSA inj.

† Antigen being eliminated by immune mechanism, but not completely removed at time of sacrifice, day 16.

‡ No detectable immune elimination of antigen, sacrificed day 14.

§ *Idem*, sacrificed day 16.

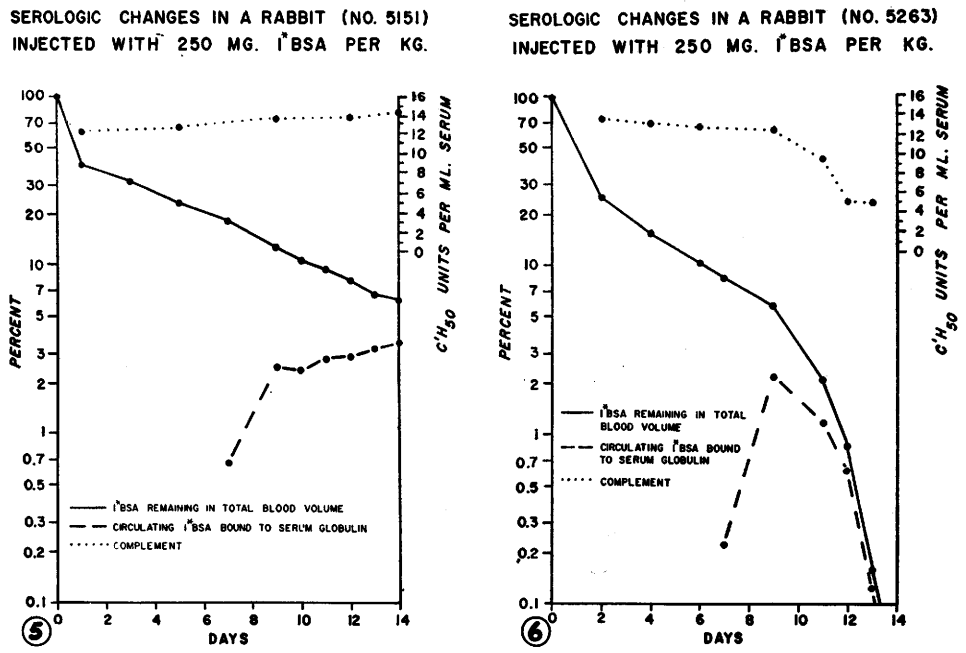

FIG. 5 & 6.

quate histologic sample of renal tissue. Arterial and to a lesser extent endocardial lesions, tend to be focal and their incidence is difficult to quantitate even on the basis of numerous histologic sections. However, in spite of this limitation, the severity of renal, vascular, and endocardial lesions recorded in the table are in fair agreement.

Results. Both histological and serological results are given in the accompanying Table. As reported(13), the most severe renal and arterial lesions developed in rabbits eliminating antigen earliest. However, of the 4 rabbits showing no immune antigen elimination, 2 (5151 and 5266) had significant glomerulitis (Fig. 2) and 1 (5151) had an extensive endocarditis and valvulitis (Fig. 4).

Since all animals (with the exception of rabbit 5268) had considerable amounts of circulating I*BSA-anti BSA complexes, no correlation could be made between presence or absence of circulating complexes in the sera and development of lesions of serum sickness. However, the animals possessing the higher levels of complexes in their sera had a tendency to develop the more severe lesions (Table). Antigen-antibody complexes often appeared in the sera a day or more before there was an increase in rate of elimination of the antigen, and in some animals they continued to circulate in the blood for a considerable time without an associated immune elimination of the antigen. The 4 rabbits which showed no immune elimination of antigen possessed antigen-antibody complexes in their sera for periods of 6 to 8 days. Forty %, or more, of the circulating I*BSA present in these rabbits was free, and not in the form of antigen-antibody complexes. Fig. 5 gives data obtained from serum of rabbit 5151, which had no immune elimination of the I*BSA, but at one time had over 50% of the circulating I*BSA bound to globulin (antibody), and developed glomerulonephritis and endocarditis. Fig. 6 shows data from serum of rabbit 5263, which had a complete immune elimination of antigen on day 13. This rabbit had a considerable portion of antigen bound in the form of antigen-antibody complexes during the immune elimination, and on autopsy it had glomerulonephritis, arteritis, and endocarditis.

The complement levels declined in all animals showing an immune elimination of antigen (Table). This decline in complement accompanied, but never preceded, immune

elimination of antigen, even when a large amount of antigen-antibody complex was present in the sera (Fig. 5 and 6). However, this utilization of complement was not correlated with development of serum sickness in all animals. Rabbit 5265 showed a fall in complement without the appearance of lesions, on observation similar to that of Moll and Hawn(9). Rabbits 5151 and 5266 developed lesions without showing a fall in serum complement levels.

Discussion. On the basis of present evidence, it would seem that while antigen-antibody complexes are probably involved in pathogenesis of serum sickness(6,13), they do not, by themselves, always induce typical lesions of this disease. Almost all rabbits respond to large injections of BSA by making antibodies capable of forming complexes with the antigen, but all animals do not develop lesions. This failure of complexes *per se* to induce lesions is in line with the difficulty experienced in producing the typical morphologic picture of serum sickness by prolonged perfusions of rabbits with soluble antigen-antibody complexes in amounts comparable to those found in active serum sickness(13). In mice repeated large injections of BSA-rabbit anti BSA complexes have given more consistent lesions of kidneys and cardiovascular systems as reported by Benacerraf, Germuth and Pollack have been able to induce arterial and to a lesser degree renal lesions by passive transfer of anti-BSA to rabbits previously injected with the specific antigen(14). It was postulated that these lesions may have been the result of complexes which formed when the transferred antibody reacted with excess circulating antigen. However, the complexes formed under these conditions may differ greatly from those formed in active serum sickness, *e.g.*, the antibody transferred by Germuth and Pollack was obtained from hyperimmune rabbits, whereas, the antibody in active serum sickness usually results from a primary antibody response. The fact that the kind of complexes formed in active immunization varies from animal to animal(7), probably as a result of differences in quantity and quality of antibody formed, may in part explain the variability in lesions among animals. It also may be that antigen-antibody complexes are only one of several elements essential in pathogenesis of serum sickness.

Those animals in which complexes form and are eliminated earliest tend to have the most severe lesions of serum sickness. However, this relationship is not absolute and in 3 animals with relatively early elimination of antigen, there were few, if any, lesions; and in 2 animals not eliminating the complexes, there were lesions. The demonstration of circulating complexes was the only serologic evidence of an immune response in these latter animals. In a few instances, Hawn and Janeway(4) observed serum sickness in humans and rabbits in the absence of an immune elimination of antigen from the blood or other evidence of an antibody response. It may have been that in these cases the host had responded with antibodies capable of forming pathogenic complexes which were not eliminated from the blood.

An insufficient production of antibody might be responsible for the ability of antigen-antibody complexes to exist, in the circulation, without being rapidly eliminated. It appears that the first antibody synthesized combines with the circulating antigen in great antigen excess and that the resulting complexes are not rapidly eliminated. This is evidenced by the presence of a considerable level of complexes in the circulation for several days prior to immune elimination of antigen in most of the rabbits. As additional antibody is synthesized, it probably combines with both the free antigen and the antigen in the complexes. A continuation of antibody synthesis would result in formation of larger complexes and, possibly, even aggregates of complexes. When complexes or aggregates of complexes reach a certain size they are probably removed from the circulation by phagocytic cells(15). In the rabbits, which possessed circulating complexes, but did not have an immune elimination of antigen, total amount of antibody produced might have been too small to permit completion of this process.

Serum complement did not appear to play a

consistent role in pathogenesis of serum sickness in the present experiment. Reduction of circulating complement has been associated with immune elimination of antigen and occurrence of glomerulonephritis in animals injected with large amounts of foreign protein (8,9). In our animals, too, complement levels declined during immune elimination of antigen. Initiation of the drop in level of circulating complement always coincided with the beginning of immune elimination of antigen. That the fall in complement levels depends upon more than interaction between antigen and antibody is evident in those animals with circulating complexes, but without a fall in complement or an immune elimination of antigen. It cannot be determined by the present data whether immune elimination might cause fixation of complement or whether fixation of complement, to circulating complexes, might result in immune elimination. Whatever the relationship between complexes and complement, utilization of serum complement was not always correlated with development of lesions of serum sickness. One rabbit showing a definite fall in complement, associated with immune elimination of antigen, had no detectable morphologic lesions. On the other hand, the development of lesions occurred without measurable participation of serum complement in 2 rabbits.

Summary. Neither the level of antigen-antibody complexes in the serum nor amount of complement utilized during the responses of rabbits to large injections of BSA could be correlated consistently with either the development or severity of the lesions of serum sickness. Almost all rabbits responded to large injections of BSA by making antibodies capable of forming complexes with circulating antigen yet only ⅔ of them developed significant lesions. On the whole those animals making the earliest responses and eliminating the complexes most rapidly had the most severe lesions; but some animals in which complexes were not eliminated rapidly from the blood also developed lesions. Likewise complement did not appear to be utilized in some rabbits which developed lesions and in some rabbits showing a fall in complement no lesions were found. Thus, it would appear that either there are other factors in addition to complexes and complement which are essential in the pathogenesis of serum sickness or that the complexes vary in their properties from animal to animal and that some of these complexes are more pathogenic than others.

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