A COMPARATIVE HISTOLOGIC AND IMMUNOLOGIC STUDY IN RABBITS OF INDUCED HYPERSENSITIVITY OF THE SERUM SICKNESS TYPE*

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Plates 14 to 19

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In the present study, attention has been directed toward the relationship between the tissue alterations of experimental hypersensitivity and the immunologic response produced by the injection of foreign protein.

The histologic changes in animals made hypersensitive to foreign protein have been extensively studied. Klinge (1) and Vaubel (2) were among the first to describe lesions attributable to hypersensitivity and they noted the similarity of these lesions to those of rheumatic fever and polyarteritis nodosa. Later, Rich and Gregory (3–6) amply confirmed and extended these observations. The demonstration by these workers that a single intravenous injection of a foreign protein (horse serum) can produce marked cardiovascular lesions in rabbits greatly facilitated studies in this field. Recently, Hawn and Janeway (7) made the important observation that lesions resulting from hypersensitivity could be induced in rabbits by the injection of highly purified proteins such as either crystallized bovine albumin or bovine gamma globulin. The use of a chemically homogeneous antigen instead of a mixture of antigens (*e.g.* serum) made possible the application of rigorous immunologic techniques in studies on experimental hypersensitivity. By employing qualitative serological methods these investigators demonstrated that the acute lesions of serum sickness develop at a time when the antigen is still present in the serum, and that healing and healed lesions are seen only after free antibody has appeared in the circulation.

In the present study, quantitative immunologic methods have been employed in an attempt to define as precisely as possible the relationship between the immune response and the development of lesions. Crystallized bovine albumin has been used as the antigen. Preliminary observations indicated that the administration of this purified protein to rabbits at a dosage of approximately 0.25 gm. per kg. of body weight regularly led to a high incidence of both cardiovascular and renal lesions. Further, hitherto unreported alterations

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in the spleen and lymph nodes were observed in a large proportion of the experimental animals.[1]

Materials and Methods

Crystallized bovine serum albumin obtained from Armour and Company was dissolved in isotonic saline to form a 10 per cent solution to which a quantity of solid sodium bicarbonate was added to give a pH of 7.4.

Albino male rabbits weighing approximately 2 kg. were used throughout this study. Each experimental animal received a single intravenous injection of 0.5 gm. of crystallized bovine serum albumin. In most instances, 5 ml. samples of blood were obtained from the marginal ear veins at varying intervals thereafter for immunologic study. Skin tests with 1 mg. of the antigen in 0.2 ml. of saline were performed on the majority of the animals. These were usually given 1 day prior to the day of sacrifice. However, in one experiment, summarized in Table IV, a total of 5 skin tests was performed at 2 day intervals prior to the termination of the experiment. In each experiment, control animals kept under conditions identical to those applying to the experimental animals were bled at comparable intervals. All the rabbits were killed by air embolism and immediately autopsied for histologic study. The tissues regularly examined included the lungs, heart, thymus, esophagus, thyroid, liver, spleen, pancreas, mesenteric lymph nodes, adrenals, kidneys, testes, intestine, psoas muscle, and skin from the site of the skin test. Usually the liver, kidneys, adrenals, and spleen were weighed. Pieces of tissue were fixed either in 10 per cent formalin, or in formalin followed by post chromation in 3 per cent potassium dichromate, or in Zenker's fluid. Hematoxylin-azure-eosin and hematoxylin-eosin were used for routine staining of tissue sections. Additional staining methods employed on sections of spleen were the periodic acid–Schiff, Wilder's reticulum, Van Gieson connective tissue, eosin-methylene blue, Goodpasture's modification of the Gram, and the Ziehl-Neelsen (8). Sections of kidneys were stained by the periodic acid–Schiff method in order to study the glomerular basement membranes.

Immunologic tests were performed as follows: Serum was removed from the specimens of clotted blood within 24 hours after collection and stored frozen at −15°C. until used. Qualitative tests for the presence of antigen were carried out on each sample of unknown serum. This was done by layering 0.2 ml. of the unknown sample over 0.2 ml. of a pooled antiserum of high titer obtained from rabbits immunized with alum-precipitated crystallized bovine albumin. When this test was positive, a quantitative determination of the antigen concentration in the unknown serum was made by the addition of 1 ml. of an appropriate dilution of the serum to 1 ml. of a pooled antiserum. The resulting precipitate obtained after 48 hours in the cold was washed twice with cold saline and its nitrogen content then determined by the Kjeldahl technique (9). A positive test for excess antibody in the supernatant fluid was accepted as an indication of complete precipitation of the antigen in the unknown. After the nitrogen content of the precipitate had been determined, the amount of antigen nitrogen in the precipitate was obtained by reference to a standard precipitin curve of the particular pooled antiserum employed. This curve was constructed from data representing the amounts of precipitate formed on the addition of varying but known quantities of antigen to 1 ml. portions of the pooled antiserum. In every determination, the dilution of the unknown serum employed was such that the nitrogen content of the precipitate formed by the addition of the pooled antiserum fell on the relatively straight midportion of the curve. At this portion of the curve small differences in the amount of antigen are reflected in large increments of total precipitate. The antigen values obtained from the curve were multiplied by the dilution factor of the original sample to obtain the antigen concentration of the unknown serum.

[1] After this manuscript had gone to press, the author's attention was directed to a recent publication by Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1952, **91**, 109, wherein similar lesions are described in the spleens and lymph nodes of rabbits receiving horse serum.

Qualitative tests for the presence of antibody were made by the addition of 4 μg. of antigen N contained in 0.2 ml. of saline to 0.5 ml. of the unknown serum. When the tests for antibody were positive, quantitative antibody determinations were performed as follows: Antigen in increments of 2 or 4 μg. of nitrogen was added to 1 ml. of unknown serum until no further precipitation was observed.[2] Antibody nitrogen was calculated by subtraction of the antigen N from the total nitrogen found by analysis of the washed precipitate. All serologic procedures were carried out at 0–5°C. and were done in duplicate.

TABLE I

Anatomic Alterations in Rabbits 8 Days after Intravenous Injection of 0.5 Gm. of Bovine Plasma Albumin

Rabbit No.	Subendothelial infiltrations	Arteritis	Endocarditis	Glomerulonephritis*	Lesion in	
					Spleen*	Lymph node
28 a	0	0	0	+	0	0
30 a	0	0	0	0	0	0
31 a	+	0	+	++	+++	+
32 a	0	0	0	0	0	0
33 a	0	0	0	0	0	0
34 a	0	0	0	+	0	0
35 a	0	0	0	0	0	0
36 a	+	0	0	0	+	0
Total.....8	2 (25%)	0	1 (17%)	3 (38%)	2 (25%)	1 (17%)

* Lesions in kidney and spleen graded 1+ to 3+ in order of increasing severity. Otherwise signs denote following: 0 = absence of lesion; + = lesion present; — = no observation made.

EXPERIMENTAL RESULTS

Each of 76 rabbits received a single intravenous injection of crystallized bovine serum albumin. 10 were killed at 6 days after injection, 8 at 8 days, 25 at 12 days, 12 at 15 days, 15 at 18 days, and 6 at 28 days. The histologic findings at each time interval are presented in Tables I to V, and summarized in Table VI. When quantitative antigen and antibody determinations were done, the animals are arranged in order of decreasing magnitude of serum antigen concentrations and increasing levels of serum antibody present at the time of sacrifice. This was done to facilitate correlation of the histologic and immunologic results.

In the experimental animals, alterations were encountered in the aorta and many of the medium sized arteries throughout the body, in the mitral and aortic valves, in the glomeruli of the kidneys, and in the lymphoid tissue of the spleen and lymph nodes. None of these alterations was observed in 24 control rabbits. Focal collections of lymphocytes and monocytes were seen in the myocardium of approximately 40 per cent of the experimental animals killed between the 12th and the 18th days after injection; similar lesions were

[2] The error involved in the precipitation of antibody by successive addition of antigen instead of by the single addition of the required amount of antigen is discussed in reference 38.

TABLE II

Anatomic Alterations in Rabbits 12 Days after Intravenous Injection of 0.5 Gm. of Bovine Plasma Albumin

Rabbit No.	Antigen or antibody nitrogen* per ml. of serum	Arthus reaction‡	Subendothelial infiltrations	Arteritis	Endocarditis	Glomerulonephritis§	Granulomatous lesion	
							Spleen§	Lymph node
	μg.							
32	95 At	0	0	0	0	0	0	0
38	68 At	0	0	0	0	0	+	−
49	51 At	0	+	0	0	0	++	0
41	45 At	0	0	0	0	0	0	−
42	43 At	0	+	0	0	++	+++	+
44	43 At	0	0	0	0	+	++	+
45	39 At	0	+	0 (phlebitis of hepatic veins)	0	++	+++	+
46	30 At	0	+	+ stomach, spleen, acute	+	++	++	−
29	30 At	0	+	+ lung, acute	+	+++	+++	0
47	26 At	0	+	+ heart, mesentery, acute	+	+	+++	−
50	25 At	0	0	0	0	0	++	+
36	15 At	0	+	0	0	+++	+++	−
34	15 At	0	+	+ lung, acute	+	+++	+++	0
28	12 At	0	+	0	+	+++	++	+
30	8 At	0	+	0	+	+++	+	0
35	5 At	0	+	0	0	+++	+++	+
31	0	0	0	0	−	+++	++	0
26	11 Ab	0	+	0	+	+++	+	0
40	29 Ab	0	0	+ heart, acute	+	+++	+++	−
37	32 Ab	1.2 h	+	+ spleen, heart, peripancreatic, acute	+	+++	++	+
27	35 Ab	3.6 h	+	0	+	+++	0	0
39	75 Ab	4.8 h	+	0	+	+++	0	−
33	86 Ab	1.7 h	+	0	+	++	+	+
48	149 Ab	1.9 h	+	0	0	++	−	−
43	—	—	+	+ heart, stomach, acute	+	+++	+++	−
Total....25		5(21%)	18(72%)	7(28%)	13(54%)	20(80%)	20(83%)	8(50%)

* At = antigen; Ab = antibody. In the absence of an accelerated rate of antigen elimination due to antibody formation, the serum antigen nitrogen concentration on the 12th day would equal approximately 47 μg. per ml. This value was calculated by projecting the second phase of antigen elimination in Text-fig. 3. Concentrations of antigen above this value indicate the relative absence of antibody formation; concentrations below this value denote the presence of antibody formation. It is apparent that the smaller the amount of antigen, or in its absence, the greater the amount of circulating antibody, the greater has been the response. The rabbit numbers are arranged in order of the degree of immune response.

‡ Product of length, width, and height measured in centimeters. h = hemorrhagic.

§ Alterations in kidney and spleen are graded 1+ to 3+. In the case of the spleen, 1+ denotes that 0–29 per cent of the follicles show alterations; 2+, 29–50 per cent; and 3+, over 50 per cent of the follicles show alterations. Otherwise signs denote following: 0 = absence of lesion; + = lesion present; − = no observation made.

observed in 8 per cent of the control group. The lesions in both groups of animals conformed to the spontaneous myocardial lesions of rabbits described

by Miller (10). The reason for the varying incidence of these lesions is not clear.

TABLE III

Anatomic Alterations in Rabbits 15 Days after Intravenous Injection of 0.5 Gm. of Bovine Plasma Albumin

Rabbit No.	Antigen or antibody nitrogen* per ml. of serum	Arthus reaction‡	Subendothelial infiltrations	Arteritis	Endocarditis	Glomerulonephritis§	Lesion in spleen§
	μg.						
12	2 At	0	0	+ heart, subacute liver kidney mesentery, acute	+	++	+
16	0	0	0	+ heart, subacute	+	++	+
17	31 Ab	2.5 h	0	+ heart, lung, subacute	0	+	+
11	37 Ab	0	0	0	0	+	0 fibrosis about central artery
15	55 Ab	0.8 pp	+	+ heart, subacute	+	+++	0 fibrosis about central artery
18	110 Ab	0.6 h	0	0	+	++	0
13	—	2.3 h	+	+ spleen, subacute	0	+++	+
19	—	0	0	0	0	+	0
20	—	0	0	0	0	0	0 fibrosis about central artery
Total...9		4 (44%)	2 (22%)	5 (56%)	4 (44%)	8 (89%)	4 (44%)

* At = antigen; Ab = antibody.

‡ Product of length, width, and height measured in centimeters. h = hemorrhagic; pp = pale pink.

§ Lesions in kidney and spleen are graded 1+ to 3+ in order of increasing severity. Otherwise, + = presence of lesion, 0 = absence of lesion, — = no observation made.

Pathologic Findings

1. Injury to the Cardiovascular System

(*a*) *Changes Confined to the Intima ("Subendothelial Leukocytic Infiltration") of Arteries and Aorta.*—Foci of edema and leukocytic infiltration were present beneath the endothelium

of one or more of the larger pulmonary arteries or ascending aortas in 72 per cent (18/25)[3] of the rabbits sacrificed on the 12th day. However, as early as 8 days after the injection of antigen, the intima of these vessels in a few animals showed varying degrees of edema and infiltration by polymorphonuclear and mononuclear cells. The lesions in the aorta were often continuous with an infiltration at the base of the aortic cusps. Occasionally, the cellular infiltrate in the aorta extended into the coronary ostia and merged with an inflammatory reac-

TABLE IV

Anatomic Alterations in Rabbits 18 Days after Intravenous Injection of 0.5 Gm. of Bovine Plasma Albumin

Rabbit No.	Antigen or antibody nitrogen per ml. of serum	Arthus reaction*	Subendothelial infiltrations	Arteritis	Endocarditis	Glomerulonephritis‡	Lesion	
							Spleen	Lymph node
	μg.							
20	27 At	0	+	0	0	0	+§	0
19	28 Ab	0	+	0	+	++	+§	—
28	47 Ab	0 p	+	0	+	0	0	0
16	83 Ab	0.9 r	+	0	0	+	0	—
24	88 Ab	0.4 h	0	0	0	+++	0	0
18	128 Ab	12 r	0	0	0	0	0	0
25	178 Ab	4.5 h	0	0	0	+	0§	—
21	180 Ab	6.3 h	0	0	0	++	0§	0
17	183 Ab	7.4 r	0	+ heart, healed	0	0	0	0
26	189 Ab	6.3 h	+	0	0	+	0	—
23	205 Ab	3.8 h	0	0	0	+	0§	0
27	235 Ab	6.4 h	0	0	0	0	0	—
30	317 Ab	6.8 h	0	0	0	+	0§	—
22	388 Ab	2.2 h	0	0	0	0	0	0
29	407 Ab	3.2 h	0	0	0	0	0	—
Total...15		13 (87%)	5 (33%)	1 (7%)	2 (13%)	8 (53%)	2 (13%)	0

* Product of length, width, and height measured in centimeters. h = hemorrhagic; r = red.

‡ Lesions in kidney and spleen are graded 1+ to 3+ in order of increasing severity.

§ Fibrosis about central artery.

tion involving the coronary arteries. On the 12th and 15th days, the infiltrate in the pulmonary arteries and aorta consisted predominately of mononuclear cells, and by the 15th day, fibroblasts were present in the lesions of some of the rabbits (Fig. 1). As the pulmonary arteries and aortas of animals sacrificed on the 28th day appeared normal, healing must have taken place without appreciable scarring.

(*b*) *Changes Involving the Media and Adventitia of Arteries* ("*Arteritis*").—The marked alterations observed in the medium sized arteries were similar to those described by Rich and Gregory (3). They were present in either the coronary, pulmonary, mesenteric, gastric,

[3] The numerator represents the number of animals showing the lesions; the denominator, the total number in the experimental group.

TABLE V

Anatomic Alterations in Rabbits 28 Days after Intravenous Injection of 0.5 Gm. Bovine Plasma Albumin

Rabbit No.	Subendothelial infiltrations	Arteritis	Endocarditis	Glomerulonephritis	Lesion	
					Spleen	Lymph node
17 a	0	0	+	0*	0	0
18 a	0	0	0	0	0	0
19 a	0	0	0	0	0	0
20 a	0	0	0	0	0	0
21 a	0	0	0	0	0	0
24 a	0	0	0	0	0	0
Total.....6	0	0	1	0	0	0
22 a‡	+	0	+	++	++	0
23 a	+	+ heart kidney	+	+++	+	—
25 a	0	+ heart kidney pancreas mesentery	+	++	+	—

* Scattered glomeruli showing adhesions between tuft and Bowman's capsule.

‡ These 3 rabbits were sacrificed at 15 days as controls for the above.

TABLE VI

Summary of Incidence of Anatomic Lesions at Various Intervals after Injection of Bovine Albumin

Time after injection	No. of rabbits	Per cent of animals with								
		Circulating antigen	Circulating antibody	Arthus reaction	Subendothelial infiltrations	Arteritis	Endocarditis	Glomerulonephritis	Lesions in spleen	Lesions in lymph node
days										
6	10	100	0	0	0	0	0	0	0	0
8	8	100	0	0	25	0	17	38	25	17
12	25	71	29	21	72	28	54	80	83	50
15	9	33	67	44	22	56	44	89	44	—
18	15	7	93	80	33	7	13	53	13	0
28	6	—	—	—	0	0	17	0	0	0

pancreatic, renal, or splenic arteries in 28 per cent (7/25) of animals sacrificed on the 12th day and 56 per cent (5/9) of animals sacrificed on the 15th day. The slightest change consisted of edema of the media and an accumulation of fluid and leukocytes beneath an intact swollen endothelium. A further degree of injury consisted of edema and beginning necrosis of the media which reduced the muscle cells to thin strands of cytoplasm with pyknotic nu-

clei. Eventually, all three coats of the artery showed varying degrees of edema, infiltration by polymorphonuclear and mononuclear leukocytes, and necrosis. Fibrinoid was encountered both in the media and adventitia and occasionally in the adjacent connective tissue (Fig. 2). Usually only a portion of the circumference of a medium sized artery showed these evidences of injury. When the involved vessel was small, often its entire wall was necrotic (Fig. 3). The internal and external elastic membranes appeared to be resistant to damage, for they could be observed as highly refractile bands within the wall of vessels in spite of necrosis and fibrinoid degeneration. On the 15th day, the cellular infiltrate in the arterial lesions was mainly mononuclear. In the few lesions encountered in the animals sacrificed on the 18th day, there was early fibrosis of the adventitia and intima in addition to mononuclear infiltration. No arterial lesions were observed in animals sacrificed on the 28th day.

(*c*) *Changes Involving the Valves* (*"Endocarditis"*).—Inflammatory changes were observed in the aortic or mitral valves of 54 per cent (13/24) of the animals sacrificed on the 12th day. The aortic surface and base of the aortic valve were the sites most frequently involved. These changes consisted of a proliferation of the endothelial cells, a subendothelial infiltration of polymorphonuclear and mononuclear cells, and edema of the adjacent connective tissue (Fig. 4). More severe lesions were occasionally encountered in the inferior aspect of the base of the aortic valve where, in addition to numerous monocytes and Anitschkow cells in the involved valve, aggregates of large basophilic cells containing a prominent nucleolus were found. Here, the connective tissue was usually swollen and, in one instance, showed deposits of fibrinoid. In animals sacrificed after the 15th day, the endocarditis was both less frequent and less severe. No alterations were observed which could be interpreted as the healed stages of these lesions.

2. *Injury to the Kidney* (*"Glomerulonephritis"*)

An unexpected result was a high incidence of glomerular changes (Fig. 6). These were observed in over 80 per cent (28/34) of animals sacrificed on the 12th and 15th days. In the kidneys showing the slightest involvement (graded as +) the glomerular epithelial cells were swollen and increased in number. However, the degree of involvement varied from one glomerulus to another. Some were entirely normal, in others there was an increased cellularity of a single loop, while still others showed a diffuse cellular hyperplasia. These slight lesions were observed as early as 8 days after injection of antigen. More severe lesions (graded ++) observed on the 12th day consisted in a uniform alteration of nearly all glomeruli so that they were distinctly larger than normal. Numerous swollen epithelial and endothelial cells occluded the glomerular capillaries, and reduced the number of blood-filled loops. The most severe lesions were observed on the 12th and 15th days. At that time, the basement membranes of some of the glomeruli were thickened and fragmented with focal areas of karyorrhexis and pyknosis in the endothelial cells of the capillaries. The cells of Bowman's capsule were sometimes swollen and increased in number. Occasionally adhesions were observed between the tuft and the capsule. A coagulum of protein was found between the capillaries and in the capsular space of the involved glomeruli. In kidneys with the most severe glomerular changes (graded +++) numerous casts and red blood cells were present in the distal convoluted tubules and collecting ducts. Hyalin droplets were prominent in the epithelium of the proximal convoluted tubules. The kidneys of animals showing advanced glomerular changes were in the gross pale and swollen. On cut surface the glomeruli appeared either indistinct or as gray dots. After the 15th day, the glomerular changes were less severe. By the 28th day, the kidneys of all the animals were normal. Recovery apparently took place with restitution of original structure and without scarring.

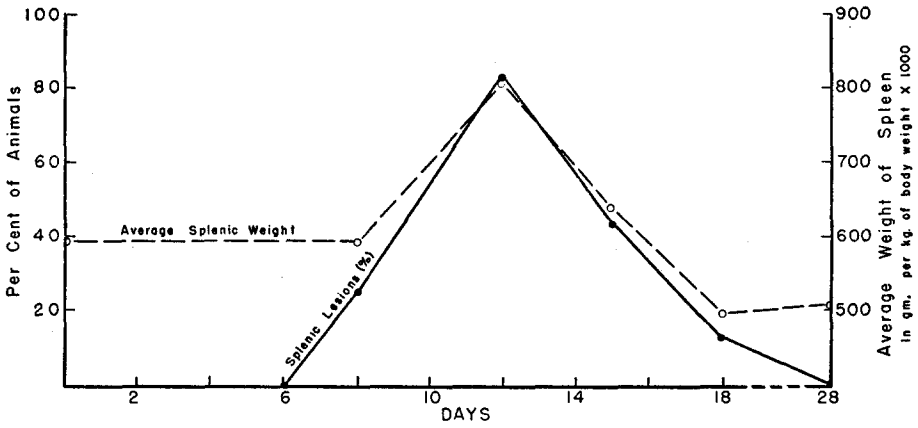
3. *Alterations in the Spleen*

In the normal rabbit spleen the central artery is covered by a continuous sheath of lymphoid tissue. The swellings of this sheath which represent the splenic follicles consist of two concentric zones of lymphoid tissue; a *central* zone of loosely packed medium sized lymphocytes and reticulum cells, and a marginal zone of closely packed medium sized lymphocytes separated from the former zone by a rim of connective tissue. In the larger splenic follicles a third zone of densely packed small lymphocytes may be encountered immediately adjacent to the central artery. The blood supply of the follicles consists of an internal and external vascular network arising from branches of the central and sheath arteries. Around the central artery and these peripheral branches, there is an accumulation of reticulum cells and reticulum.

In the spleens of rabbits receiving bovine albumin and sacrificed on the 6th day, there were changes in the splenic follicles similar to those seen after the administration of other foreign proteins (11, 12). Numerous large lymphocytes, lymphoblasts, and occasional reticulum cells were present in the marginal zone of the follicle and in the adjacent pulp. Many of these cells were in mitosis. After the 6th day there appeared in the splenic follicles a distinctive alteration due to a replacement of the normal lymphoid tissue by numerous macrophages. This alteration was present in 25 per cent (2/8) of the spleens taken on the 8th day and in 83 per cent (20/24) of those taken on the 12th day. The earliest phase of this change occurred as multiple foci of macrophages within the marginal zone of the follicle, often about the branches of the central and sheath arteries. As these foci became larger and more numerous, they formed a more or less continuous cuff of macrophages around the central zone (Fig. 10). In some instances numerous macrophages were seen about the central artery. In early lesions these cells were about twice as large as lymphocytes and possessed a moderate amount of colorless, agranular cytoplasm, and a single, oval, leptochromatic nucleus with one or two prominent chromatin dots (Fig. 7). Later, the predominant cell of the infiltrate in the follicles possessed abundant eosinophilic cytoplasm and thus resembled the so called "epithelioid" cell. Simultaneously with the appearance of the epithelioid cells, numerous foreign body giant cells were encountered. Some of the giant cells were enormous and contained as many as 100 nuclei. In the most extensive lesions, epithelioid and giant cells completely replaced the lymphoid tissue of the central zone and much of the marginal zone of the follicles (Figs. 8 and 11). At this stage the spleens were soft, enlarged (Table VII and Text-fig. 1), and on cut section showed prominent, bulging follicles (Fig. 9). These severe changes were observed predominately on the 12th day when the incidence of splenic lesions was highest.

With every section of spleen a careful search was made for evidence of damage to blood vessels. In the early lesions, the blood vessels of involved follicles appeared normal. However, in the spleens with follicles showing the most extensive cellular reactions, vascular alterations were occasionally encountered. In the spleen of one animal (No. 37, Table II), a single trabecular artery showed necrosis and infiltration of the wall by polymorphonuclear leukocytes. In another spleen (No. 46, Table II) there was segmental necrosis and leukocytic infiltration of the wall of a single central artery. However, the lymphoid tissue adjacent to this partly necrotic central artery appeared normal (Fig. 12). 4 of the 24 spleens removed on the 12th day contained a rare central artery or branch showing focal deposits of fibrinoid in its wall. Fairly abundant fibrinoid was sometimes present between the individual epithelioid cells adjacent to those arteries containing focal fibrinoid deposits. Finally, in three spleens taken at the 12th day, numerous central arteries showed segmental fibrinoid deposits and, occasionally, necrosis with fibrinoid. In some of the follicles containing damaged arteries, the perivascular infiltrate of epithelioid cells showed both fibrinoid and frank necrosis, possibly secondary to ischemia (Fig. 13).

Healing of the splenic follicles and arteries was first noted in a few of the animals killed on the 12th day. Each of the animals in which the splenic follicles appeared to be healing showed detectable antibody, considered indicative of antigen elimination. Healing was heralded by the disappearance of epithelioid and giant cells and by the reappearance of lymphocytes, first in the midzone of the follicle between the central artery and the periphery (Fig.



TEXT-FIG. 1. Weight of spleen in relation to occurrence of splenic lesions. After data of Table VII and column 8 of Table VI. Solid line represents the per cent of animals with splenic lesions at various intervals after injection of bovine albumin. Broken line represents average splenic weight in relation to body weight of same group of rabbits.

TABLE VII

Changes in Weight of Spleen Following Injection of Bovine Albumin

Time after injection of antigen	No. of rabbits	Average weight of rabbits	Average weight of spleen ± S.D.	Weight of spleen per kg. body weight × 1,000 ± S.D.
days		*kg.*	*gm.*	*gm.*
0	24	2.02	1.21 ± 0.28	0.598 ± 0.138
8	8	1.85	1.09 ± 0.23	0.590 ± 0.124
12	24	2.03	1.65 ± 0.64	0.812 ± 0.315
15	9	2.73	1.75 ± 0.53	0.641 ± 0.194
18	15	2.12	1.05 ± 0.35	0.496 ± 0.166
28	6	2.50	1.26 ± 0.54	0.504 ± 0.216

14), then at the periphery, and finally about the central artery. With the reappearance of lymphocytes, numerous plasma cells were encountered in the loose stroma of the follicles. The fact that the follicles were smaller and less cellular suggested that a reduction in lymphoid tissue had occurred. The weight of the spleen in these animals was smaller (Text-fig. 1). The spleens of certain animals killed on the 12th and 15th days showed broad bands of loose reticular and fibrous connective tissue extending along the central artery from one follicle to another (Fig. 15). After the 15th day, with condensation of the fibrous tissue and the return of the lymphoid tissue to its normal status, this perivascular fibrosis was much less striking.

TABLE VIII

Character and Frequency of Lesions in the Spleens of Rabbits Sacrificed 12 Days after Receiving Bovine Albumin

Rabbit No.	Antigen or antibody nitrogen per ml. of serum	Per cent of splenic follicles* showing					Presence of giant cells in lesions	Presence of vascular damage in spleen	Per cent of follicles showing fibrosis about central artery
		Infiltration of peripheral zone by epithelioid cells	Infiltration of central zone by epithelioid cells	Extensive infiltration of both zones by epithelioid cells	Palisade of epithelioid cells about arteries without other alterations	Total			
	μg.								
32	96 At	0	0	0	0	0	0	0	0
38	68 At	2	0	0	0	2	0	0	0
49	51 At	14	10	15	0	39	+++	Ed., fib., 1 central artery‡	0
41	45 At	0	0	0	0	0	0	0	0
42	43 At	8	8	0	46	62	0	Fib., 1 central artery	0
44	43 At	18	11	0	7	36	++	0	0
45	39 At	15	6	7	24	52	++	0	0
46	30 At	22	12	0	5	39	+	Nec., inf., 1 central artery	0
29	30 At	6	18	7	67	98	+	Nec., 1 sheath artery	0
50	25 At	27	9	6	0	42	0	0	0
36	15 At	19	17	10	34	80	+++	Fib., num. cent. arteries	0
34	15 At	5	9	5	65	84	+++	Fib., num. cent. arteries	0
28	12 At	17	26	0	0	43	+	Fib., num. cent. arteries	0
30	8 At	0	1	0	6	7	0	0	0
31	0	19	15	0	4	38	+	0	0
40	29 Ab	49	0	18	4	71	0	0	0
37	32 Ab	27	3	0	7	37	0	Nec., inf., 1 trab. artery; fib., 1 cent. artery	0
27	35 Ab	0	0	0	0	0	0	0	23
39	75 Ab	0	0	0	0	0	0	0	50
33	86 Ab	6	5	0	9	20	0	0	13

* Results based on observations made on 50 to 80 splenic follicles. Only 20 of 25 rabbits sacrificed are included here.

‡ Ed. = edema of media; nec. = necrosis; inf. = infiltration with inflammatory cells; fib. = fibrinoid; trab. = trabecular; num. cent. = numerous central.

In addition to the marked changes in the splenic follicles, slight alterations were observed in the red pulp. A diffuse hyperplasia of the reticulo-endothelial cells of the sinusoids and a slight increase in number of plasma cells were noted in many of the spleens removed on the 12th day. Occasionally clumps of epithelioid cells surrounded small arteries in the pulp. In sections of four spleens taken on the 12th day, portions of the trabeculae and capsule were swollen and intensely eosinophilic, but no definite fibrinoid alteration was observed. A summary of the numerous changes encountered in the pulp and follicles of spleens of animals sacrificed on the 12th day is presented in Table VIII.

4. Alterations in the Mesenteric Lymph Nodes

By the 6th day after the injection of bovine albumin, marked hyperplasia of the mesenteric lymph nodes had occurred. The cortex of the nodes was widened and contained numerous large follicles, and the medullary cords were swollen by many medium and large lymphocytes, some in mitosis. Beginning on the 8th day but occurring most frequently on the 12th day, numerous macrophages were encountered in the lymph nodes. This change observed in the nodes was morphologically similar to that occurring simultaneously in the lymphoid tissue of the spleen. As in the case of the splenic follicle, one of the earliest alterations consisted of a cuff of epithelioid cells surrounding the periphery of a densely cellular cortical follicle (Fig. 16). In a single lymph node, several follicles that were completely infiltrated by epithelioid cells (Fig. 18) showed focal areas of necrosis. Although it was considered that the necrosis may have resulted from ischemia, no changes in the blood vessels were observed. In many lymph nodes epithelioid cells were more numerous in the medullary cords than in the cortex. In the medullary portions of several lymph nodes these cells together with an occasional foreign body giant cell formed small granuloma-like lesions (Fig. 19). In the involved lymph nodes, the sinuses were crowded with macrophages, epithelioid cells, and lymphocytes of all varieties. Changes of varying degrees of severity were present in 50 per cent of the lymph nodes taken on the 12th day. Lymph nodes examined on the 18th day were normal. Unfortunately, lymph nodes were not examined in the animals sacrificed on the 15th day.

Immunologic Findings

In order to determine the relationship between the histologic changes and the immunologic response of the host, the rate of elimination of bovine albumin (antigen) from the blood and the subsequent development of its specific antibody were determined in 30 of the 76 experimental rabbits. 15 of these 30 rabbits were killed on the 12th day and the remaining 15 on the 18th day. In addition, the serum antigen or antibody concentration present at the time of sacrifice was measured in each rabbit killed on the 12th, 15th, and 18th days. These concentrations are tabulated with the histologic findings from the same animals in Tables I to V.

The complete serologic results on the 30 rabbits are recorded in Tables IX and X. As shown by these two tables, antigen remained in the sera of all the animals for as long as 10 days. By the 12th day, approximately 30 per cent (12/37) of the rabbits had eliminated the antigen and produced measurable quantities of circulating antibody. By the 18th day 7 per cent (1/14) of the animals continued to show traces of antigen while 93 per cent (13/14) had developed considerable levels of antibody (Table X).

The immunologic results in the 25 animals killed on the 12th day are recorded

in Table IX and summarized graphically in Text-fig. 2. In the figure, the median blood clearance of antigen is represented by the center solid line. In

TABLE IX

Serum Antigen and Antibody Levels Following a Single Intravenous Injection of 0.5 Gm. of Bovine Plasma Albumin

Rabbit No.	Antigen or antibody nitrogen per ml. of serum*						
	5 min.	2 days	4 days	6 days	8 days	10 days	12 days
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
32	0.840	0.346	0.309	0.220	0.166	0.121	0.096
38	1.06	0.399	0.285	0.208	0.150	0.100	0.068
49							0.051
41							0.045
42							0.043
44							0.043
45							0.039
46							0.030
29	0.885	0.311	0.231	0.170	0.131	0.086	0.030
47							0.026
50							0.025
36	0.890	0.288	0.204	0.150	0.105	0.063	0.015
34	0.940	0.307	0.242	0.179	0.125	0.079	0.015
28	0.710	0.254	0.202	0.143	0.088	0.049	0.012
30	0.750	0.317	0.237	0.151	0.108	0.053	0.008
35	0.750	0.232	0.188	0.141	0.090	0.064	0.005
31	0.650	0.252	0.213	0.138	0.086	0.036	0.000
26	0.950	0.340	0.250	0.181	0.094	0.052	0.011 Ab
40	0.860	0.368	0.242	0.196	0.134	0.076	0.029 Ab
37	1.12	0.304	0.207	0.182	0.101		0.032 Ab
27	0.735	0.284	0.160	0.146	0.043	0.000	0.035 Ab
39	0.775	0.307	0.231	0.171	0.111	0.002	0.075 Ab
33	0.815	0.300	0.206	0.176	0.098	0.036	0.086 Ab
48							0.149 Ab
Median	0.840	0.307	0.231	0.171	0.105	0.053	0.015
Per cent of injected antigen remaining in serum	66.6	24.4	18.3	13.6	8.3	4.2	0.4

Animals are listed in order of decreasing magnitudes of serum antigen concentrations or increasing levels of antibody present at time of sacrifice (12 days).

* Values represent antigen N unless otherwise specified as antibody by abbreviation Ab.

addition the antigen elimination curves of 2 animals, one showing the highest level of antigen at the time of sacrifice and another showing the highest level of free antibody are presented. It is evident that the median curve contains 3 components; an early phase of rapid loss of antigen from the circulation, a

second phase of gradual disappearance of antigen occurring between the 2nd and 6th days after injection, and a final phase of rapid elimination of antigen beginning with the 6th day. However, in some animals (*e.g.* No. 32), the third phase of rapid elimination of antigen had not appeared by the time of sacrifice;

TABLE X

Serum Antigen and Antibody Levels Following a Single Intravenous Injection of 0.5 Gm. of Bovine Plasma Albumin

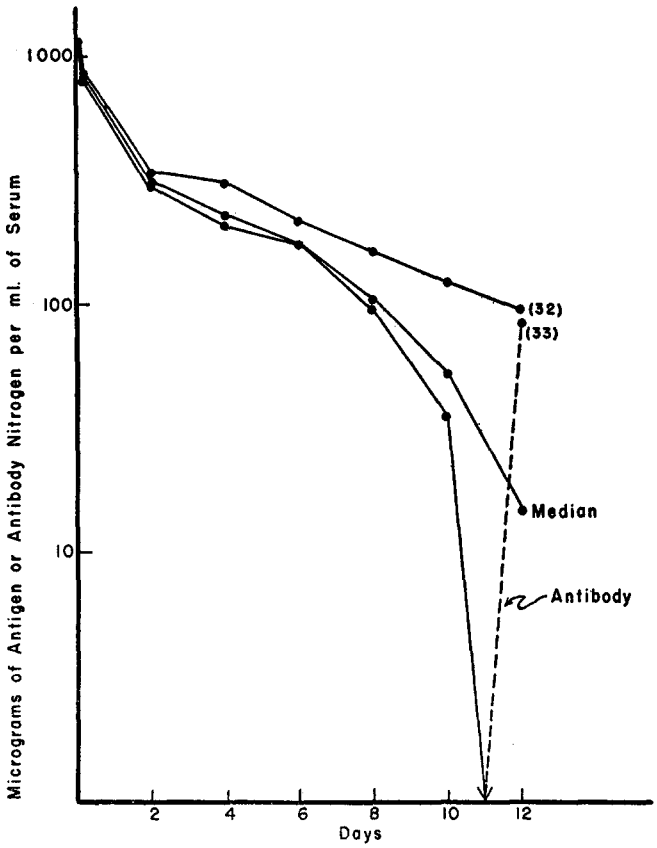
Rabbit No.	Antigen or antibody nitrogen per ml. of serum on following days after injection*							
	1 day	4 days	8 days	10 days	12 days	14 days	16 days	18 days
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
20	0.358	0.229	0.148	0.125		0.051	0.041	0.027
19	0.395	0.204	0.102	0.067	0.054	0.030	0.006	0.028 Ab
28	0.312	0.224	0.144	0.084	0.072	0.031	0.001	0.047 Ab
16	0.400	0.257	0.148	0.116		0.026		0.083 Ab
24	0.299	0.208	0.115	0.070	0.005	0.039 Ab	0.064 Ab	0.088 Ab
18	0.268	0.179	0.097	0.048	0.027 Ab	0.095 Ab	0.125 Ab	0.128 Ab
25	0.394	0.224	0.132	0.083	0.043	0.008 Ab	0.125 Ab	0.178 Ab
21	0.344	0.288	0.140	0.091	0.028	0.060 Ab	0.126 Ab	0.180 Ab
17	0.271	0.220	0.125	0.083	0.015	0.060 Ab	0.156 Ab	0.183 Ab
26	0.322	0.205	0.096	0.032	0.024 Ab	0.099 Ab	0.143 Ab	0.189 Ab
23	0.429	0.249	0.140	0.073	0.006 Ab	0.089 Ab	0.172 Ab	0.205 Ab
27	0.407	0.226	0.120	0.070	0.000	0.224 Ab	0.198 Ab	0.235 Ab
30	0.390	0.231	0.140	0.079	0.004	0.202 Ab	0.279 Ab	0.317 Ab
22	0.411	0.257	0.155	0.088	0.072 Ab	0.251 Ab	0.388 Ab	Dead
29	0.352	0.216	0.065	0.042	0.058 Ab	0.151 Ab	0.327 Ab	0.407 Ab
Median.....	0.358	0.224	0.125	0.079	0.004	0.060 Ab	0.143 Ab	0.178 Ab
Per cent of injected antigen remaining in serum.....	28.4	17.8	9.9	6.3	0.3			

* Values represent antigen N unless otherwise specified as antibody by abbreviation Ab.

in others as previously indicated (*e.g.* No. 33), the antigen had been completely eliminated and circulating antibody had developed.

The temporal relationship of the arterial, glomerular, and splenic changes to the three phases of antigen elimination and the appearance of circulating antibody is shown graphically in Text-fig. 3. Here, the median serum antigen and antibody concentrations obtained from composite data of Tables IX and X are plotted semilogarithemically against time. Arithmetic plots of the incidences of the lesions (Table VI) are presented in the same figure. The tissue lesions were found to occur at the time of the third or so called "immune" phase of

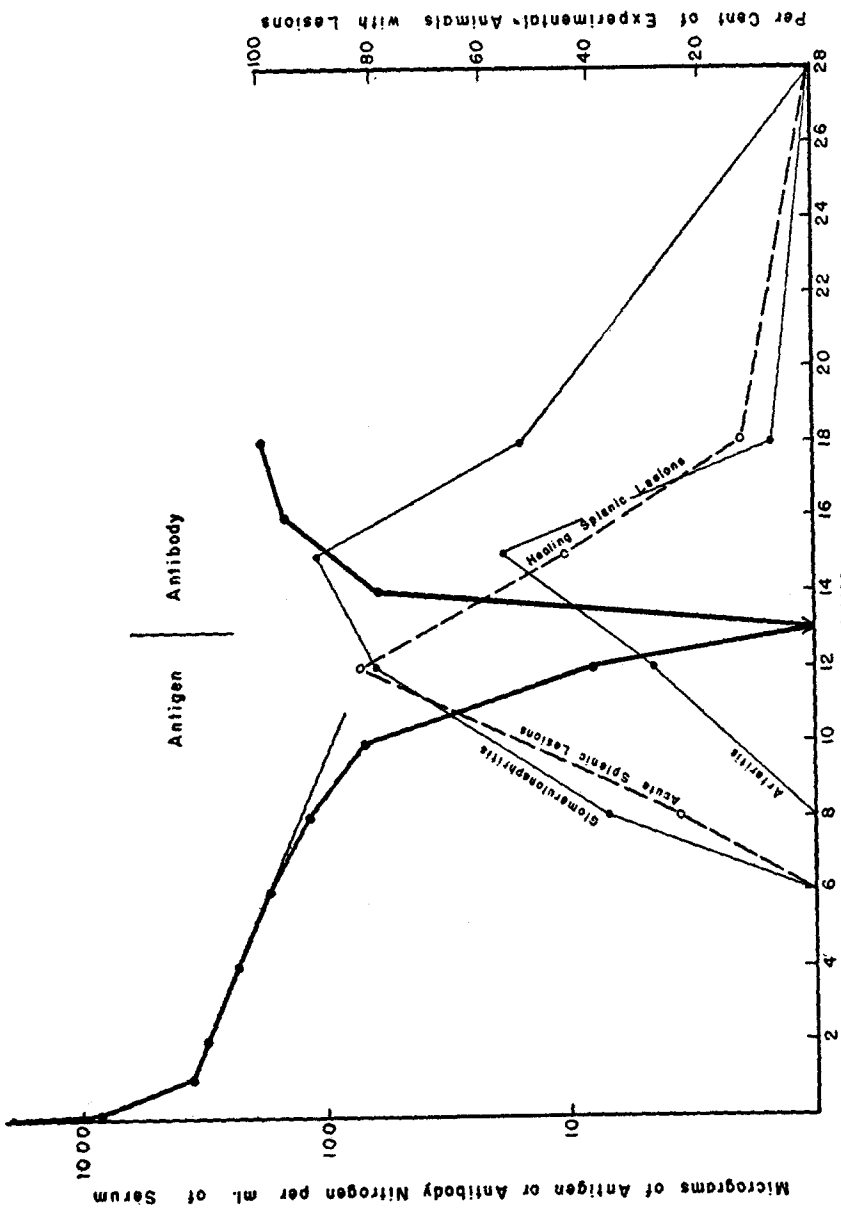
antigen elimination. As shown in the figure, lesions first appeared after the immune phase had begun; they were present in greatest severity and in highest



TEXT-FIG. 2. Blood clearance of bovine plasma albumin. After data of Table IX. Values for serum antigen and antibody nitrogen are plotted semilogarithmically with respect to time after injection of antigen. The median blood clearance of antigen calculated from values obtained from 15 rabbits is represented by the center solid line. The curve of the rabbit showing the slowest rate of elimination of antigen (No. 32) and that of the rabbit with the fastest elimination of antigen (No. 33) are also presented. By the time of sacrifice the antigen had been completely eliminated in rabbit 33, and circulating antibody (broken line) had appeared.

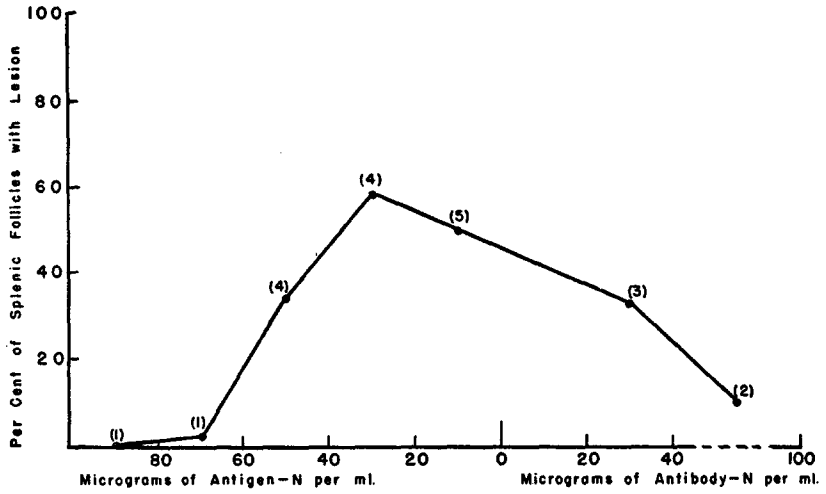
incidence about the time the immune phase was completed; and they were encountered in decreasing frequency and in the process of healing shortly after the development of circulating antibody.

To investigate further the relationship of the splenic lesions to the immune phase of antigen elimination, a comparison was made between the extent of



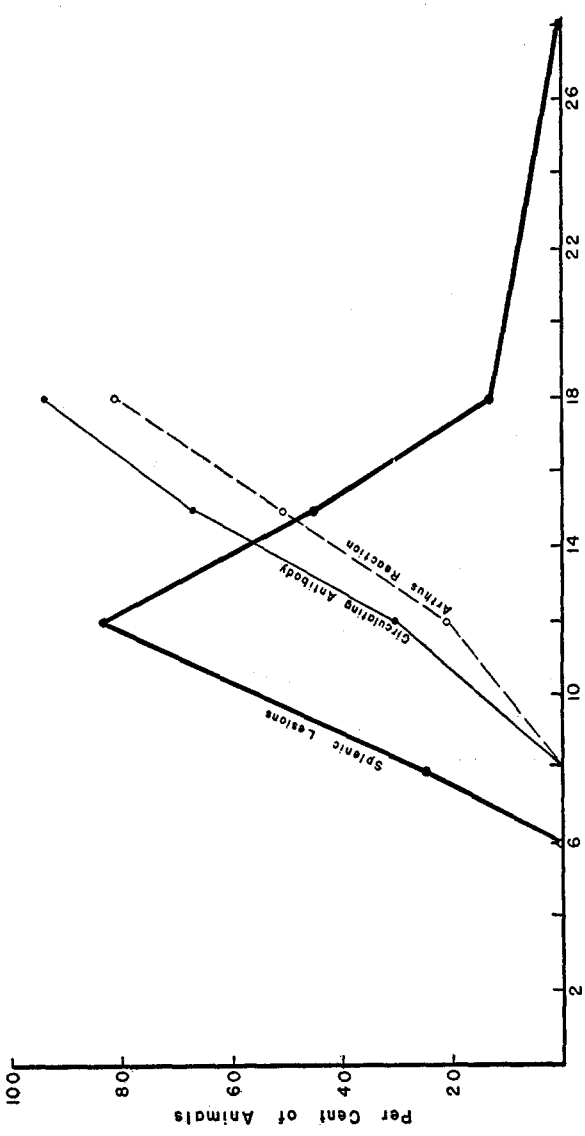
TEXT-FIG. 3. Relation of time of occurrence of tissue lesions to blood clearance of antigen and development of antibody. After combined data of Tables IX and X and data of Table VI. The curve showing the median blood clearance of antigen and the appearance of circulating antibody is represented by the thick solid line and refers to the ordinate on the left. Values after the 13th day represent antibody. The thin solid line projected from the antigen clearance curve at the 6th day shows the course the antigen would follow in the absence of an increased elimination of antigen by antibody. The incidence of splenic lesions at various times is represented by the broken line and incidence of arteritis and glomerulonephritis by the thin solid lines. These values refer to the ordinate at the right of the figure.

the lesion and the magnitude of the serum antigen or antibody concentration present in each of 20 animals when sacrificed on the 12th day. It is apparent from Text-fig. 2 that the persistence of large amounts of serum antigen until the 12th day indicates an absent or delayed immune phase of antigen elimination; small amounts of antigen signify the presence of a substantial immune response at this time; on the other hand, presence of free antibody denotes completion of the immune phase of antigen elimination. If the splenic lesions



TEXT-FIG. 4. Relationship of severity of splenic lesion to serum antigen or antibody concentration at time of sacrifice. After data of column 7 of Table VIII. Rabbits are grouped according to their serum antigen or antibody concentrations at the time of sacrifice. Those with concentrations falling within increments of 20 μg. of nitrogen are grouped together. The average severity of the splenic lesions of each group of animals as measured by the per cent of splenic follicles with granulomatous lesions is plotted against the serum antigen or antibody concentration. The number of animals in each group is found in parentheses at each point on the curve.

are a consequence of the immune phase of antigen elimination, then the severest changes should occur in those rabbits with small amounts of serum antigen or antibody. Conversely, those animals with large amounts of antigen should show few or no lesions. The finding of large amounts of antibody is believed to indicate that the immune phase has occurred at some earlier time. Therefore, the splenic lesions of animals with large amounts of free antibody might show varying degrees of healing. The actual relationship between the extent of the lesion and the magnitude of the serum antigen or antibody concentration is plotted in Text-fig. 4. As shown there, the extent of the splenic lesions in rabbits killed on the 12th day was inversely proportional to the magnitude of the serum antigen or antibody concentrations. In light of the foregoing considera-



TEXT-FIG. 5. Time of occurrence of splenic lesion, Arthus reaction, and circulating antibody after a single intravenous injection of bovine albumin. After data of Table VI. The per cent of animals sacrificed at varying intervals after injection of bovine albumin showing splenic lesions, containing circulating antibody, and producing a positive Arthus response respectively are plotted against time.

tions, this correlation supports the interpretation that the splenic lesions result from the immune phase of antigen elimination.

The question naturally arises as to whether an analysis of other tissue lesions results in the same conclusion. Briefly, the glomerular lesions can be correlated with the immunologic findings in much the same way. However, the incidence of cardiovascular lesions was insufficiently high to permit accurate analysis. The time at which the splenic lesions occurred, in relation to skin sensitivity (Arthus reaction) is shown graphically in Text-fig. 5. As indicated previously, the highest incidence of Arthus reactions occurred after most of the rabbits had circulating antibody and at a time when the splenic lesions had undergone healing.

DISCUSSION

Cardiovascular and Renal Lesions

The present study demonstrates that a high incidence of the various types of lesions generally attributed to hypersensitivity can result from a single intravenous injection of bovine albumin. These tissue changes include a focal inflammatory reaction in the intima of the larger pulmonary arteries and aorta and arteritis, endocarditis, and a glomerulonephritis. Since these lesions were reversible or reparable, their incidence varied with the time interval between the injection of antigen and sacrifice of the animals. The tissue lesions were first observed 8 days after the administration of the antigen, were present in highest incidence on either the 12th or 15th day, and were absent by the 28th day.

Subendothelial accumulations of leukocytes were present in either the pulmonary arteries or aorta of 72 per cent of the animals sacrificed on the 12th day. Similar lesions have been described by Apitz (13), Ehrich, Seifter, and Forman (12), and Schwab and coworkers (14) in rabbits treated with horse serum or bovine gamma globulin. An arteritis morphologically similar to polyarteritis nodosa was found in 51 per cent of the animals sacrificed on the 15th day. The arterial lesions were as frequent and as severe as those reported in animals given whole serum (1–3, 12, 15–17). Although the lesions were found in a wide variety of organs, the vessels of the heart were most frequently affected. An interesting complication of the arteritis was found in one animal dying 11 days after the injection of bovine albumin. In this animal (No. 43, Table II) there was extensive ulceration of the stomach, apparently due to widespread necrosis of arteries. One of the ulcers perforated and resulted in an acute peritonitis.

Changes in the heart valves were seen in 54 per cent of animals killed on the 12th day. The basic similarity between the morphology of the experimental lesions and those seen in rheumatic fever has already been emphasized by others (18).

An unexpected result was the high incidence of glomerular changes in over 80 per cent of animals sacrificed on the 12th and 15th day after the injection of bovine albumin. The renal lesions were transient in nature; no late lesions were encountered. Hawn and Janeway (7) observed a high incidence of glomerular lesions after the injection of bovine gamma globulin but not after bovine albumin. It may be of importance that these workers used four times the dose of albumin that was employed in the present study. On the basis of these findings, Hawn and Janeway suggested a possible relationship between the organs involved and the chemical nature of the antigen. The present results demonstrate the need for studies on the influence of dose size alone before the effects of various antigens can be compared.

Histologic and Immunologic Correlations

The characteristic tissue changes following the injection of large amounts of antigen are considered by most workers to result from hypersensitivity, that is, antigen-antibody reaction. This concept is based on (*a*) the occurrence of tissue lesions at about the same time that antibody makes its appearance (12), (*b*) the occurrence of skin hypersensitivity (Arthus reaction) at the same time that tissue changes develop (3, 12), (*c*) regression of lesions following the disappearance of antigen (7), and (*d*) prevention of the lesions by agents that inhibit antibody formation (14).

Interpretation of the earlier work on experimental hypersensitivity is made difficult by the fact that mixtures of antigens (for example, horse serum) were employed. It is known that different antigens vary in the time at which an antibody response appears. For example, antibody following the intravenous administration of the gamma globulin fraction of bovine serum develops at the end of the 1st week whereas antibody to the albumin fraction appears at the end of the 2nd week (7). It is apparent that a correlation of the time of occurrence of the tissue lesions with the immune response to whole serum has little or no significance, and to make a correlation with each of the separate antigens in whole serum would be, of course, a considerable undertaking. The difficulties inherent in whole serum can be eliminated by the use of a single purified antigen such as bovine albumin or globulin.

In the present experiments, the application of quantitative immunologic techniques permitted determination of the rate of disappearance of bovine albumin from the circulation after its injection. The elimination of bovine albumin occurred in three phases and was usually followed by the appearance of serum antibody. Free antibody and antigen were never present in the blood simultaneously. The significance of the three phases of antigen elimination has been studied by Dixon and coworkers (19, 20). According to these investigators, the first phase of rapid loss of antigen results from equilibration of the antigen between the vascular and extravascular fluids. The second phase represents

the normal rate of catabolism of the antigen by the body after equilibrium has been established. Dixon and coworkers have accumulated considerable evidence indicating that the third or so called "immune" phase of antigen clearance is the result of a more rapid catabolism and elimination of antigen following its combination with newly formed antibody in the tissues.

The present studies have permitted a precise correlation between the development of the tissue lesions and the three phases of antigen elimination and circulating antibody. None of the tissue lesions ordinarily attributed to hypersensitivity was encountered during the first and second phases of antigen elimination. However, tissue lesions were first seen during the third or so called "immune" phase of antigen clearance. The greatest number of lesions was observed at about the end of the "immune" phase. With the complete removal of antigen and the appearance of circulating antibody, the number and severity of lesions declined. These temporal relationships support the hypothesis that the tissue lesions result from antigen-antibody combination.

The disappearance of antigen during the third phase of elimination has been considered to result from the combination of circulating antigen with antibody as it forms in the tissues. However, it is generally believed that antibody formation is not a function of all cells. If the tissue lesions are the result of the interaction of antigen and antibody, then one must explain how antibody gets to the tissue sites in the presence of circulating antigen. It has been suggested that the antigen becomes fixed to tissue and that antibody is carried to the tissue in cells such as lymphocytes or monocytes (7). Direct evidence for this is lacking. However, it is known from *in vitro* studies that soluble antigen-antibody complexes can occur when antibody is added to excess antigen (9). If such soluble complexes occur *in vivo*, then an intracellular transport mechanism to explain the dissemination of antibody may be unnecessary.

Almost all the animals sacrificed during the immune phase of antigen elimination showed one or more types of histologic change (Table II). As has been indicated, a glomerulonephritis was more frequently encountered than was an arteritis. However, the difference in the frequency of these two lesions may be more apparent than real for the focal character of the vascular lesions makes their presence easily overlooked when only a single section of each organ is examined.

It has been repeatedly observed that the presence of tissue lesions is not paralleled by the occurrence of the Arthus reaction (3, 21). The reason for this is now clear. The Arthus reaction and the tissue lesions develop at two different time intervals after the intravenous administration of antigen (Table VI and Text-fig. 5). The tissue lesions, which presumably result from the combination of circulating antigen with antibody in the tissues, appear while the antigen is still present in the circulation. The Arthus reaction results from the combination of circulating antibody with antigen introduced into the tissues (22–24)

and, therefore, occurs after the appearance of free antibody, or at a time when the tissue lesions are regressing.

Splenic and Lymph Node Lesions

Minor changes in the spleens and lymph nodes of animals treated with antigens have been described by numerous workers (11, 12, 25–28). The histopathology of these changes has in general consisted of (*a*) an activation of the germinal centers and (*b*) an accumulation of plasma cells and, to a slight extent, reticulo-endothelial cells in the red pulp or medulla. The alterations mentioned have been speculatively related to antibody formation but not to hypersensitivity. In fact, it has been claimed that hypersensitivity is not accompanied by any detectable alterations in the spleen or lymph nodes (7). The frequent occurrence in these experiments of lesions in the spleen and lymph nodes that are qualitatively different from any of the changes ordinarily related to antibody formation is believed noteworthy.

Usually, the lesions consisted of an accumulation of epithelioid cells and, less often, giant cells. In the spleen, this alteration was almost wholly confined to the follicles; in the lymph nodes, both the cortex and medulla were involved. Occasionally the follicular granulomas of the spleen contained a central core of fibrinoid material sometimes associated with a central artery showing fibrinoid necrosis. Such structures bore a superficial resemblance to tubercles. Both focal granulomas and a diffuse granulomatous reaction were encountered in the lymph nodes. In one lymph node, foci of necrosis were seen.

The frequent occurrence of the splenic and lymph node lesions in the experimental animals and their absence in the control animals clearly indicated that the leisons had developed in response to the administration of bovine albumin. In the absence of other findings, several possible explanations for the mechanism of development of these lesions might be entertained. One might consider (*a*) that the splenic and lymph node lesions resulted from infection introduced by the bovine albumin; (*b*) that the lesions resulted from a toxic effect of bovine albumin; (*c*) that the lesions represented cells active in antibody formation; or (*d*) that the lesions resulted from antigen-antibody combination. The distribution of the splenic lesions with respect to the follicles argues against an infectious etiology. Furthermore, it should be stated that numerous attempts to stain bacteria and fungi were unsuccessful. With respect to a toxic effect of bovine albumin, it should be noted that the animals which maintained the highest levels of circulating antigen did not develop lesions. There is no adequate evidence that the cells participating in the lesions are capable of antibody formation. Against this possibility was the observation that regression of the lesions occurred while increasing amounts of antibody were appearing in the circulation.

Considerable evidence was found to support the conclusion that the splenic

and lymph node lesions resulted from hypersensitivity or antigen-antibody combination. First, the earliest lesions occurred soon after the onset of antigen-antibody interaction as evidenced by the immune phase of antigen elimination. Secondly, regression of the lesions took place when the antigen had been eliminated and the reaction of antigen with antibody had ceased. Finally, the extent and age of the splenic lesions in individual animals sacrificed on the 12th day after the administration of the antigen more or less paralleled the degree and stage of the immune response.

In the case of the cardiovascular and renal lesions it was postulated that antibody was transported to the tissue sites. However, since the spleen and lymph nodes are generally considered sites of antibody formation, the possibility arises that the splenic and lymph node lesions result from the reaction of circulating antigen with antibody as it is formed in these organs. Such a mechanism might account for the observation that the peak incidence of the splenic and lymph node lesions tended to be earlier than that of the cardiovascular and renal lesions (Text-fig. 3).

The mechanism by which antigen-antibody reactions induce this type of tissue alteration is obscure. It is well known that certain insoluble agents foreign to the body can promote an epithelioid and giant cell reaction (29). Whether insoluble antigen-antibody precipitates act in this manner requires further study. The possibility that the splenic and lymph node lesions may have arisen as a result of vascular damage alone seems unlikely. The first lesions developed before any change in the blood vessels was discernible and only a few of the lesions were associated with any vascular damage. Furthermore, changes in the blood vessels in other organs were not accompanied by an epithelioid and giant cell reaction.

It should be noted that regression of the splenic lesions took place very rapidly. 12 days after the injection of antigen, the granulomatous lesions in the spleen were as frequent as the renal lesions, and at least twice as frequent as the arterial alterations. However, 3 days later, that is, on the 15th day, the splenic lesions were infrequent and slight, while the arterial and glomerular changes were very prominent. That a granulomatous alteration had previously been present in some of the negative spleens was indicated by the presence of considerable fibrosis about the central artery of some of the splenic follicles. It is well known both in the human being and in the experimental animal that there are marked individual differences in the response to a given antigen (3). For instance, in the present study, although the animals were all treated similarly, they varied considerably in their tendency to develop the arterial lesions. However, the results of the present experiments make it clear that the time of sacrifice of an animal in relation to its immune response is an important factor in determining the extent and, perhaps, type of tissue lesion which will be encountered.

In relation to the present experimentally induced splenic and lymph node lesions, it is of interest that similar granulomatous lesions have been observed in association with polyarteritis nodosa in man (30–35). The occurrence of these lesions in the spleens of patients with polyarteritis nodosa has been described in detail in a recent report by Ball and Davson (34). These workers noted that granulomatous lesions in the Malpighian bodies might be present when the vascular lesions of polyarteritis nodosa were inconspicuous or even absent. Granulomatous lesions in the lymph nodes have only occasionally been encountered in cases of polyarteritis nodosa (33).

Although in the present experiments, granulomatous lesions were observed only in the spleen and lymph nodes, some cases of polyarteritis nodosa in man show granulomatous foci in many organs (30–33, 35–37). These foci usually occur in relation to necrotic collagen or adjacent to or within the wall of a damaged vessel. Their occurrence in cases of polyarteritis nodosa resulting from hypersensitivity to drugs (32, 35, 37) or associated with asthma (33) has established that granulomata can result from hypersensitivity. The present experimental demonstration that a granulomatous reaction may be a manifestation of hypersensitivity supports this interpretation. In experiments now in progress in this laboratory, granulomatous lesions have been occasionally encountered in the lymphoid tissue of the bone marrow (Fig. 17) as well as in the spleen and lymph nodes of animals injected with bovine albumin. The association of the granulomatous reaction with lymphoid tissue has suggested the possibility that the widespread granulomatous foci encountered in some cases of polyarteritis nodosa might represent a response of the focal lymphocytic infiltration to repeated or prolonged exposure to antigen. Experimental studies are now in progress to explore this possibility.

SUMMARY

A histologic and quantitative immunologic study was made on a large group of rabbits injected intravenously with a single dose of crystallized bovine albumin and bled and sacrificed at various intervals after injection. Both cardiovascular and renal lesions were encountered in high incidence. The various stages in the development of these lesions were observed from their first appearance until their complete regression. The cardiac, vascular, and renal alterations were morphologically similar to those of rheumatic fever, polyarteritis nodosa, and acute glomerulonephritis, respectively, in man.

In addition to those tissue changes ordinarily attributed to hypersensitivity, peculiar granulomatous lesions consisting of epithelioid and foreign body giant cells were encountered in the follicles of the spleen and in the lymph nodes of a large proportion of the animals receiving the antigen. Similar granulomas in the spleen and lymph nodes as well as in other tissues have been described in polyarteritis nodosa. The present experimental demonstration that an epithe-

lioid and giant cell reaction may result from hypersensitivity provides evidence for the allergic origin of those cases of polyarteritis nodosa of unknown etiology containing this type of lesion.

In the present study the time of development of the cardiovascular, renal, and granulomatous lesions was determined in relation to the blood clearance of antigen and the time of appearance of circulating antibody. All the tissue lesions developed during the "immune" phase of antigen elimination and regressed after the antigen had been completely eliminated and free antibody had appeared in the circulation. These temporal relationships indicate that the tissue lesions which occur after the intravenous administration of foreign protein are the result of antigen-antibody combination.

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BIBLIOGRAPHY

1. Klinge, F., *Beitr. path. Anat. u. allg. Path.*, 1929–30, **83,** 185.
2. Vaubel, E., *Beitr. path. Anat. u. allg. Path.*, 1932, **89,** 374.
3. Rich, A. R., and Gregory, J. E., *Bull. Johns Hopkins Hosp.*, 1943, **72,** 65.
4. Rich, A. R., and Gregory, J. E., *Bull. John Hopkins Hosp.*, 1943, **73,** 239.
5. Rich, A. R., and Gregory, J. E., *Bull. Johns Hopkins Hosp.*, 1944, **75,** 1.
6. Gregory, J. E., and Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1946, **78,** 1.
7. Hawn, C. V. Z., and Janeway, C. A., *J. Exp. Med.*, 1947, **85,** 571.
8. Lillie, R. D., Histopathologic Technic, Philadelphia, The Blakiston Co., 1948.
9. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, Charles C. Thomas, 1948.
10. Miller, C. P., *J. Exp. Med.*, 1924, **40,** 543.
11. Rich, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1935, **32,** 1349.
12. Ehrich, W., Seifter, J., and Forman, C., *J. Exp. Med.*, 1949, **89,** 23.
13. Apitz, K., *Virchows Arch. path. Anat.*, 1933, **289,** 46.
14. Schwab, L., Moll, F. C., Hall, T., Brean, H., Kirk, M., Hawn, C. V. Z., and Janeway, C. A., *J. Exp. Med.*, 1950, **91,** 505.
15. Hopps, H. C., and Wissler, R. W., *J. Lab. and Clin. Med.*, 1946, **31,** 939.
16. Fox, R. A., and Jones, L. R., *Proc. Soc. Exp. Biol. and Med.*, 1944, **55,** 294.
17. More, R. H., and McLean, C. R., *Am. J. Path.*, 1949, **25,** 413.
18. More, R. H., Waugh, D., and Kobernick, S. D., *J. Exp. Med.*, 1949, **89,** 555.
19. Dixon, F. J., Bukantz, S. C., and Dammin, G. J., *Science*, 1951, **113,** 274.
20. Dixon, F. J., Bukantz, S. C., Dammin, G. J., and Talmadge, D. W., *Fed. Proc.*, 1951, **10,** 553.
21. Berthrong, M., Rich, A. R., and Griffith, P. C., *Bull. Johns Hopkins Hosp.*, 1950, **86,** 131.
22. Opie, E. L., *J. Immunol.*, 1924, **9,** 231.
23. Culbertson, J. T., *J. Immunol.*, 1935, **29,** 29.
24. Germuth, F. G., Jr., Oyama, J., and Ottinger, B., *J. Exp. Med.*, 1951, **94,** 139.

25. Fagraeus, A., Antibody Production in Relation to the Development of Plasma Cells, Stockholm, Esselte Aktiebolag, 1948.
26. Bjorneboe, M., and Gormsen, H., *Acta path. et microbiol. scand.*, 1943, **20,** 649.
27. Ehrich, W. E., and Harris, T. W., *J. Exp. Med.*, 1942, **76,** 335.
28. Ringertz, N., and Adamson, C. A., *Acta path. et microbiol. scand.*, 1950, suppl. 86, 1.
29. Rich, A. R., The Pathogenesis of Tuberculosis, Springfield, Illinois, Charles C. Thomas, 1951.
30. Ophüls, W., *Arch. Int. Med.*, 1923, **32,** 870.
31. Rössle, R., *Virchows Arch. path. Anat.*, 1933, **288,** 780.
32. Rich, A. R., *Bull. Johns Hopkins Hosp.*, 1945, **77,** 43.
33. Churg, J., and Strauss, L., *Am. J. Path.*, 1951, **27,** 277.
34. Ball, J., and Davson, J., *J. Path. and Bact.*, 1949, **61,** 569.
35. Waugh, D., *Am. J. Path.*, 1952, **28,** 437.
36. Bergstrand, H., *J. Path. and Bact.*, 1946, **58,** 399.
37. More, R. H., McMillan, G. C., and Duff, G. L., *Am. J. Path.*, 1946, **22,** 703.
38. Germuth, F. G., Jr., and Ottinger, B., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 815.

EXPLANATION OF PLATES

Photographs by K. Cramer Lewis, Washington University School of Medicine.

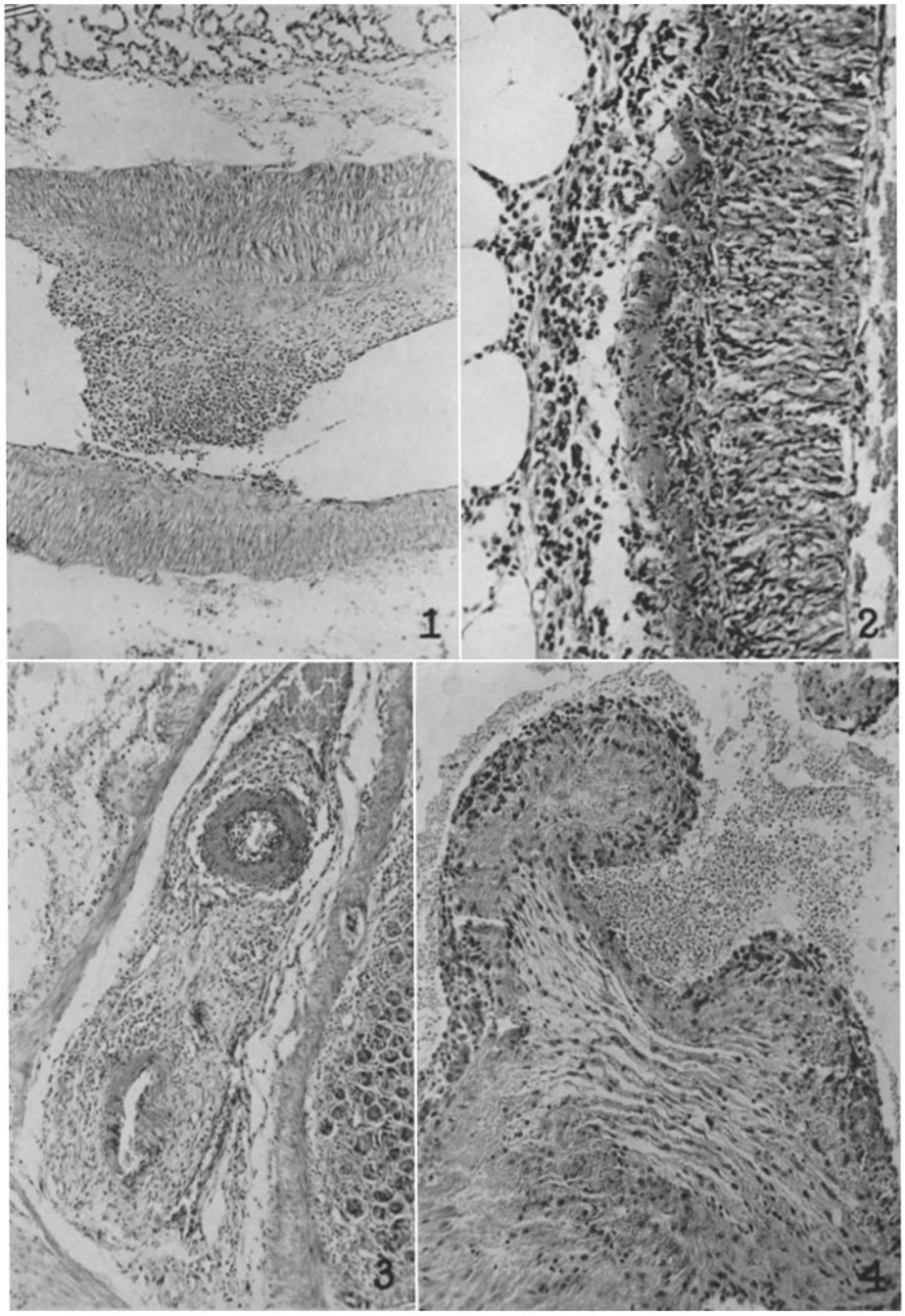
PLATE 14

FIG. 1. Rabbit 15, sacrificed at 15 days. Segment of a branch of the pulmonary artery showing intimal proliferation and subendothelial infiltration with mononuclear cells. The media is edematous but otherwise normal. Azure, eosin, and hematoxylin. × 75.

FIG. 2. Rabbit 37, sacrificed at 12 days. Section of pancreatic artery. Edema, polymorphonuclear leukocytic infiltration and necrosis of media, fibrinoid in adventitia, and leukocytic infiltration of the surrounding tissue. Azure, eosin, and hematoxylin. × 200.

FIG. 3. Rabbit 46, sacrificed at 12 days. Section of wall of stomach showing necrosis and fibrinoid degeneration of two small arteries. Azure, eosin, and hematoxylin. × 70.

FIG. 4. Rabbit 23 a, sacrificed at 15 days. Section of mitral valve and valve ring showing endothelial proliferation and subendothelial infiltration with mononuclear leukocytes. The ground substance is edematous and contains numerous large mononuclear cells and Anitschkow myocytes. Hematoxylin and eosin. × 120.



(Germuth: Induced hypersensitivity of serum sickness type)

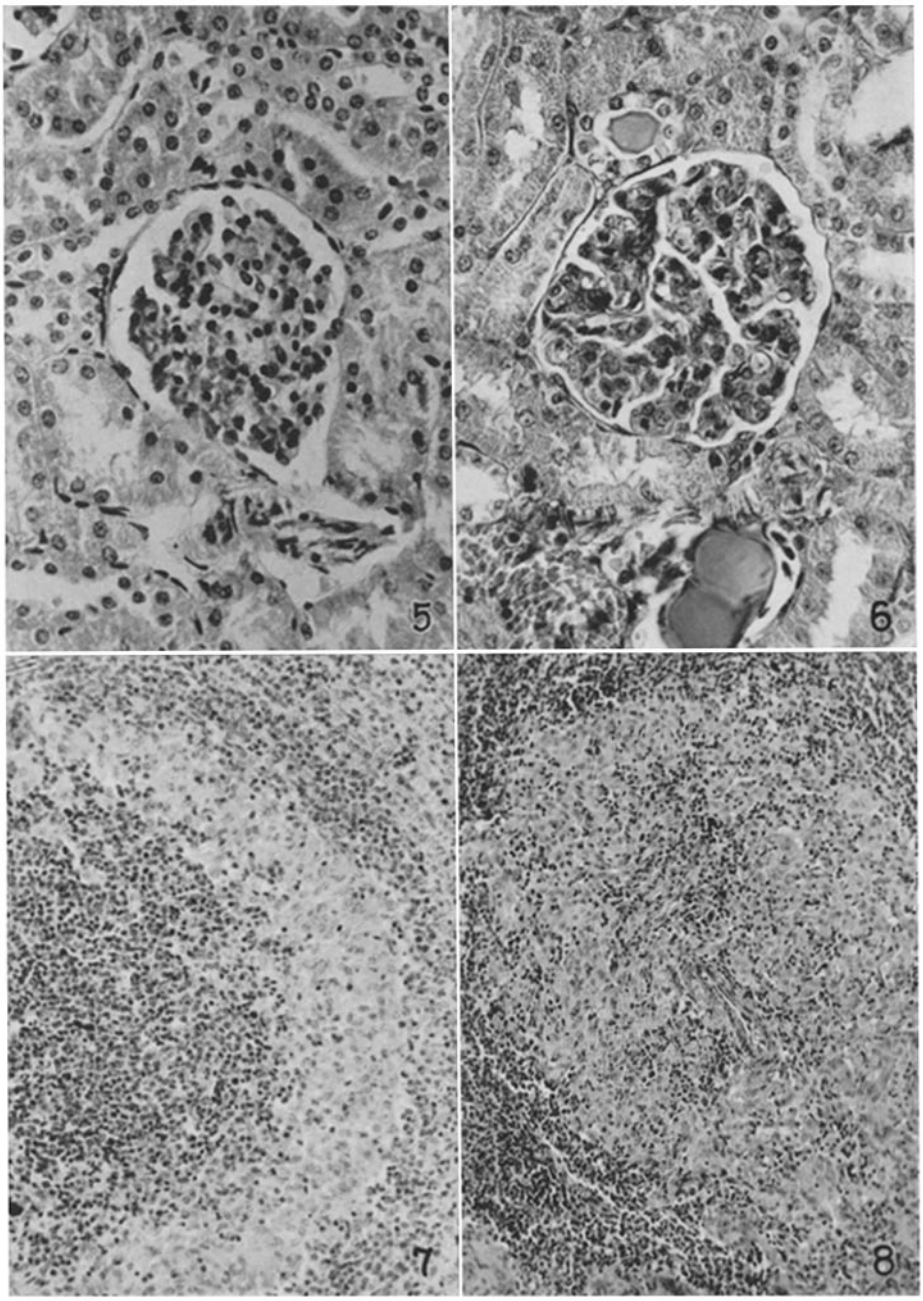
PLATE 15

FIG. 5. Normal rabbit. Glomerulus with delicate and patent capillary loops. Azure, eosin, and hematoxylin. × 340.

FIG. 6. Rabbit 35, sacrificed at 12 days. Diffuse glomerulonephritis, graded +++. Numerous swollen epithelial and endothelial cells occlude the glomerular capillaries. Two protein casts can be seen in the distal convoluted tubules. Droplets of protein are present in the epithelium of the proximal convoluted tubules. Azure, eosin, and hematoxylin. × 340.

FIG. 7. Rabbit 31 a. Early lesion in the spleen showing compact layer of macrophages with epithelial appearance in the marginal zone of a follicle. Hematoxylin and eosin. × 170.

FIG. 8. Rabbit 49, sacrificed at 12 days. Epithelioid and multinucleated cells replacing almost all of the lymphoid tissue of a splenic follicle. Note that the central artery is normal. Hematoxylin and eosin. × 130.



(Germuth: Induced hypersensitivity of serum sickness type)

PLATE 16

FIG. 9. Rabbit 31 a, sacrificed at 8 days. Low power magnification of spleen containing granulomatous lesions in the follicles. Wilder's reticulum stain. × 15.

FIG. 10. Rabbit 31 a. Early lesion in a splenic follicle. The follicle is considerably larger than normal due to a proliferation of medium sized and large lymphocytes in the marginal zone. A prominent layer of pale staining macrophages extends around the central zone and about the central artery. Hematoxylin and eosin. × 75.

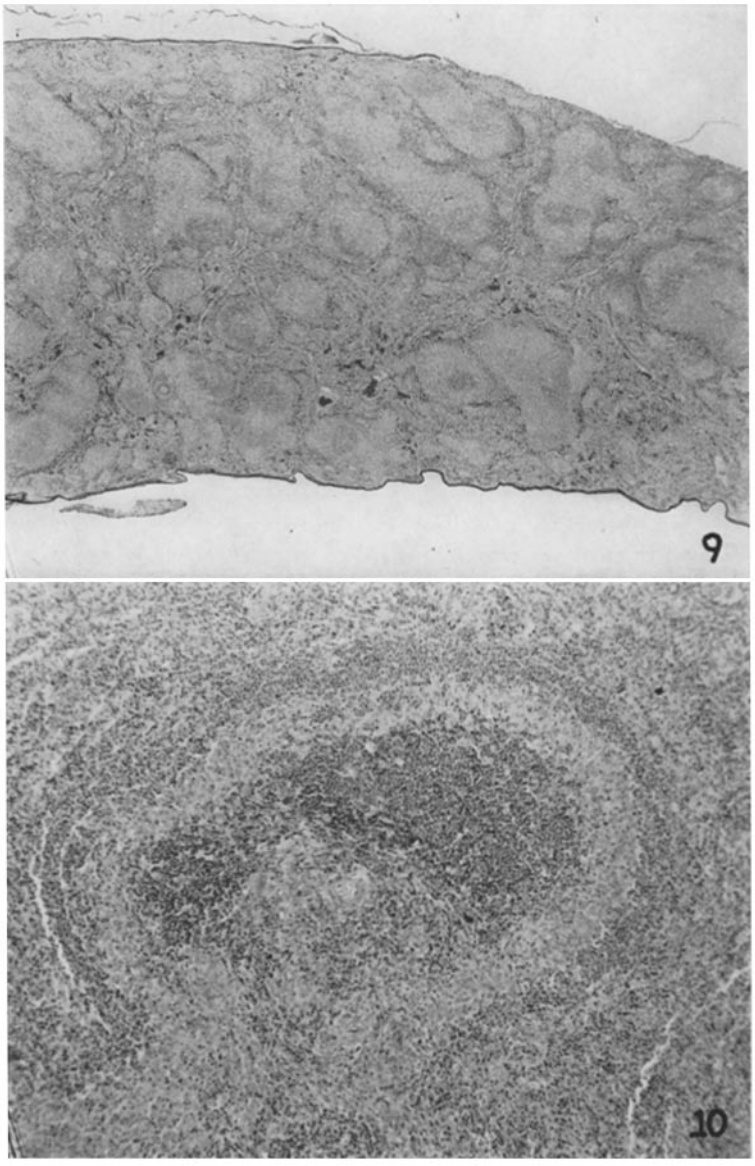


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PLATE 17

FIG. 11. Rabbit 49, sacrificed at 12 days. Granulomatous lesion in a splenic follicle consisting of numerous large foreign body giant cells with few epithelioid cells. Hematoxylin and eosin. × 145.

FIG. 12. Rabbit 37, sacrificed at 12 days. Splenic follicle showing a peripheral zone of epithelioid cells. The central artery shows segmental necrosis. Note that the peripheral lesion and the damaged artery are separated by a zone of normal lymphocytes. Hematoxylin and eosin. × 140.

FIG. 13. Rabbit 34, sacrificed at 12 days. Necrosis of a central artery and adjacent epithelioid cells with the deposition of fibrinoid. Hematoxylin and eosin. × 330.

FIG. 14. Rabbit 27, sacrificed at 15 days. Healing lesion in a splenic follicle. There are two distinct zones of epithelioid cells. Fibrinoid is present in the wall of the central artery. Hematoxylin and eosin. × 230

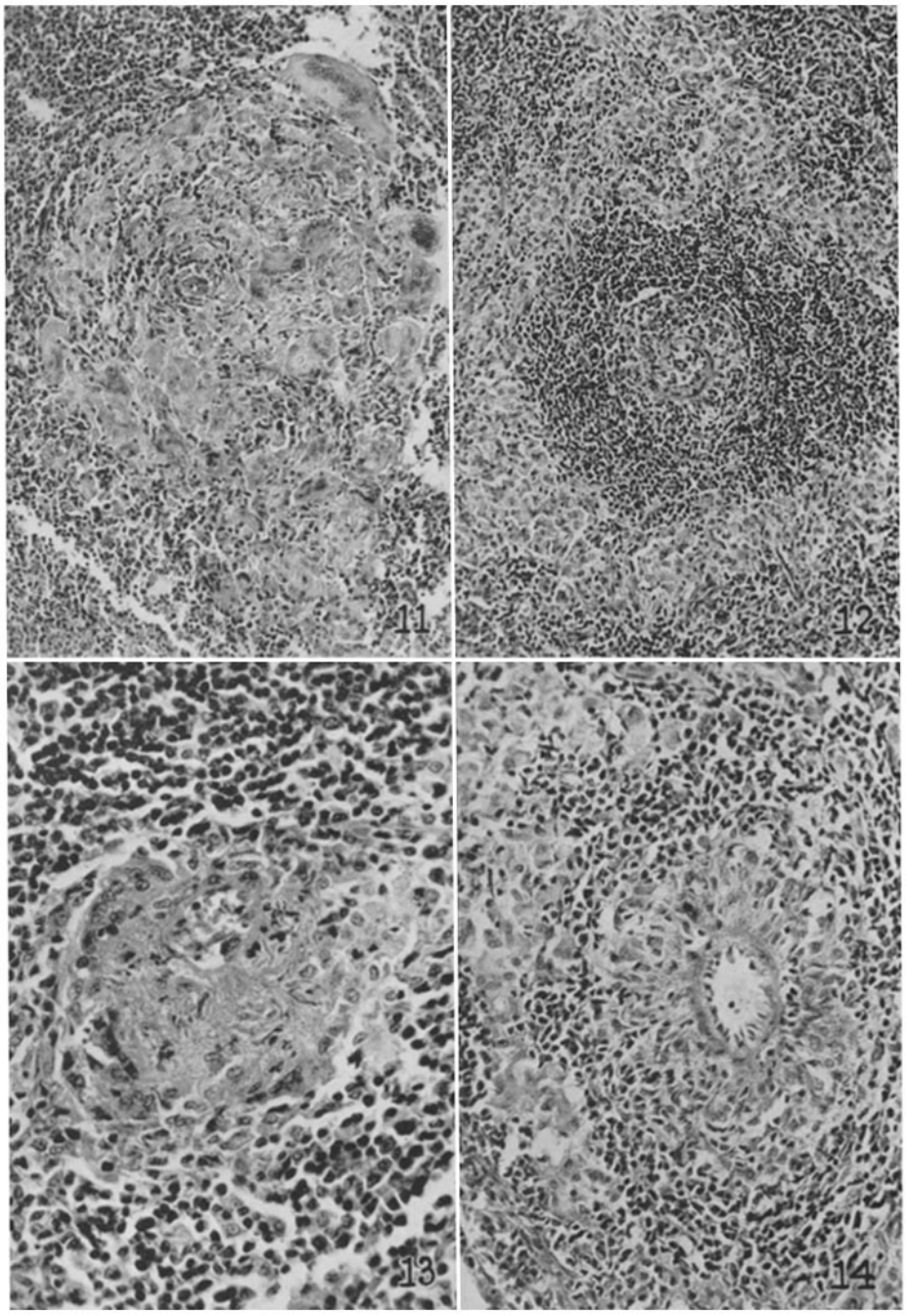

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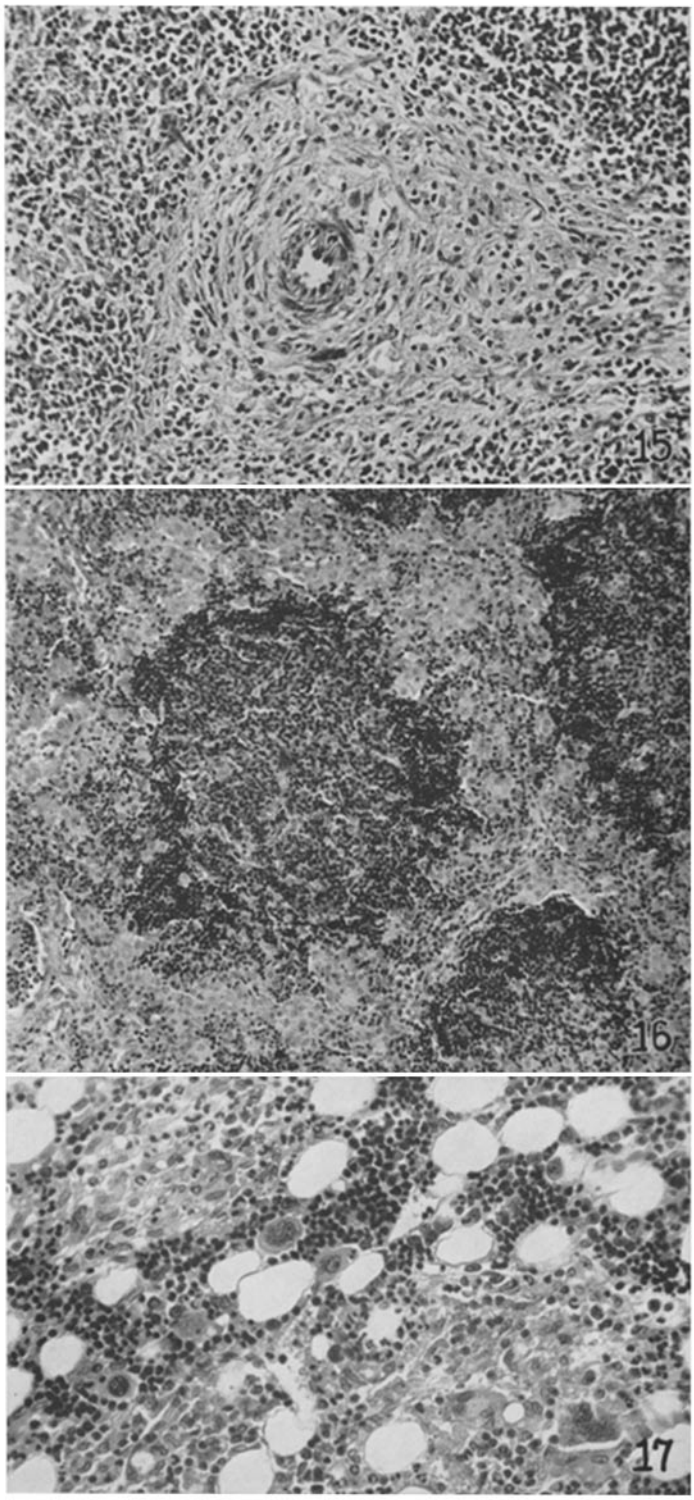
PLATE 18

FIG. 15. Rabbit 39, sacrificed at 12 days. Healed splenic lesion. There has been a proliferation of reticular and fibrous connective tissue about the central artery. This animal had developed circulating antibody. Hematoxylin and eosin. × 180.

FIG. 16. Rabbit 44, sacrificed at 12 days. Epithelioid cells surrounding the periphery of the follicles of a mesenteric lymph node. Hematoxylin and eosin. × 120.

FIG. 17. Focal accumulations of epithelioid and giant cells in the femoral marrow of a rabbit sacrificed at 10 days. Hematoxylin and eosin. × 230.

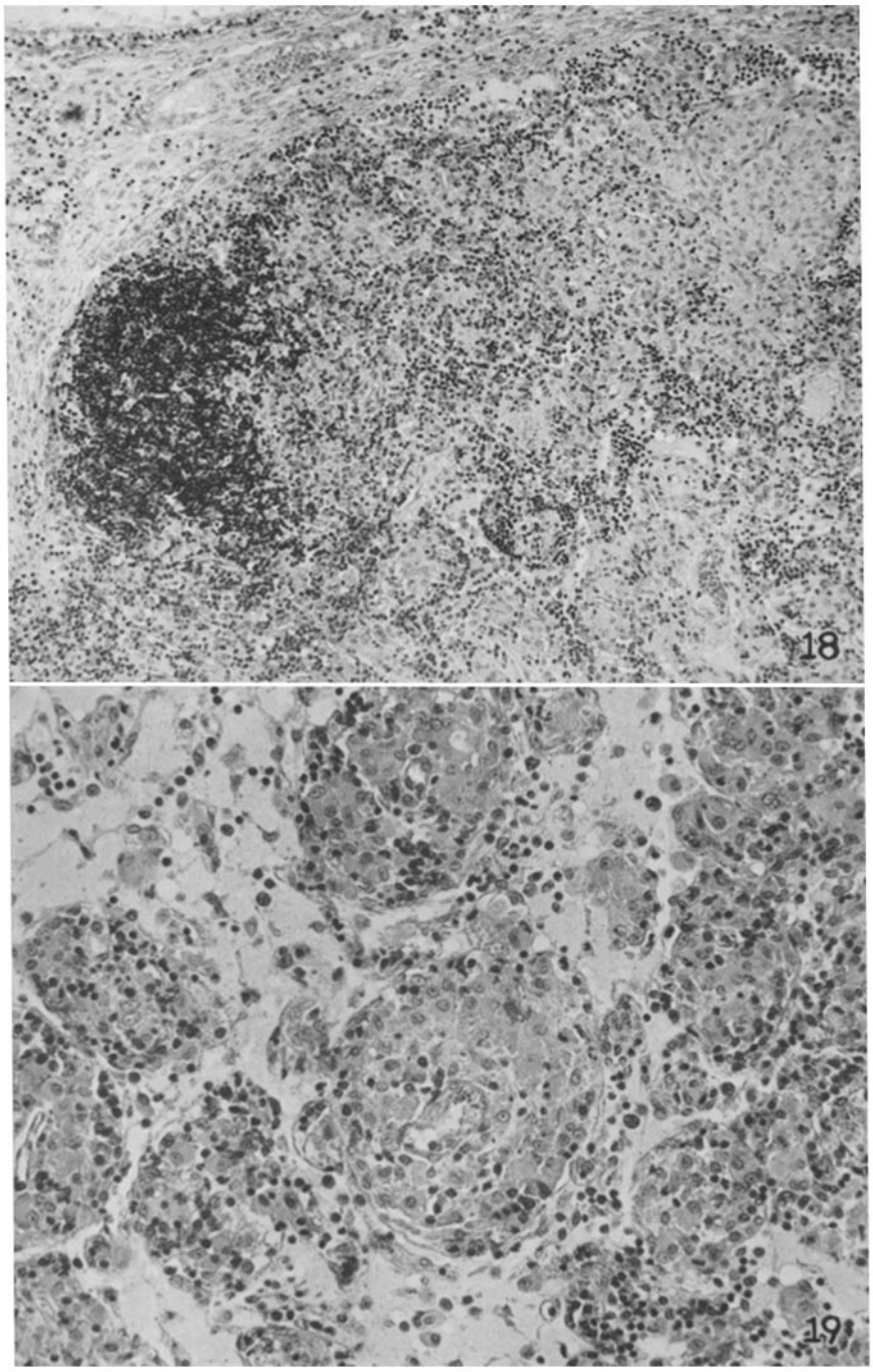


(Germuth: Induced hypersensitivity of serum sickness type)

PLATE 19

FIG. 18. Rabbit 44. Section of the cortex of a mesenteric lymph node. The follicle on the left has been partly infiltrated by epithelioid cells; on the right, the follicle has been completely replaced by these cells. Hematoxylin and eosin. × 130.

FIG. 19. Rabbit 44. Section of the medullary cords of a mesenteric lymph node showing more or less complete replacement of lymphoid tissue by aggregates of epithelioid cells. Hematoxylin and eosin. × 250.



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