EXPERIMENTAL SERUM DISEASE

A Pathogenetic Study

By W. E. EHRICH, M.D., JOSEPH SEIFTER, M.D., and CAROLYN FORMAN, M.D.

(From the Graduate School of Medicine of the University of Pennsylvania, the Wyeth Institute of Applied Biochemistry, and the Philadelphia General Hospital, Philadelphia)

Plates 1 to 3

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It is well established that large quantities of antigen injected intravenously cause characteristic tissue changes. If whole bacteria are injected, the recipient develops mesenchymal reactions especially in lungs, spleen, and liver. If serum is used, the recipient may acquire serum disease with special involvement of skin, lymph nodes, joints, heart, lungs, spleen, and kidneys. The mesenchymal reactions produced by bacteria and other corpuscular antigens have been made the topic of a recent report of the Council on Pharmacy and Chemistry of the American Medical Association (1). The present discussion is limited to the reactions caused by dissolved antigens.

The various tissue changes caused by large doses of serum or other dissolved antigens given intravenously were first[1] described by Klinge and his associates (1932) (2, 3), by Apitz (1933) (4), and by Masugi and his group (1934) (5–7). The cardiac lesions were well illustrated by Apitz (4), the glomerular lesions by Masugi and Sato (5), and the arterial lesions by Miura (7). The experimental lesions closely resemble those of human serum disease, rheumatic fever (or rheumatoid arthritis), glomerular nephritis, and periarteritis nodosa.

The observations of these early workers have been confirmed and extended by Rich and Gregory (8–12) and by Hawn and Janeway (13). Rich and Gregory have furnished many excellent illustrations, notably of the arterial and cardiac lesions.

It is noteworthy that the literature contains no attempt to distinguish between the various lesions from the pathogenetic point of view. Rich (12) commented on the variety of organs involved saying that the differences in organs affected in different rabbits were possibly due to heredity. Hawn and Janeway (13) who used pure albumin and globulin fractions in their experiments, found that albumin affected the arteries especially, whereas globulin damaged the

[1] It is of historical interest that experimental periarteritis and other mesenchymal reactions to antigens were well described and illustrated by Harris and Friedrichs in this *Journal* in 1922. The lesions were produced with filtrates of tissues from a case of human periarteritis. They were interpreted as caused by an infectious agent.

glomeruli and the myocardium in special. When commenting on these results they expressed the view that the organ differences were possibly due to chemical differences in the antigens used.

The various tissue changes caused by serum have all been explained by allergy, or more precisely, by the anaphylactic type of hypersensitivity. It has been assumed that the antibodies elicited by the serum became fixed in or on the cells of the vascular connective tissue, and that the lesions were due to an Arthus phenomenon, that is to say to an antigen-antibody reaction in or on these cells. Such a reaction could be brought about either by a second injection of antigen after abundant antibody had been fixed after a first injection, or by a single injection if antigen was still in circulation when antibody was formed and laid down. The simultaneous occurrence of antigen and antibody after intravenous injection of serum has been demonstrated repeatedly.

It had been observed previously, however, that many of these lesions appear within 24 hours, and reach considerable intensity within 3 days, after a single injection of antigen (14–17). As they appeared before significant quantities of antibody could have formed, they were interpreted as "primary reactions of the organism to bacteria or their products" (14) or as representing "the nonspecific reaction of the animals in the process of disposing of certain foreign materials injected into the blood stream" (15). It is of interest that some of these reactions have recently been interpreted as morphologic expressions of antibody production (Bjørneboe and Gormsen, 18).

These various observations seemed to indicate a need for more detailed analysis of the various tissue reactions that follow the intravenous injection of serum. The results of this study were illuminating for it was found that there are normergic as well as allergic reactions; that there are at least two Arthus phenomena, a subacute and acute; and also that the various antigenic diseases, first produced experimentally 16 years ago, are distinct entities from the pathogenetic point of view.

EXPERIMENTAL

All experiments were performed in rabbits. With few exceptions they were males of the Chinchilla variety; however some albinos and females were used.

1. A first series of experiments was designed to study the effect on young and old rabbits of a single large dose of horse serum obtained from different commercial sources (Wyeth (serum A), Lederle (serum B), and Squibb (serum C)).[2] The sera contained 3.4 to 4.0 per cent of globulin.[3] Ten ml. of serum per kg. body weight was administered intravenously to 32

[2] We are indebted to Dr. B. Scott Fritz and Dr. J. H. Brown of the Wyeth Incorporated Biological Laboratories at Marietta, Pennsylvania, and to Mr. George F. Squibb and the Squibb Institute for Medical Research in New Brunswick, New Jersey, for supplying most of the horse serum used in this study.

[3] We are indebted to Dr. J. Reinhold for determining the globulin concentrations.

animals divided into 4 groups, 3 of which consisted of young rabbits averaging 2.3 or 2.4 kg. in body weight, and the fourth of old rabbits averaging 3.4 kg. A fifth group of 6 rabbits weighing 3.4 kg. served as controls. Ten animals were sacrificed after 11 days, 14 after 14 days, and 8 after 19 days.

The weight of the rabbits did not change significantly during the first 2 weeks of the experiment. Thereafter, it increased in the 8 young animals that were allowed to live until the 19th day. The rectal temperature remained fairly constant between the 4th and 7th day in 18 rabbits studied in relation to this point. The ears of the rabbits showed hyperemia and edema on the 5th day in the one experiment in which they were observed. The injection of 0.1 ml. of horse serum intracutaneously on the 12th day caused a marked reaction at the site of injection in 12 of 16 rabbits studied in relation to this point.

Urinalysis revealed small amounts of protein (trace to +) in 3 of 8 animals in which the urine was tested before the administration of horse serum. On the 5th day after injection, 6 of these showed some protein (trace to ++) and 5 some erythrocytes (5 to numerous per field). On the 13th day, only 4 revealed some protein (trace to ++) and 3 some erythrocytes (1 to 5 per field). Of 8 rabbits examined on the 7th day only, 4 showed some protein (trace to +++) and 1 numerous erythrocytes. Of 9 tested on the 8th day only, 1 showed a trace

TABLE I

Precipitin Titers in Rabbits Injected with 1 Dose of 10 Ml. of Horse Serum per Kg. of Body Weight

	Days	No. of animals			
		1:1	1:10	1:40	1:100
Young rabbits	5	16*	15*	9*	4*
	11	26	26	24	12
Old rabbits	11	6	6	2	0

* The figures in these columns represent the number of rabbits showing a positive precipitin test in the dilutions indicated. Undiluted rabbit serum (1:1) precipitated horse serum in all animals tested. The numbers in the first column under "No. of animals" thus represent the total number of rabbits tested on the days indicated.

of protein and 2 some erythrocytes (25 to 75 per field). These figures seem to show that increased leakage from the glomeruli occurred early during the experiment, but at the time of sacrifice this had disappeared.

Precipitin tests with horse serum diluted with 9 parts of saline showed good antibody response in the young rabbits treated with sera A or C, but poor antibody formation in the old rabbits treated with serum B (Table I).

2. A second series of experiments was undertaken to study the effect of duck serum instead of horse serum on a group of rabbits with an average weight of 2.3 kg. Six animals received single doses of 1.8 to 4.2 ml. per kg., 2 received 3 doses on 3 successive days totalling 8.5 or 9 ml. per kg., and 3 received a single injection of 10 ml. per kg. All were sacrificed 15 or 17 days after the first injection. There had been no significant change in weight in these animals. The rectal temperature was slightly elevated on the 6th day in the 5 animals that received the largest doses. All showed a reaction in the skin at the site of injection of 0.1 ml. of duck serum 12 days after the first injection.

3. A third series of experiments was performed to study the early development of the various tissue changes of serum disease. As the preceding experiments had shown that doses under 10 ml. per kg. were not effective, the 13 rabbits of this series were given 20 ml. of horse erum C per kg. in 2 doses administered within 1 hour. Four animals were sacrificed after 3s days, 4 after 5 days, 2 after 7 days, and 3 after 14 days.

4. In a last series of experiments the effect of 2 doses of 10 ml. of horse serum per kg. was tested in 2 groups of 5 rabbits each. Five of these animals were utilized at St. Louis,[4] the other 5 at Philadelphia. The latter had an average weight of 2.0 kg., the former weighed between 2.0 and 2.5 kg. Three of these animals showed a moderate rise in temperature on the 5th day following the first injection, and 1 on the day following the second injection. All showed flushing of the ears from the 5th to the 7th day and 4 showed a reaction in the skin at the site of injection of 0.1 ml. of horse serum 11 days after the first injection. Three of each group were sacrificed 26 days after the first injection (7 days after the second) and 2 after 34 days (15 days after the second).

Morphological Findings

The weights of spleens and adrenals were recorded in 2 experimental groups and in the controls, and of the thymus in all groups, of the first series of experiments. While spleen and thymus were considerably enlarged, the adrenals were significantly smaller than those of normal controls.

The histologic changes were most marked in heart, lungs, kidneys, and spleen. With 20 ml. of serum per kg. of body weight the liver was also affected.

Heart.—As pointed out by Apitz (4), the portions of the heart most commonly affected were auricle and ventricle of the right heart. With 20 ml. of serum per kg. the left heart was also involved though less severely than the right. The tissue of the right heart most severely affected was the myocardium. The first change observed was focal activation of mesenchymal cells around small blood vessels and elsewhere in the myocardium. On the 5th day mitotic figures were encountered in these cells. This was followed by widespread proliferation of mesenchymal cells including immature plasma cells on the 7th day. Polymorphonuclear leukocytes and broken or pyknotic nuclei were occasionally seen during the first week of experimentation.

Animals sacrificed on the 11th day or later often showed focal hyaline degeneration or disintegration of muscle fibers surrounded by proliferating mesenchymal cells including plasma cells and a few eosinophilic leukocytes (Fig. 1). This lesion was seen by Longcope (19) and was fully described and well illustrated by Apitz (4). There may have been some disintegration of a few muscle cells before the 11th day, but if it occurred, it was not conspicuous and possibly of a different nature from the hyaline degeneration and disintegration which occurred later.

Occasionally perivascular accumulations of mesenchymal cells resembling Aschoff bodies were observed. These were noted by Junghans (3), but were fully described only by Rich and Gregory (10).

The degree of the myocardial lesions varied considerably in individual animals. In the more severe cases the mural endocardium (Fig. 2) and the pericardium were affected as well.

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[4] We are indebted to Dr. Margaret Henry and Dr. W. Barry Wood for permission to use their animals.

The lesions in the endocardium appeared to be covered by well preserved endothelial cells as noted by Apitz (4).

Ten of the 47 animals with myocardial lesions showed valvular lesions also. These were most marked at the base of the valve (Fig. 3), as described by Junghans (3), but in some instances the leaflets were involved as well (Fig. 4). Histologically these lesions consisted of elements similar to those of the myocardial and endocardial lesions. There were mesenchymal cells of various descriptions, often covered with well preserved endothelium. The valvular lesions have been fully described by Rich and Gregory (9, 10).

It is noteworthy that no correlation was found between myocardial and valvular lesions. In fact, the animals which had the most severe valvular lesions, showed only mild myocardial lesions and *vice versa*.

Considering the time of appearance of the cardiac lesions, it is apparent from Table II, series 3, that some arose during the first week of the experiment. Mesenchymal proliferation in the myocardium was present 3 days after the first injection of serum (the first day when the animals were sacrificed). In Apitz' (4) experiments they were noted as early as on the 2nd day after the injection of 15 to 20 ml. of horse serum, but his animals had been sensitized previously. Lesions resembling Aschoff bodies and valvular lesions were observed in our material only 11 days after the first injection.

Lungs.—The pulmonary lesions were among the most conspicuous of the various tissue changes. The structures affected most severely were the small arteries and veins, and the alveolar septa. A typical lesion consisted, as previously described (14–16), of proliferating mesenchymal cells and plasma cells together with lymphocytes and mononuclear cells in the intimal and adventitial layers of the small vessels (Fig. 5), in the septa of the alveoli, and in the connective tissue surrounding the bronchi. These lesions were present in 17 of 26 young rabbits and in 1 of 6 old rabbits sacrificed 11 to 19 days after 10 ml. of horse serum per kg. of body weight; they were present in all rabbits that received 1 injection of 20 ml. per kg. or 2 injections of 10 ml. per kg. (Table II).

In the more severe cases the large arteries and veins were affected as well, the former more than the latter. The lesions in the large arteries were chiefly intimal (Fig. 6), while in the large veins the adventitial tissue was also affected (Fig. 7). The intimal changes, which had been observed already by Apitz (4), closely resembled those of the heart valves.

There was some relationship in frequency between the lesions in the lungs and those in the myocardium if whole groups were compared. But in individual animals, correlation did not exist; in fact, in some of the most severe cases of pulmonary reaction there was nothing in the heart, and *vice versa*.

As to the histogenesis of the pulmonary lesions, it was pointed out elsewhere (16) that they arose as early as within the first 24 hours after the injection of antigen. Accordingly, they were well developed on the 3rd day of the present experiments. There were at this time many disintegrating granulocytes and large basophilic round cells, often with mitotic figures. Where the septa were thickened, many macrophages often containing many granules of disintegrated granulocytes were seen in the septa and alveoli. After 5 and 7 days fresh infiltrations with intact eosinophilic or pseudoeosinophilic granulocytes were noted in the various lesions within the small vessels, the large vessels, and the interstitial tissue (Fig. 8). Sometimes these infiltrations appeared quite independently of preexisting lesions (Fig. 9). The granulocytic infiltration was associated with a greater breakdown and phagocytosis of lymphocytes within the germinal centers of the bronchial mucosa.

Kidneys.—The structures most severely affected in the kidneys were the glomeruli. The typical lesion consisted of the proliferative or "intracapillary" type of diffuse glomerular nephritis, showing all transitions from a mild increase in cellularity to marked proliferation

TABLE II

Frequency (a) and Intensity (b)‡ of the Various Lesions Caused by Injected Serum*

	Days after first injection	No. of animals	Av. wt.	Heart				Lungs		Glomerulitis				Spleen		Arteritis			
				Myocardium		Valves				Intracap.		Extra-cap.		Activity of germinal centers	Reaction in pulp	Sub-acute		Recurrent	
				(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)			(a)	(b)§	(a)	(b)§
			kg.																
Series 1																			
Salicylate control (serum A)‖	—	6	2.4	0	0	0	0	33.5	+	0	0	0	0	+	+++-++++	0	0	0	0
Group IV (horse serum C)	11	10	2.4	100	0-+	50	++++	50	+-++	90	+	0	0	+++	++-+++	50	1-2	0	0
Group II (horse serum A)	14	8	2.4	100	++-+++	50	++	87.5	+-++	100	+-++	0	0	++++	+++-++++	50	1-2	0	0
Group I (horse serum A)	19	8	2.3	100	++-+++	0	0	62.5	+-++	100	+-++	0	0	++++	++++	25	1	0	0
Untreated control	—	6	3.4	67	+	0	0	50	+-++	0	0	0	0	0-+	+++-++++	0	0	0	0
Group III (horse serum B)	14	6	3.4	17	+	0	0	17	++	33	+	0	0	+++-++++	+++-++++	33	1-2	0	0
Series 2																			
Duck serum, 1.8–4.2 ml./kg.	15	6	2.4	50	0-+	0	0	17	+	17	0-+	0	0	++	+++	0	0	0	0
Duck serum, 8.5–10 ml./kg.	17	5	2.1	80	+	0	0	80	+	100	+	0	0	+++	+++-++++	20	1	0	0
Series 3																			
Horse serum C, 20 ml./kg.	3	4	—	75	++	0	0	100	+++-++++	25	++++	0	0	+-++	++-+++	0	0	0	0
	5	4	—	100	++	0	0	100	++++	50	0-+	0	0	+++	++++	0	0	0	0
	7	2	—	100	++-+++	0	0	100	++++	100	++	0	0	++++	+++-++++	0	0	0	0
	14	3	—	100	++-+++	33	+++	100	+	100	++	0	0	+++-++++	+	67	2	0	0
Series 4																			
Horse serum, 2 inj. of 10 ml./kg.,	26	3	—	33	++-+++	—	—	100	++	0	0	0	0	—	—	33	2	100	1-2
St. Louis	34	2	—	0	0	—	—	100	++-+++	0	0	0	0	—	—	0	0	0	0
Horse serum A, 2 inj. of 10 ml./kg.,	26	3	2.0	100	+	—	—	100	++	0	0	67	+	+-++**	+-++**	100	1-2	33	1
Philadelphia	34	2	2.0	100¶	—	—	—	100	++	0	0	0	0	++**	+++-++++**	0	0	0	0

* The frequency has been expressed in per cent of animals affected, in order to facilitate comparison.

‡ The plus signs refer to the positive cases only.

§ Number of organs involved.

‖ This group of rabbits was treated with salicylates as well as with serum.

¶ These hearts showed fibrosis rather than cellular proliferations. Their intensity was not graded.

** Only 1 spleen available for microscopic study.

of mesenchymal cells (Figs. 10 and 11). This experimental lesion was described and illustrated first by Masugi and his associates in animals injected with antikidney serum (20, 21) and in rabbits receiving horse serum or vaccines (5, 22, 7); but no distinction was made between intracapillary and extracapillary glomerular nephritis in their studies or subsequent ones (23).

Diffuse intracapillary glomerular nephritis was observed in 35 of 46 animals sacrificed 11 to 19 days after the introduction of serum. It was most marked in the animals that received the largest dose of serum, and mildest in the old animals (Table II). Unlike the pulmonary proliferations, the glomerular lesions showed good correlation with the myocardial ones, both in frequency and intensity, if whole groups were compared. But here too the correlation was incomplete, if individual animals were considered. It is noteworthy that Hawn and Janeway's (13) animals also showed close correlation between renal and myocardial lesions, whereas the arterial changes were independent of both.

As bearing upon the histogenesis of the intracapillary glomerular nephritis, it can be stated that increase in cellularity of the glomeruli was observed in 1 of 4 animals sacrificed on the 3rd day, and in 2 of 4 sacrificed on the 5th (Table II, series 3). Seven days after the injection of 20 ml. of serum per kg. proliferation was mild in one, and marked in the other. The proliferation was preceded by swelling of the loops with considerable pyknosis of nuclei (Fig. 12). The latter change was described by Longcope (19), and swelling with doubling of the number of nuclei was seen by Hawn and Janeway (13) 7 days after the injection of bovine gamma globulin.

In the 10 animals that were sacrificed 26 to 34 days after the first of two injections of horse serum, intracapillary glomerular nephritis was not observed. Instead, we found the extracapillary variety, namely partial necrosis of loops with a fibrinous exudate in the glomerular space and early adhesions (Fig. 13). This lesion has been described and illustrated repeatedly by Masugi and his group (20–22); it has been illustrated once by Rich and Gregory (8). The difference in pathogenesis between these two types of nephritis, however, was not recognized.

Spleen.—The reaction of the spleen to intravenous injection of antigen has often been described. At least two reactions have been observed, namely, activation of germinal centers in the white pulp (14, 15, 2, 4, 17) and proliferation of plasma cells and other mesenchymal elements in the red pulp (14, 15, 17, 24). In the present series of animals, activation of germinal centers was moderate 3 days after the injection of horse serum, and marked after 5 to 19 days. It was moderate after the injection of 1.8 to 4.2. ml. per kg. of duck serum, but marked after 8.5 to 10.0 ml. per kg. The mesenchymal reactions in the red pulp varied considerably from individual to individual. On the whole they were most marked in the animals showing greatest activation of germinal centers and *vice versa*. There was some correlation of the activation of germinal centers and the mesenchymal reactions with the proliferations in the myocardium and in the kidneys, but again the relationship was incomplete.

In addition to the reactions in the germinal centers and red pulp, some animals developed marked reticuloendothelial proliferation in the lymph sheaths around the arteries. This lesion seemed to be distinct from those already mentioned, and from the allergic arteritis to be described later. The reticuloendothelial proliferation was most marked in the rabbits which received serum C (Table II, series 1 IV, and series 3) though it occurred also in some animals of other series.

The histogenesis of the changes in the germinal centers and red pulp has been described in a previous communication (17). The lesions in the red pulp resembled those in the lungs, both in time of appearance and in cellular composition and therefore do not need to be described again. The reticuloendothelial reaction in the lymph sheaths was conspicuous 3 days after the injection of 20 ml. of horse serum per kg., that is to say at a time when numerous large

mesenchymal cells with many mitotic figures were found surrounding the arteries. After 5 days there were many macrophages with abundant somewhat acidophilic cytoplasm, and there were giant cells as well.

In the spleens of the animals sacrificed after a second injection, the germinal centers were quite inactive while the pulp in 1 animal revealed marked myeloid metaplasia, and in another, marked reticuloendothelial hyperplasia. It may be significant that the animal with the myeloid metaplasia showed both a marked necrotizing arteritis and an extracapillary glomerular nephritis.

Arteries.—The lesions to be discussed under this head have been uniformly described as allergic arteritis. They are those first described and illustrated by Klinge and his associates (2, 3) and Masugi and his group (5, 22, 7), and they were clearly illustrated by Rich and Gregory (8–10), Hopps and Wissler (25), McKeown (26), and others. They were found in our animals most often in heart, spleen, and liver, though kidneys, lungs, and thymus were also involved.

Histologically the arteritis was distinct from the arterial changes described above which consisted merely of proliferating intimal cells (Fig. 6). It showed all the histologic earmarks of an allergic inflammation as described and illustrated recently by Goddard (27). It differed also from the above mentioned changes in that all layers of the artery were involved.

Two distinct varieties of allergic arteritis were noted. One was characterized, as McKeown (26) put it, first by edema of the wall with swelling of the muscle fibers and loss of nuclear definition, and later by proliferation of mesenchymal cells in all layers of the artery (Figs. 14 and 15). The lesion was segmental and at times associated with the intimal proliferation discussed above.

In contrast to the mesenchymal reactions elsewhere, allergic arteritis was not observed before the 11th day of experimentation (Table II). It was seen as well in animals sacrificed 26 days after the first of two injections of serum. At this time the adventitial tissue appeared to be more cellular than at earlier dates (Fig. 16). As this arteritis arose in an insidious fashion, we have called it subacute allergic arteritis.

The other variety of allergic arteritis resembled the first in that it showed mesenchymal proliferation in all layers of the artery. But in addition there was necrosis and deposition of fibrin (Fig. 17). As this arteritis exhibited both subacute and acute changes, we have termed it recurrent allergic arteritis. It is of considerable interest that this arteritis occurred only in animals that received two injections of serum. The acute changes were obviously superimposed on subacute changes resulting from the first serum injection.

A third variety of lesion, a truly acute arteritis (Fig. 18), was not observed in the present series of animals. It occurred once in a group of rabbits treated with serum and salicylates as will be shown in a subsequent paper.

There was no consistent relationship between allergic arteritis and the other mesenchymal reactions. But it was noted that subacute arteritis occurred most often in those animals which showed severe mesenchymal reactions in myocardium and glomeruli.

DISCUSSION

The experiments here reported have shown that the intravenous injection of large doses of serum causes a great variety of reactions in the vascular connective tissue, notably of the right heart, the lungs, the kidneys, and the spleen. The frequency and intensity of these reactions varied with the dose of serum injected. Twenty ml. of serum per kg. was more effective than 10 ml., while

smaller doses were quite ineffective. The development of the lesions depended also on the age of the animals. Old rabbits with an average weight of 3.4 kg. reacted distinctly less, both serologically and morphologically, than young ones weighing 2.0 to 2.4 kg. Some of the old rabbits also showed mild spontaneous mesenchymal reactions in heart and lungs, obviously as a result of previous infections. These differences may explain some of the variations and failures obtained by previous observers.

The most pronounced lesions developed along the path of the injected antigen from the ear vein through the right heart into the lungs, while beyond the lungs the spleen and kidneys were most markedly affected. In this respect, serum disease differs from the reactions to corpuscular antigens which appear chiefly in lungs, spleen, and liver (14, 15). The two differ also in that allergic arteritis has not been observed following single or repeated doses of corpuscular antigen.

The differences in reaction to serum and corpuscular antigen are obviously due to the differing particle sizes of the antigenic materials, for if whole bacteria or other corpuscular antigens are injected intravenously, they are taken up by the granulocytes and macrophages, which accumulate particularly in lungs, spleen, and liver. It appears that after they have been broken up by these cells, antigen is gradually released and induces antibody formation (28) or is metabolized or excreted, especially by liver and kidneys. Antibody formation under these conditions takes place particularly in the spleen (29).

If dissolved antigens such as horse serum are used, some molecules reach the spleen where they induce antibody formation. Others on their way from the injected vein, infiltrate the right heart, the lungs, and other tissues, by reason of their much smaller molecular weight. Here they enter the lymph vessels and on their way through the regional lymph nodes reach the antibody-forming cells in these nodes. Still others go to the kidneys and liver to be excreted and metabolized. That this is the course of events, seems evident from recent work with radioactive antigen[5] and from work with heparin and hepinoids, the fate of which is easily studied with the aid of toluidine blue.[6] In the case of dissolved antigens, antibody formation seems to take place in the lymph nodes as well as in the spleen and most likely also in the heart, lungs, and other tissues.

It seems obvious that many of the various lesions here reported are identical with those encountered by Clark and Kaplan (30) in human serum disease. These authors concluded that the changes did not correspond with those of early rheumatic fever, but were uncertain of their character.

Subsequently, Rich and Gregory (8–12) stated their belief that the myo-

[5] Reported before the American Society for Experimental Pathology and the American Association of Immunologists at Atlantic City on March 18, 1948.

[6] This work is in progress and will be presented elsewhere.

cardial and valvular lesions now in question were the same as those of rheumatic fever. It is true that in certain animals we have obtained lesions that closely resembled Aschoff bodies. But while some of these may well be an equivalent of rheumatic lesions, it should not be overlooked, that the common myocardial changes in animals and man following the intravenous injection of large doses of serum are not usually seen in rheumatic fever, nor is allergic arteritis a usual feature of rheumatic fever. In fact, it may be worth asking whether the experimental lesions do not correspond to those of rheumatoid arthritis in man rather than of rheumatic fever.

It is clear that the allergic arteritis of our experiments corresponded to human periarteritis nodosa, as generally contended, and that the two varieties of glomerular nephritis resembled the intra- and extracapillary types of glomerular nephritis in man.

Concerning the pathogenesis of the various lesions, it has been well known since the turn of the century that in man the intravenous injection of a large dose of serum may be followed, mostly within 8 to 12 days (31), by fever, urticaria, some proteinuria, enlargement of lymph nodes, and joint pains. In rabbits it may be followed, generally within 5 to 6 days (32, 7), by fever, erythema and edema of the ears, and transient proteinuria. It was observed by Derrick, Hitchcock, and Swift (33) that in man urticaria and adenopathy preceded arthritis. It has also long been known that in animals 10 to 12 days have to elapse before skin hypersensitivity can be demonstrated.

As concerns the serological aspects of these phenomena, it is common knowledge that precipitins become demonstrable in the circulating blood only when serum disease is in progress, that severe serum disease develops only in the presence of a good antibody titer (34), and that recovery from this disease is conditioned by the disappearance of antigen (35). It is also known that early in precipitin formation antigen and antibody may be present simultaneouly in the blood without reacting with one another (35–37).

In the present series of experiments, fever, erythema and edema of the ears, and transient proteinuria were observed 5 to 7 days after 1 injection of serum, antibodies were demonstrated after 5 days, and skin sensitivity after 11 to 12 days. This falls in with previous findings already mentioned.

Fox and Jones (38) and Hopps and Wissler (25) have noted that there is no consistent correlation in frequency and intensity between ear reaction, skin sensitivity, precipitin titers, and morphologic changes in the rabbit, other than a gross correlation between precipitin titers, mesenchymal reactions, and allergic arteritis. However, the time of appearance of the various phenomena in the present experiments has made it evident that the common mesenchymal reactions in heart, lungs, and spleen commence before the appearance of serum disease, that is to say, when antibodies are not yet demonstrable, that serum

disease develops at the time of maximum antibody formation which in rabbits occurs on the 5th and 6th days (39), and that subacute allergic arteritis occurs only after serum disease has passed and skin sensitivity has appeared, a happening which indicates that considerable quantities of antibody have been laid down in the vascular connective tissue. Necrotizing myocardial lesions, and valvular ones were observed only after the animals had become sensitive. Similarly, severe proliferative glomerular nephritis was observed only at this later stage, while degenerative changes and mild proliferation were already to be seen during the stage of serum disease. Necrotizing arteritis and extracapillary glomerular nephritis were seen only after a second injection of serum in sensitized animals.

It thus appears that allergic arteritis is a true Arthus phenomenon, and that there are at least two Arthus phenomena, namely, a subacute and a recurrent one. Similarly extracapillary glomerular nephritis is to be interpreted as an acute Arthus phenomenon.

We have no evidence that the mesenchymal reactions in myocardium, lungs, and spleen, which commenced before the onset of serum disease, were allergic. Since they occurred when the first antibodies were produced in the tissues (39), and the lesions contained abundant lymphoid cells, notably plasma cells, it seems likely that Bjørneboe and Gormsen (18) were correct in their assertion that these proliferative reactions had to do with the production of antibodies rather than that they were the results of antigen-antibody union. The validity of this view has been further substantiated recently by Fagraeus (24).

As to the necrotizing lesions in the myocardium, and the more severe proliferative changes in the glomeruli, their time of appearance as well as their frequent association seems to indicate that they too were subacute Arthus phenomena, at least in part.

SUMMARY

The intravenous injection of one large dose of serum into rabbits caused a variety of changes of considerable complexity.

1. There was an immediate proliferation of mesenchymal cells, including plasma cells, particularly in heart, lungs, and spleen.

2. The signs of serum disease developed only at the time of abundant antibody formation, before significant quantities of antibody were laid down in the vascular connective tissue.

3. Allergic arteritis, marked glomerular nephritis, myocardial necrosis, and Aschoff body–like structures were seen only after hypersensitivity had developed. It appears that most, if not all, of these pathological alterations were true Arthus phenomena.

4. There were at least two distinct varieties of allergic arteritis and glo-

merular nephritis, namely a proliferative one, following the first injection, and a a necrotizing one, seen only after 2 injections. It appears that the first was a subacute Arthus phenomenon, while the latter was an acute Arthus phenomenon superimposed on a subacute one. The subacute experimental glomerular nephritis resembled the intracapillary glomerulonephritis in man, while the acute variety was like human extracapillary glomerular nephritis.

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EXPLANATION OF PLATES

PLATE 1

FIG. 1. Hyaline disintegration of muscle fiber with mesenchymal proliferation in myocardium of right heart. 19 days after 1 injection of 10 ml./kg. of horse serum. × 460.

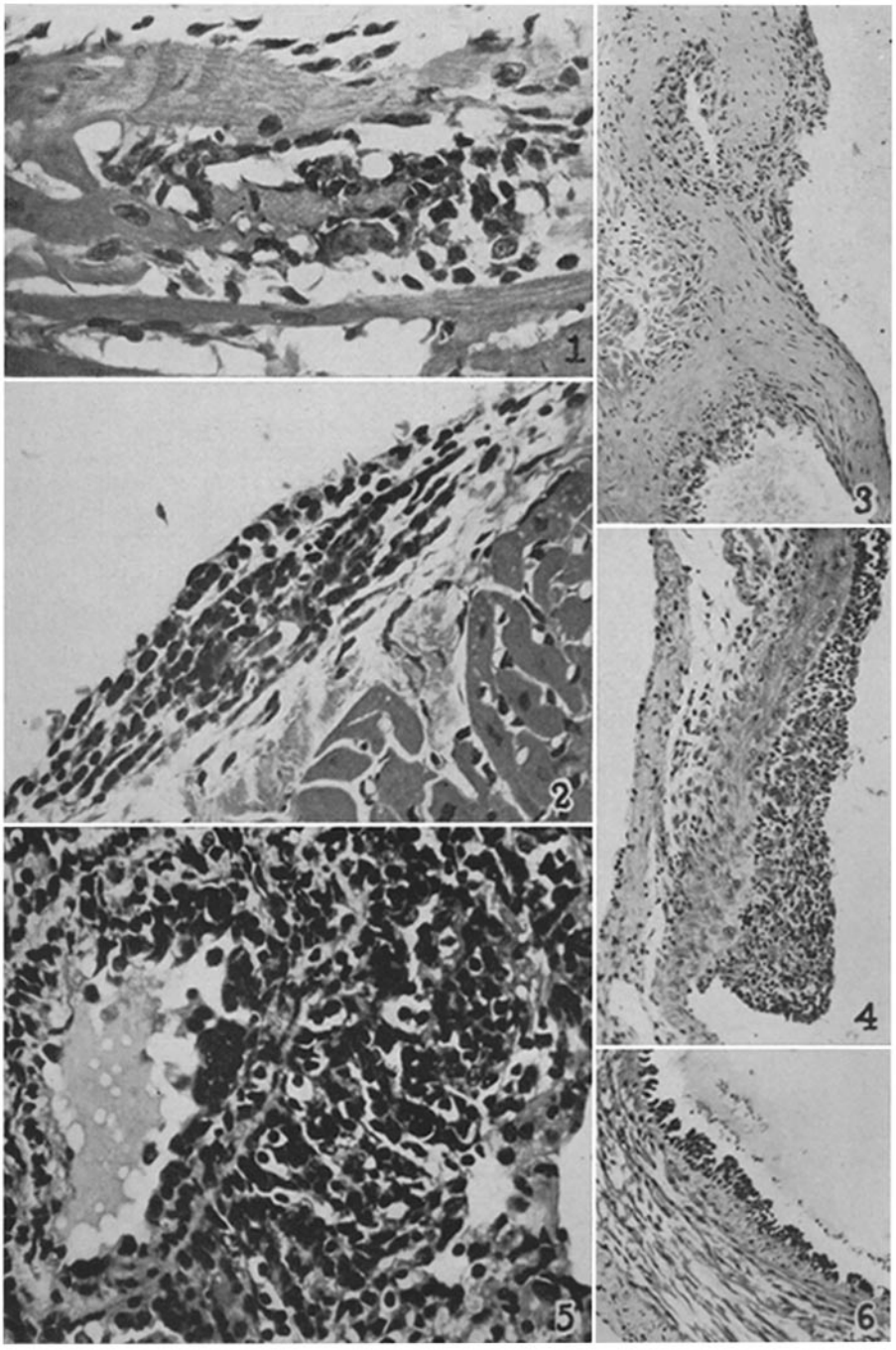
FIG. 2. Mesenchymal proliferation in endocardium. 19 days after 1 injection of 10 ml./kg. of horse serum. × 460.

FIG. 3. Mesenchymal proliferation at base of valve. 11 days after 1 injection of 10 ml./kg. of horse serum in a rabbit treated with salicylates. × 460.

FIG. 4. Mesenchymal proliferation in valve. 14 days after 1 injection of 10 ml./kg. of horse serum. × 115.

FIG. 5. Mesenchymal proliferation in small pulmonary vessel. 14 days after 1 injection of 10 ml./kg. of horse serum in a rabbit treated with salicylates. × 115.

FIG. 6. Mesenchymal proliferation in intima of large pulmonary artery. 14 days after 1 injection of 10 ml./kg. of horse serum. × 115.



(Ehrich *et al.*: Serum disease)

PLATE 2

FIG. 7. Mesenchymal proliferation in intima and adventitial layer of large pulmonary vein. 14 days after 1 injection of 10 ml./kg. of horse serum. × 460.

FIG. 8. Marked eosinophilic infiltration of intima of large pulmonary artery. 11 days after 1 injection of 10 ml./kg. of horse serum. × 115.

FIG. 9. Eosinophilic infiltration of intima of large artery. Note intact endothelium. Rabbit treated with heparin for 12 days. × 460.

FIG. 10. Normal glomeruli. × 460.

FIG. 11. Severe intracapillary glomerulonephritis. 14 days after 1 injection of 10 ml./kg. of horse serum in a rabbit treated with salicylates. × 460.

FIG. 12. Acute glomerular damage with swelling of loops and pyknosis of nuclei. 3 days after 1 injection of 20 ml./kg. of horse serum. × 460.

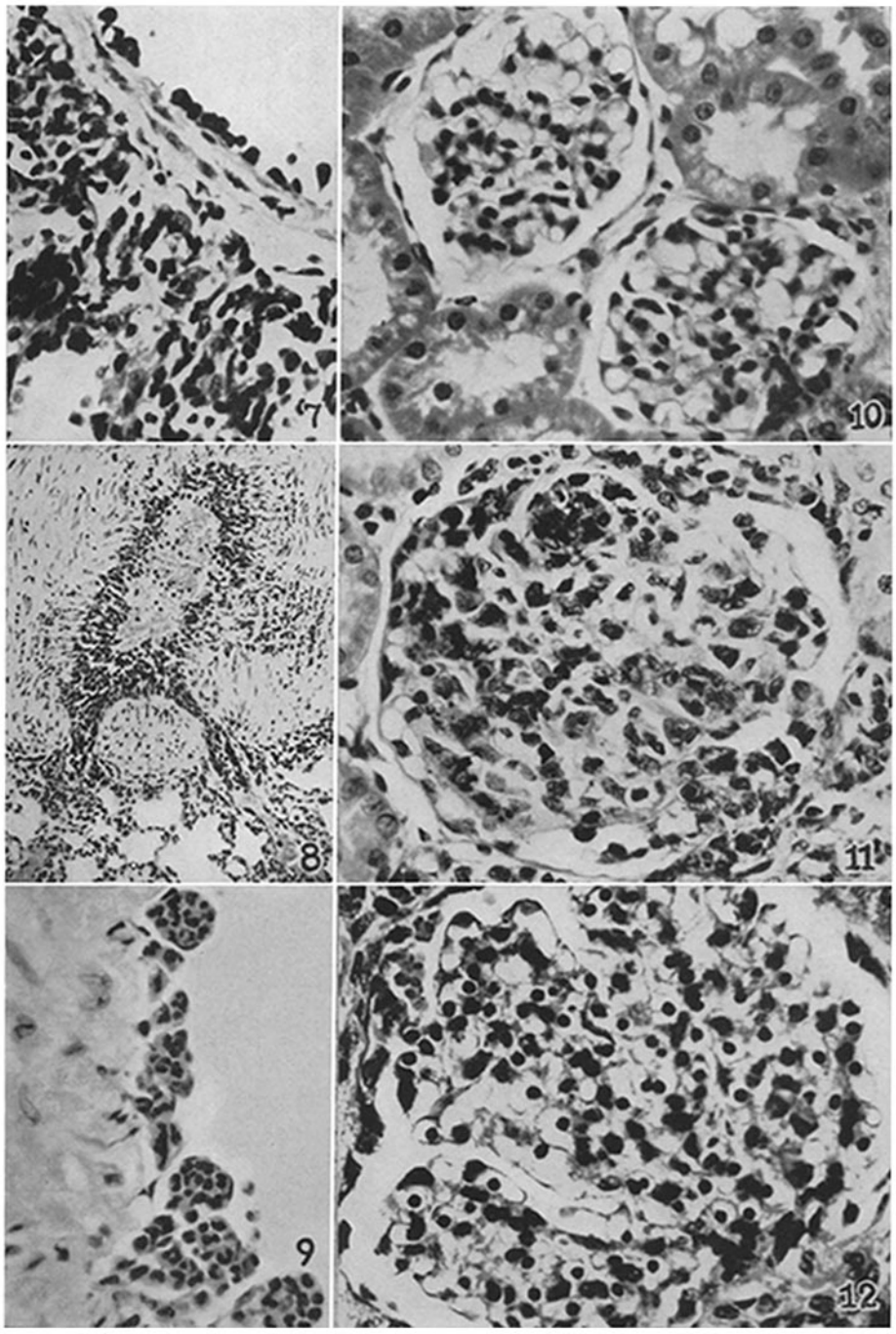


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PLATE 3

FIG. 13. Extracapillary glomerular nephritis. 26 days after the first of 2 injections of 10 ml./kg. of horse serum each. × 460.

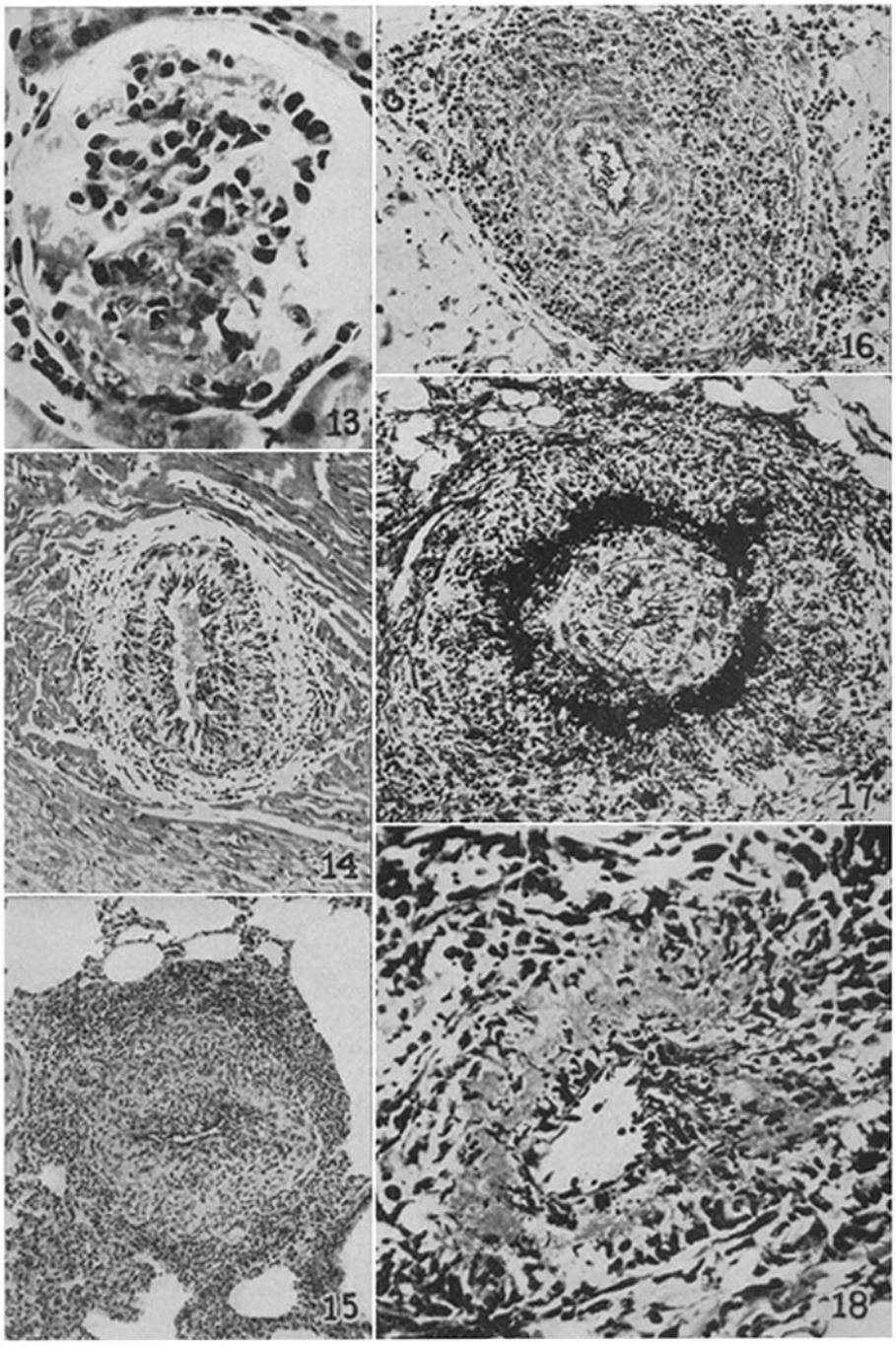
FIG. 14. Subacute allergic arteritis of coronary artery. 14 days after 1 injection of 10 ml./kg. of horse serum. × 115.

FIG. 15. Subacute allergic arteritis of pulmonary artery. 14 days after 1 injection of 10 ml./kg. of horse serum in a rabbit treated with salicylates. × 115.

FIG. 16. Subacute allergic arteritis of coronary artery. 26 days after the first of 2 injections of 10 ml./kg. of horse serum each. × 115.

FIG. 17. Acute and subacute (recurrent) allergic arteritis of coronary artery. 26 days after the first of 2 injections of 10 ml./kg. of horse serum each. × 115.

FIG. 18. Acute allergic arteritis of coronary artery. 11 days after 1 injection of 10 ml./kg. of horse serum in a rabbit treated with salicylates. × 230.



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