

THE ARTHUS PHENOMENON

LOCAL ANAPHYLACTIC INFLAMMATION IN THE RABBIT PERICARDIUM, HEART, AND AORTA

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PLATES 10 AND 11

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The Arthus phenomenon has been studied most extensively in subcutaneous tissue but has also been produced in certain viscera. Unpublished work¹ on the routes of absorption of diffusible and non-diffusible substances from the rabbit and dog pericardial sac led us to believe that this might be a site which was especially suitable for the study of the Arthus phenomenon. The advantage in using this closed space is due to the ease with which antigen can be applied to sensitized tissue without significant trauma. The present paper reports the results of such experiments upon the rabbit pericardium, heart, and aorta.

After repeated parenteral injections of a foreign protein into rabbits Arthus (1) observed that a subsequent subcutaneous injection of the same protein resulted in a local, severe, sterile, inflammatory reaction. If the protein was injected into a normal untreated animal in much greater quantities it produced no demonstrable effect. This destructive lesion produced in a sensitized animal by the subcutaneous injection of the specific antigen has since been known as the "Arthus phenomenon." Gerlach (2) has given a careful comparative histological description of this sequence of events in guinea pigs, rabbits, rats, dogs, and in man. By excising, in the sensitized animals, a portion of the subcutaneous tissue at different intervals after reinjection of the specific antigen he could accurately trace the different stages of the inflammatory process. As early as 1 hour after inoculation,

¹ This work was carried out in the Department of Pathology, Harvard Medical School, while one of us (D. S.) held the James Jackson Cabot Student Fellowship.

swelling of the connective tissue and edema with compression of the vessels and disappearance of the capillaries was evident. This was followed by a leucocytic infiltration, hemorrhage, and necrosis of the involved tissue, leading eventually to the formation of a sterile abscess. At the end of 8 days, new connective tissue was found at the periphery and healing proceeded with the formation of scar tissue covered by a hairless epidermis. In contrast to this, control animals which had not previously been sensitized, showed only a slight infiltration of leucocytes at the site of injection, lasting about 24 hours. Certain immunological studies, notably those of Opie (3), have demonstrated that the union of antigen and antibody in or on the fixed tissue cells is necessary for the appearance of the Arthus phenomenon.

Several investigators have shown that this local anaphylactic inflammation is not peculiar to the subcutaneous tissues, but can occur in other tissues of the body, as for example, the viscera and the endothelial cavities. It has been produced in the lungs, Busson (4); von Friedberger (5); Ishioka (6); Schlecht and Schwenker (7); and Opie (8); in the testicle, Long and Seyfarth (9); in the joints, Landouzy, *et al.* (10); Schlecht and Schwenker (7); and Klinge (11); and in the peritoneum, Longcope (12); Roessle (13). Shapiro and Ivy (14) have produced gastric ulcers by injection of the homologous antigen into the gastric submucosa of sensitized rabbits. Hepler and Simonds (15) have studied the inflammation which resulted when the specific antigen was injected into the kidney parenchyma of a sensitized rabbit. Such injections produced hemorrhage, necrosis of the tubules, and a leucocytic exudate while similar injections of the antigen into normal rabbits' kidneys produced hemorrhage only. Alessio (16) and Choi (17) have reported similar necrosis and leucocytic exudate in the livers of immune rabbits following the direct injection of the specific antigen into this organ. The findings of Coulter and Pappenheimer (18) and of Long and Finner (19) although accomplished by a different technique are nevertheless significant in this connection. Coulter and Pappenheimer obtained injury to the glomeruli of rabbits' kidneys by injecting either bacterial products or egg white into the renal artery of animals that had been sensitized by previous parenteral injections of the specific antigen. Long and Finner produced a glomerulonephritis in tuberculous swine by the injection of tuberculin into the renal artery. Our own experience with the occurrence of anaphylactic inflammation in the kidney and liver of sensitized rabbits after direct introduction of the antigen into these organs agrees with the observations of Hepler and Simonds, Alessio and Choi.

EXPERIMENTAL DATA

A group of seventeen rabbits received daily subcutaneous injections of 1 cc. of a 20 per cent solution of fresh egg white. After the sixth to the tenth injection, most of these animals developed a characteristic local inflammation similar to that described by Arthus. They were then allowed to rest from 4 to 5 days, at which time their precipitin titer against egg white ranged from 1:20,000 to 1:160,000.

At this time 1 cc. of a 20 per cent solution of fresh egg white was injected into the pericardial sac of each of these animals in the following manner: Under ether anesthesia a parasternal thorcotomy was performed and upward traction was exerted on the medial margin of the wound. This procedure stretched the pericardium so that the direct injection into its sac could be accomplished with accuracy and without injury to the myocardium. Artificial respiration was unnecessary since the rabbit mediastinum is complete. A control series of fifteen normal rabbits, whose sera contained no precipitins against egg white, received similar intrapericardial injections of the egg white solution. The sensitized animals with their controls were sacrificed at intervals from 2 to 18 days after the operation by the intravenous injection of 5 to 8 cc. of 95 per cent alcohol. Four sensitized animals died within the first 4 days. Routine smears and cultures were taken from all the pericardial sacs and the tissues were studied histologically after Zenker fixation.

Sensitized animals sacrificed 2 to 4 days after the intrapericardial injection showed a characteristic gross picture. On resection of the anterior chest wall, the heart appeared dilated and its surface was colored a deep purplish red. The parietal pericardium was slightly thickened and in a few instances was lightly adherent to the epicardium. In about one-fourth of the seventeen experimental animals the pericardial fluid was serosanguineous in character, increased in amount, and contained free bits of fibrin. The epicardium in such instances was deeply congested and on its violaceous surface a finely granular deposit was present. This fibrinous epicarditis was frequently present even when there were no free masses of fibrin in the pericardial fluid. In addition to the deep congestion of the outer aspect of the heart there were also many tiny fresh subepicardial hemorrhages scattered between the dull, stippled, grey areas. These hemorrhagic areas were frequently present in the adventitia of the intrapericardial portion of the great vessels. A striking edema in the loose supracardiac tissue about the thymus gland was sometimes present. On opening such hearts the chambers appeared markedly dilated and occasionally petechial hemorrhages were apparent on the cut surface of the muscle. A picture of the heart of an experimental animal as compared with a control is shown in Fig. 1.

These obvious gross changes were present in thirteen of the seventeen sensitized animals (76 per cent). Of the fifteen controls no gross changes were noted following the injection of the egg white solution into the pericardial cavity.

Microscopic studies were made of many sections of tissue selected from the pericardial sac, the four chambers of the heart and the intrapericardial and extrapericardial portions of the great vessels. Fifteen of the seventeen experimental animals (88 per cent) showed characteristic microscopic lesions in some portions of the heart muscle and pericardium. Changes in the myocardium occurred more frequently in the auricles than in the ventricles but in the more striking instances the entire heart muscle participated in the process. In half of the animals there were changes in the adventitia of the aorta and in three there was a peculiar lesion involving the intima. The characteristic pathology in the heart muscle consisted of focal necroses involving muscle cells, connective tissue, and blood vessels. In these areas of necrosis there was striking hemorrhage and a cellular infiltration made up chiefly of polymorphonuclear leucocytes in the animals sacrificed as early as 2 days after the intrapericardial injection. In the animals sacrificed at 4 days the polymorphonuclear cells were largely replaced by lymphocytes and large mononuclear cells. Frequently, these cells were found assuming a perivascular arrangement. These changes are shown in Fig. 2.

The lesions of "spontaneous interstitial myocarditis" described by Miller (20) could be found after careful study in about half of these animals. In most cases there was no difficulty in distinguishing the hemorrhagic necrotic areas from the "spontaneous lesions" which consist of interstitial collections of lymphocytes, endothelial cells, and fibroblasts. When the inflammatory changes in the sensitized animals were marked, it was impossible to determine whether they were superimposed upon a "spontaneous" lesion.

The pathology in the aortae was confined to the adventitia in most instances and consisted of edema, hemorrhage, a peculiar hyalinoid deposition, and a cellular infiltration comparable to that in the myocardium. This picture is shown in Fig. 3. In three aortae there were changes in the intima consisting chiefly, in two instances, of a sub-endothelial collection of polymorphonuclear cells unaccompanied by cholesterol collections or other evidence of spontaneous arteriosclerosis. In the third animal the changes in the intrapericardial aorta were striking. Here there was complete destruction of the intima and a dense polymorphonuclear leucocytic infiltration invaded

the inner half of the blood vessel. Portions of this necrotic area were covered by an early thrombus. Gram stains of this area failed to disclose the presence of any organisms.

Comparable microscopic sections of the control animals showed no such lesions. In none of the sections was there any evidence of pericarditis, adventitial or intimal changes in the great vessels, or the hemorrhagic focal necroses found in the myocardium of the sensitized group. In 55 per cent of this control group the characteristic lesions of "spontaneous interstitial myocarditis" were present. They could not be confused with the type of histopathology present in the sensitized animals. The routine direct smears and cultures of the pericardial fluids were uniformly negative except for a culture of *Staphylococcus aureus* obtained from one of the control animals. However, there was no microscopic change demonstrable in the sections of this animal.

Of the four sensitized animals which died during the first few days after the operation, a rabbit which was found dead $1\frac{1}{2}$ days after injection had the most striking cardiac pathology of any animal in the series and may have died as a result of this lesion, the only other contributing factor being a marked liver coccidiosis. Another rabbit is noteworthy which died 2 days after the intrapericardial injection. At autopsy the heart was greatly dilated and its epicardial surface was studded with hemorrhages. The liver was of a deep purple color and appeared congested. There was about 100 cc. of straw-colored ascitic fluid. Although there were some old scars of coccidiosis infection in the liver no cysts were found in the peritoneal cavity. Microscopically, the heart showed a striking myocarditis and it would appear that the cause of death in this instance was secondary to myocardial insufficiency with failure of the right portion of the heart.

Of two sensitized rabbits sacrificed at later intervals, one rabbit killed on the 8th day after the operation showed no demonstrable lesions but an animal sacrificed 18 days later had a definite though slight myocarditis.

Although no absolute correlation was found between the height of the precipitin titer, the subcutaneous Arthus phenomenon and the subsequent cardiac lesion, the protocols favor the impression that when the precipitin titer is high and the subcutaneous Arthus phe-

nomenon is marked, there will be a more pronounced lesion in the pericardium, heart, and aorta. The converse of this is not always true. This finding agrees with our previous observations on the relation between the height of the precipitin titer in sensitized rabbits and the extent of liver and kidney lesions after direct introduction of the antigen into the particular organs.

DISCUSSION

An intense inflammatory reaction in the pericardium, heart, and intrapericardial aorta can regularly be produced in a sensitized rabbit by the intrapericardial injection of the homologous antigen. The pathology described in this paper may be considered to be of an allergic nature. It is apparent that the appropriate union of antigen and antibody in such an area as the pericardial cavity may lead to striking changes. The lesions are not confined to the pericardial sac since a striking myocarditis is present in most of these animals.

A possible explanation for the development of myocarditis in sensitized animals subjected only to an intrapericardial injection of the antigen may be found in previous work in which we had shown that such a substance as trypan blue would extend back through the rabbit heart after intrapericardial injection. At the end of 15 minutes the endothelial cells between the muscle fibres would be deeply stained with the dye, whereas none of the pigment could be found in the liver and spleen. The trypan blue also deeply stained the great vessels around the base of the heart. Particulate matter such as carmine when injected into either the rabbit or the dog pericardial sac did not penetrate the deeper portions of the cardiac wall, probably on account of the larger size of the carmine particles. Most of this pigment was found in macrophages in the subepicardial zone and in the dilated lymph channels and enlarged lymph glands of the anterior, posterior, and superior mediastinum. It is reasonable to suppose that the diffusible antigen in our present experiments might pass through the myocardium and produce the changes noted in the heart.

Myocardial lesions following repeated intravenous injections of foreign protein have been described by Longcope (21), Boughton (22), and Klinge (11). Longcope found extensive focal degeneration in the cells of the heart muscle following the repeated intravenous

injection of egg white or horse serum in rabbits. These areas were characterized by dense infiltration of small round cells which later gave way to scar formation. However, he found similar changes in 25 per cent of twenty-two animals which had received only one to three intravenous injections of the same foreign protein. He fails to report any pericarditis or aortitis in his series of animals. None of his protocols mention any hemorrhage or striking gross changes in the hearts. Boughton reported degeneration and regeneration of the endothelium of the smaller arteries of the hearts of guinea pigs which he had repeatedly shocked with egg white or beef serum. He described similar findings in the blood vessels of the liver, spleen, and kidney of his animals. Klinge repeatedly injected horse serum into the joints and skin of rabbits. The hearts of such animals showed small focal necroses and areas of perivascular infiltration of round cells. The photographs of these lesions closely resemble those of "spontaneous interstitial myocarditis" described by Miller. Myocardial lesions following anaphylactic shock in the guinea pig have been reported by Gay and Southard (23). They described hemorrhages into the myocardium and also demonstrated fatty changes both in the muscle cells and in the endothelial cells of the capillaries in this organ.

CONCLUSION

An intense inflammatory reaction in the pericardium, heart, and intrapericardial aorta can regularly be produced in a sensitized rabbit by the intrapericardial injection of the homologous antigen.

REFERENCES

1. Arthus, M., *Compt. rend. Soc. biol.*, 1903, 55, 817.
2. Gerlach, W., *Virchows Arch. path. Anat.*, 1923, 247, 294; *Verhandl. deutsch. path. Ges.*, 1923, 19, 126.
3. Opie, E. L., *J. Immunol.*, 1924, 9, 231.
4. Busson, B., *Wien. klin. Woch.*, 1911, 24, 1492.
5. von Friedberger, E., *Deutsch. med. Woch.*, 1911, 37, 481.
6. Ishioka, S., *Deutsch. Arch. klin. Med.*, 1912, 107, 500.
7. Schlecht, H., and Schwenker, G., *Deutsch. Arch. klin. Med.*, 1912, 108, 405.
8. Opie, E. L., *J. Immunol.*, 1924, 9, 259.
9. Long, E. R., and Seyfarth, M., *Am. Rev. Tuberc.*, 1924, 9, 254.

10. Landouzy, L., Gougerot, H., and Salin, H., *Rev. méd.*, 1910, **30**, 857.
11. Klinge, F., *Beitr. path. Anat. u. allg. Path.*, 1929, **83**, 185.
12. Longcope, W. T., *J. Exp. Med.*, 1913, **18**, 678.
13. Roessle, R., *Verhandl. deutsch. path. Ges.*, 1914, **17**, 281.
14. Shapiro, P. F., and Ivy, A. C., *Arch. Int. Med.*, 1926, **38**, 237.
15. Hepler, O. E., and Simonds, E. P., *Am. J. Path.*, 1929, **5**, 473.
16. Alessio, F., *Pathologica*, 1929, **21**, 121.
17. Choi, C. Y., *Tr. Jap. Path. Soc.*, 1930, **20**, 587.
18. Coulter, C. B., and Pappenheimer, A. M., *Proc. New York Path. Soc.*, 1916, **16**, 80.
19. Long, E. R., and Finner, L. L., *Am. J. Path.*, 1928, **4**, 571.
20. Miller, C. P., Jr., *J. Exp. Med.*, 1924, **40**, 543.
21. Longcope, W. T., *Arch. Int. Med.*, 1915, **15**, 1079.
22. Boughton, T. H., *J. Immunol.*, 1917, **2**, 501.
23. Gay, F. P., and Southard, E. E., *J. Med. Research*, 1907, **16**, 143.

EXPLANATION OF PLATES

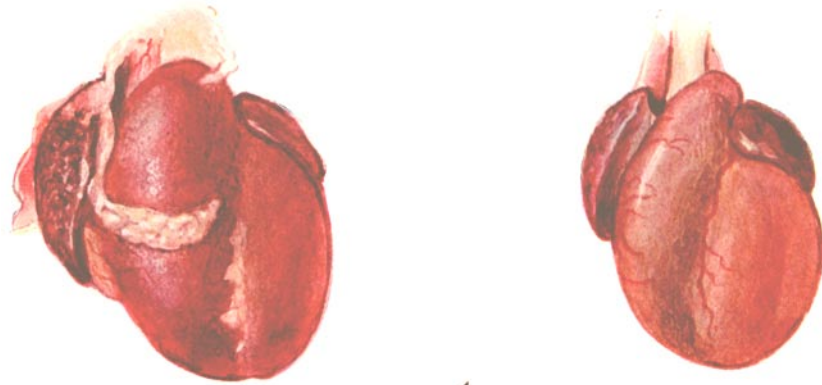
PLATE 10

FIG. 1. The hearts of two rabbits sacrificed 4 days after the intrapericardial injection of 1 cc. of a 20 per cent solution of fresh egg white. The figure on the left is that from an egg white-sensitized animal; that on the right is a normal control. These animals were approximately of the same weight. In the heart of the experimental animal note the dilated right ventricle and auricle and the deposition of large masses of fibrin over the dull epicardial surface. The injection and hemorrhages of the epicardial surface are evident in the drawing. $\times 1$.

PLATE 11

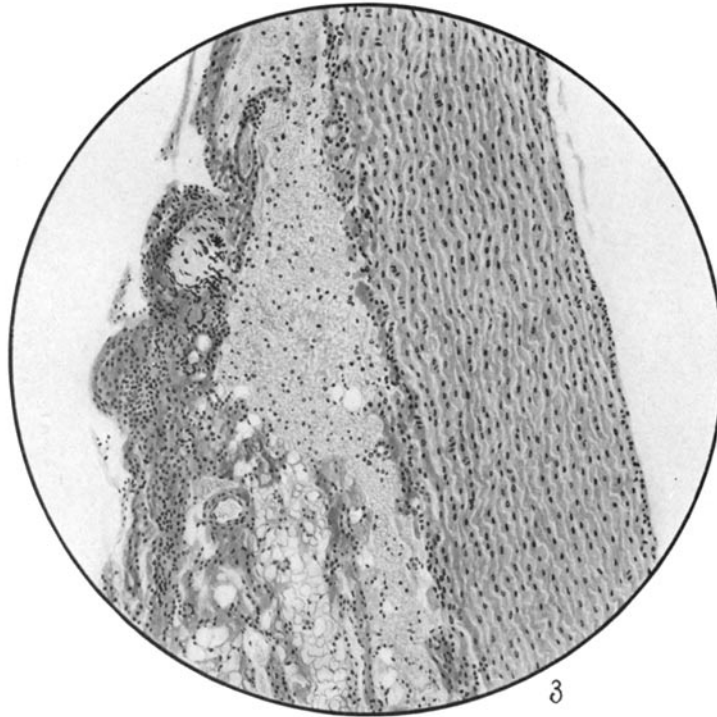
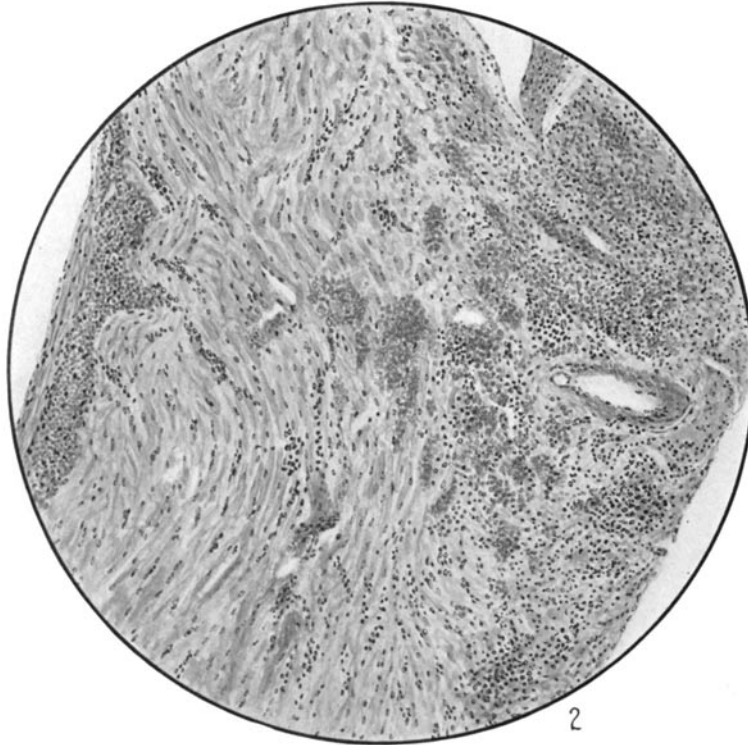
FIG. 2. A section of the right auricle of a sensitized animal dying 2 days after the intrapericardial injection of 1 cc. of a 20 per cent solution of fresh egg white. Note the loss of striations in the muscle cells, the hemorrhage between these pale fibres, and the infiltration of polymorphonuclear leucocytes and round cells. Hematoxylin and eosin. $\times 300$.

FIG. 3. The intrapericardial aorta of a sensitized rabbit sacrificed 4 days after the intrapericardial injection of 1 cc. of a 20 per cent solution of fresh egg white. Note in the adventitia the intense hemorrhage, and the cellular infiltration of leucocytes. Hematoxylin and eosin. $\times 300$.



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(Seegal *et al.*: Arthus phenomenon)



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