ACUTE VASCULAR LESIONS IN MICE FOLLOWING INJECTIONS OF PNEUMOCOCCI.*

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Plates 55–58.

In a previous report by one of us (1) on the comparative virulence of pneumococci at different stages of pneumonia, it was noted that mice dying after an injection of living pneumococci showed hemorrhage in the pleural or pericardial cavities, consisting of fresh blood clots varying in size from a wheat grain to a large pea. Among eighty-four mice that succumbed, twenty-four showed such a blood clot. The duration of life after inoculation varied from one to twenty days. The cause of the hemorrhage was traced by serial sections of one case to the rupture of a large branch of the pulmonary artery, besides which a markedly degenerated area in the transverse arch of the aorta and the base of one of the large branches arising from it (case I of this report, figures 1 and 2) was found.

The question then arose as to the constancy of these findings and whether the arterial changes were caused by the pneumococci injected or whether the vessels were ruptured by some trauma during life or some accident at autopsy, or possibly whether they were the result of some preëxisting disease among the mice.

Twenty-one mice were studied with reference to these points. Fourteen had been injected with living pneumococci, of which ten showed the hemorrhagic condition at autopsy; four showed no hemorrhage but were examined for lesions of the vessels; three died spontaneously, never having been inoculated, and one mouse was presumably healthy, having been killed by a blow on the head.

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Finally, three succumbed to inoculation of dead pneumococci, among which one showed hemorrhage.

We have examined one hundred and eighteen mice inoculated with pneumococci during the past year with reference to the hemorrhage described. Of these, eleven, or 9.3 per cent., showed the large hemorrhages, while among our previous series 28.5 per cent. had shown the condition. The discrepancy is probably to be explained by the difference in dosage. In the recent work small doses of highly virulent cultures have been given, so that a large proportion of these animals developed septicemia and died acutely. With larger doses of less virulent strains the animals often survived for many days. It is among these examples of delayed deaths occurring suddenly after a period in which the mice appear well that the hemorrhages are usually found.

The bacteriological methods have been described in the paper referred to (1) and need not be repeated here. The autopsies were conducted with great care, and in cases of intrathoracic hemorrhages special precautions were observed to prevent any injury or disturbance of the relations of the thoracic viscera. The ventral wall of the thorax was removed and the whole body immersed in fixative which was in some cases Zenker's fluid, in others 10 per cent. formalin. After the tissues were thoroughly fixed and hardened, the viscera were removed *en masse* and embedded in celloidin. In about half the cases only the organs above the diaphragm were so treated; in the others the upper abdominal organs were also included. Sections were cut twelve to eighteen microns thick and every tenth or fifteenth section was stained and mounted. In some cases every sixth section from the important part of the block was studied. The series were usually stained in hematoxylin and eosin and extra sections were colored by Weigert's method for elastic tissue. In a few instances the series was first stained in orcein by Unna's method and subsequently in hematoxylin and eosin. In one case frozen sections were cut from formalin-fixed tissue and stained for fat by Herxheimer's scarlet R method.

HISTOLOGY.

The findings in each case are summarized in the accompanying table (table I). The general features may be described as follows:

1. Ten mice injected with living pneumococci showed the hemorrhages. Seven of these showed on section rupture of the ascending aorta. In two of these seven the pulmonary artery was ruptured also. In the eighth mouse the hemorrhage arose from rupture of the pulmonary artery alone and in the ninth the source of hemorrhage was not precisely discovered, but the position of the blood clot indicated an origin from the pulmonary artery. The source of the hemorrhage in the tenth mouse was not determined.

2. Four mice injected with living pneumococci succumbed, but without showing the hemorrhage. One of these showed in section rupture of the transverse arch of the aorta with hemorrhage into the mediastinal tissues. Two showed small hemorrhage into the pleural cavity and lungs, and the other one showed no hemorrhage at all.

3. The three mice succumbing spontaneously showed no hemorrhage.

4. Three mice succumbed to injections of dead pneumococci. Two showed no hemorrhage, while one mouse exhibited rupture of the ascending aorta attended with hemorrhage.

The lesion appears usually merely as a break with rather ragged edges in one side of the vessel, about which a platelet thrombus has formed, and the whole is embedded in a dense mass of extravasated blood (figures 3 and 4). In extreme cases there is complete disappearance of the aorta through many sections, the site of the vessel being indicated by a ring of agglutinated platelets.

Above and below the area of rupture the vessel may show no microscopical change. The lumen and thickness of wall remain unaltered and the muscle cells and elastic fibers stain well. The space left by the disappearance of the vessel wall is usually larger in the center, becoming gradually smaller toward each extremity until the broken ends unite. At this point it is found that the elastic fibers remain visible and stainable, although perhaps ruptured and stretched (figure 5), forming an irregular bridge across the breach, while the muscle cells have entirely disappeared. In the most ex-

TABLE I.

Case no.	Material injected.	Duration of life.	Autopsy.	Vascular lesions.	Other lesions.
I	20,000,000 pneumococci subcutaneously.	10 days	Hemorrhage, pleural cavity.	Ruptured pulmonary artery; degenerated and ruptured transverse arch and carotid; degenerated vein.	
II	Mouse killed.		No hemorrhage.	Slight hemorrhages in pericardium and pleura; vessels and heart normal.	Peribronchial lymphoid infiltration.
III	20,000,000 pneumococci intraperitoneally.	5 days	Hemorrhage, right pleura; cultures −.	Ruptured pulmonary artery (small).	Hemorrhages in lungs.
IV	80,000,000 pneumococci intraperitoneally.	5 days	No hemorrhage; cultures −.	Small hemorrhages around pulmonary vessels; no definite vascular lesion.	Hemorrhages in lungs.
V	20,000,000 pneumococci intraperitoneally.	2 days	No hemorrhage; peritonitis; cultures +.	No vascular lesion; no hemorrhage.	Pulmonary congestion.
VI	?	6 days	No hemorrhage; congestion-lung.	Erosion of pulmonary vein by broncho-pneumonia.	Pneumonia; pleuritis; mediastinitis.
VII	80,000,000 pneumococci intraperitoneally.	6 days	No hemorrhage; smears −; cultures +.	Rupture of transverse aortic arch (small).	Hemorrhages in lungs; pericarditis; pleuritis; mediastinitis.
VIII	200,000 pneumococci subcutaneously.	5 days	Hemorrhage, pericardium.	Rupture of ascending aorta.	Peribronchial lymphoid infiltration.
IX	Spontaneous death.		No hemorrhage.	Slight hyaline change in aorta; myocarditis.	Liver parasites.
X	Spontaneous death.		No hemorrhage.	No lesion; no hemorrhage.	Peribronchial lymphoid infiltration; peritonitis.
XI	2 injections of 100,000 and 20,000,000 pneumococci.	10 days	Large hemorrhage, pericardium; cultures −.	Rupture of ascending aorta (extreme).	Focal necroses in liver.
XII	24,000 pneumococci subcutaneously.	5 days	Small hemorrhage, pericardium.	Rupture of ascending aorta; dissecting aneurysms; rupture of jugular vein.	Peribronchial lymphoid infiltration; focal necroses in liver.
XIII	100,000 pneumococci subcutaneously.	5 days	Small hemorrhage, pericardium.	Ruptures of ascending aorta and pulmonary artery.	
XIV	5,000 pneumococci.	5 days	Small hemorrhage, pleura.	No lesion (partial examination, frozen sections).	

Case no.	Material injected	Duration of life.	Autopsy.	Vascular lesions.	Other lesions.
XV	30,000,000 pneumococci subcutaneously.	4 days	Very large hemorrhage.	Rupture of ascending aorta (extreme); dissecting aneurysms; pericarditis, endocarditis; myocarditis.	Pleuritis; peritonitis; focal necroses in liver.
XVI	Dead pneumococci.	2 days	Small hemorrhage, pericardium.	Rupture of ascending aorta; degenerated vein.	Peribronchial lymphoid infiltration;
XVII	2 injections of dead pneumococci.	7 days	No hemorrhage.	No lesion.	Widespread acute inflammation.
XVIII	2 injectious of dead pneumococci.	7 days	No hemorrhage.	No lesion. Pericarditis.	Peribronchial lymphoid infiltration focal necroses in liver.
XIX	Spontaneous death.		No hemorrhage.	No lesion.	Tuberculosis (?) of liver.
XX	80,000,0000 pneumococci subcutaneously.	5 days	Large hemorrhage.	Rupture of ascending aorta; dissecting aneurysm; myocarditis.	Peribronchial lymphoid infiltration; focal necroses in liver; parasite.
XXI	320,000,000 pneumococci intraperitoneally.	2 days	Hemorrhage, pericardium and pleura.	Rupture of pulmonary artery (?).	

treme part of the lesion, remnants of elastic fibers may sometimes be demonstrated by special stains, although no sign of the arterial wall can be brought out with the ordinary stains.

Occasionally, above or below the point of rupture, small dissecting aneurysms are found. In a few cases the aortic wall shows marked degeneration, but preserves its continuity. There is a sharp line of demarcation between the normal tissues and the thinner, almost structureless membrane in which elastic remnants may be found. In case XII this portion was bulged out to form a small sac (figure 6).

The heart muscle appeared normal except for an acute myocarditis that occurred in a number of cases.

The veins are usually entirely normal. In a few instances changes strongly suggestive of degeneration occurred. Sometimes for a short arc of its circumference the vein wall stained poorly and the cell striations and outlines were lost. One such lesion had led to a definite rupture attended with a small hemorrhage.

In and about the vascular lesions there are no indications of an

inflammatory reaction. No organisms could be stained in the sections, and cultures taken from the blood at autopsy are usually sterile. In a number of cases inflammatory exudates in the pleura, pericardium, mediastinum, and lungs existed, but in no case could the pathological condition of the vessels be attributed to an extension of an inflammatory process, save, perhaps, in one instance of the pulmonary vein (case VI).

ETIOLOGY.

We have up to the present found these acute vascular lesions in those mice only which had been inoculated with living or dead pneumococci. None of the mice dying from other causes and examined histologically showed any such lesions. Of the two hundred or more mice used in the medical laboratory this year, not more than a dozen have died spontaneously, so called. The autopsies made on several of these showed no gross hemorrhage. Twelve mice were injected with streptococci in the course of other investigations and no blood clots were found at autopsy.

The evidence would therefore seem, as far as our observations are concerned, to determine the pneumococcus as the cause of the lesions. The negative cultures at autopsy, the absence of bacteria and microscopic inflammatory reaction, and the fact that the same conditions may be produced by injection of the dead microorganisms suggest that the pathological condition produced is not an arteritis but a toxic degeneration of the vessel wall produced by the poisons of the injected pneumococci.

That bacteria and their products have a definitely harmful effect upon the arteries of certain laboratory animals, for example rabbits, has been shown by Klotz (2), Duval (3), Saltykow (4), and others. Saltykow claims that by the use of bacterial poisons (staphylococci) which are injected repeatedly into rabbits over considerable periods of time, lesions can be produced resembling closely those found present in human arteriosclerosis. Mice, we believe, have not been used before in the study of experimental arterial disease nor have pneumococci received much attention from this point of view. Those who have used mice for the deter-

mination of virulence of pneumococci have probably not been in the habit of making such detailed autopsies as are here carried out.

Although the findings in mice bear some resemblance to the traumatic arterial ruptures and to the spontaneous rupture of the aorta, so called, in man, yet a traumatic origin cannot be assigned for them. The affected vessels are very deeply placed in the mouse, and definite degenerations have been discovered in the thin walls that attend the rupture and extravasation. Besides this, the hemorrhagic condition has been noted in mice inoculated with pneumococci and not in the numerous control animals.

The localization by preference in the ascending aorta may be analogous to the well known predilection of syphilitic aortitis for the same region. We should, perhaps, consider the possibility that the definite, localized degeneration is not of regular occurrence, that the walls of arteries and veins are weakened generally all over the body but without histological change, and that the rupture occurs when the blood pressure exceeds the resistance offered by the weakened vessel and most frequently in the ascending aorta where the pressure is highest. Their occurrence, however, in the pulmonary arteries and in the veins is strongly against this view.

This work may serve to emphasize the importance of the rôle played by infectious diseases and their bacterial poisons in the etiology of arterial and vascular diseases in general. Different writers (5) on arteriosclerosis have called attention from time to time to the factor, but it can scarcely be said to have obtained general acceptance. The poisonous substances are the same, as far as we know, in mouse and man, and the tissues of the arterial walls at least are composed of the same histological elements.

SUMMARY.

Mice dying several days after injections of pneumococci, both living and dead, frequently show at autopsy large intrathoracic hemorrhages.

The histological study of the thoracic organs indicates that there occurs in each case a sharply circumscribed, acute degeneration of the wall of some large vessel, usually the ascending aorta or one of the pulmonary arteries. This degenerated portion is torn out by

the pressure of the blood with almost complete disappearance of the vessel wall, leading to a gross hemorrhage.

A similar change is occasionally found in the walls of the veins which contain cardiac instead of smooth muscle.

We have found this lesion only in mice which had been recently inoculated with pneumococci. Negative cultures at autopsy, the lack of inflammatory reaction, and the occurrence of the conditions after injection of dead pneumococci suggest the cause to be a toxic degeneration of the vessel wall brought about by the poisons of the injected organisms.

We are indebted to Dr. P. W. Clough for several of the animals which died of hemorrhages in the course of his investigations in the medical laboratory.

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EXPLANATION OF PLATES.

PLATE 55.

FIG. 1. Case I. Aortic wall at the right; elastic remnants shown in degenerated wall of carotid at the left. Weigert's elastic tissue stain.

FIG. 2. Case I. Pulmonary artery. The light area at the right is a platelet thrombus in which may be seen elastic remnants. Weigert's elastic tissue stain.

FIG. 1.

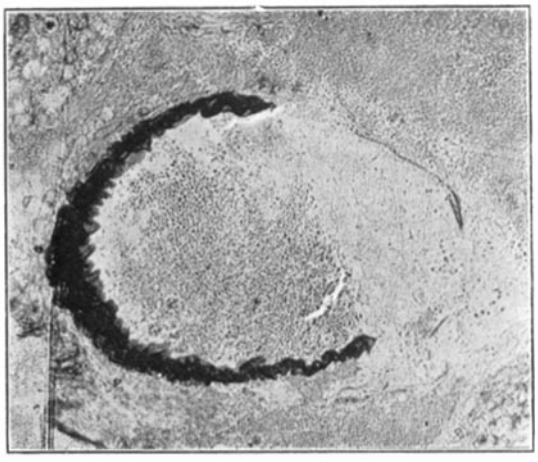

FIG. 2.

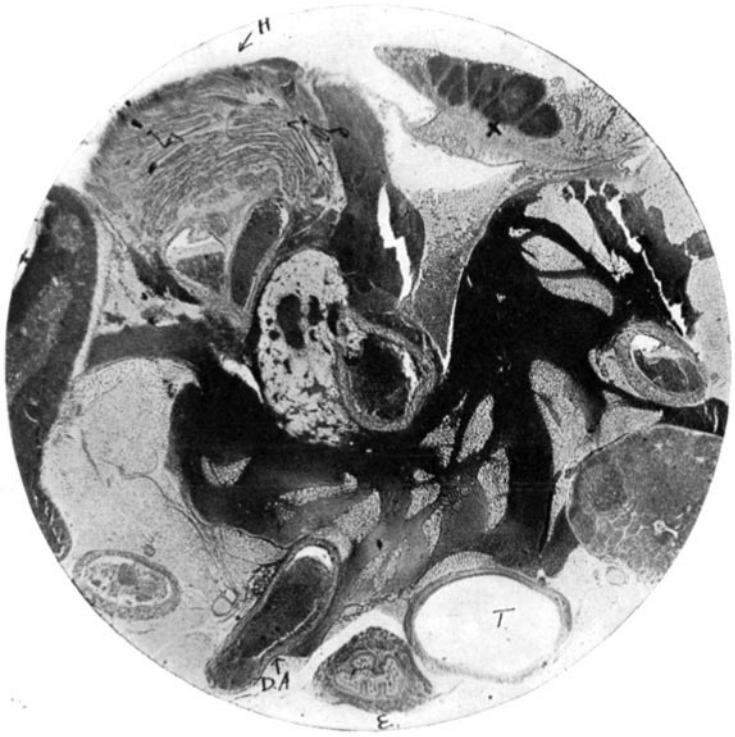

FIG. 3.

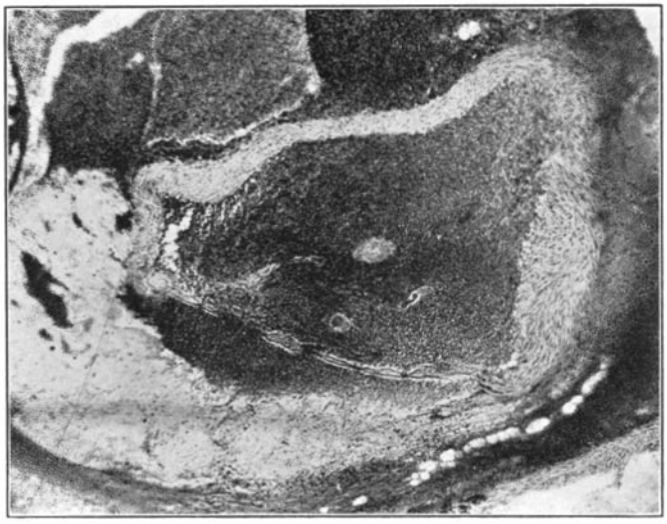

FIG. 4.

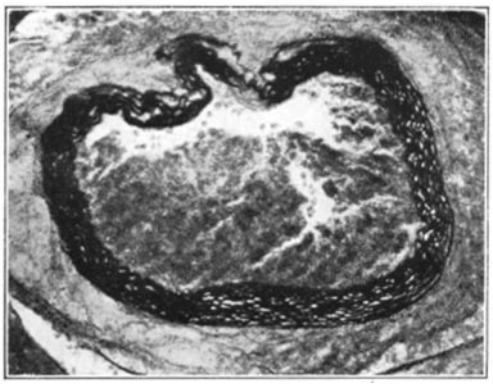

FIG. 5.

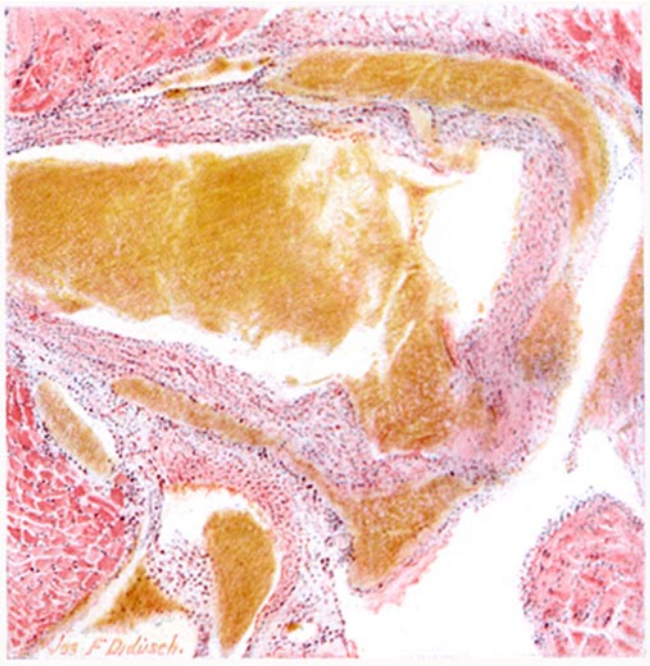

FIG. 6.

PLATE 56.

FIG. 3. Case XX. Thoracic organs. Low power; hematoxylin and eosin. H = heart; x = thymus; T = trachea; e = esophagus; $D.A.$ = descending aorta. The ruptured ascending aorta is seen in the center with a light staining platelet thrombus. The dark material throughout the section is extravasated blood.

PLATE 57.

FIG. 4. Case XX. Ascending aorta. Hematoxylin and eosin. Ruptured and stretched elastic fibers bridge the opening in the wall. A thrombus is seen in the lower left corner.

FIG. 5. Case VIII. Ascending aorta. Orcein stain. Below the main lesion.

PLATE 58.

FIG. 6. Case XII. Lower part of the ascending aorta. Hematoxylin and eosin. The degenerated, hyaline portion of the wall is bulged out to form a small aneurysm. Above there is a sharp break in the vessel wall. The blood is held in check by the adventitia.