THE EXPERIMENTAL VASCULAR LESIONS PRODUCED BY BACILLUS MALLEI.

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PLATES VII AND VIII.

In the course of an experimental study of the histological lesions of glanders, the result of which I hope to publish at a later date, I was impressed with the nature and regular occurrence of vascular changes in rabbits and guinea pigs following the injection of *Bacillus mallei* and its poison. The work was not undertaken primarily for the study of the blood vessels, but as it was early observed that the vascular lesions were similar to those produced by the administration of adrenalin and occurring in glanders in man, I considered this part of the histological study sufficiently important to warrant a special communication.

METHODS.

The animals used in this series of experiments were full grown rabbits and guinea pigs. The inoculation was made subcutaneously, intravenously, or intra-peritoneally. The same strain of *Bacillus mallei*[1] was used throughout the work. The original culture was attenuated in virulence by frequent transplantation during a period of eighteen months. Three series of animals were inoculated with definite amounts (1) of an attenuated culture, (2) of a highly virulent culture, and (3) of the cultures killed by heat. The attenuated cultures were obtained as noted above. The highly virulent cultures were obtained by frequent passage of the bacilli through guinea pigs. The killed suspensions were prepared by heating the virulent culture in salt solution to 55° C. The bacilli were prepared for inoculation by growing cultures on slanted

[1] The culture was obtained through the kindness of Dr. Frothingham of Boston.

1 per cent. dextrose-agar for from 24 to 48 hours at a temperature of 37° C.; afterwards washing down the growth and thoroughly emulsifying it in 10 c.c. of normal saline or bouillon. The dosage of the three emulsions was as follows: (*a*) Attenuated culture, 1 c.c. for guinea pigs, 5 c.c. for rabbits. (*b*) Virulent culture, 0.5 c.c. for guinea pigs, 5 c.c. for rabbits. (*c*) Killed culture, 0.5 c.c. for guinea pigs, 1.0 to 1.5 c.c. for rabbits.

Although these were the usual doses employed, another dose of the attenuated bacilli was found useful for the production of a partial immunity in guinea pigs which was to prepare them for larger doses of the virulent culture. These large doses were used to produce certain proliferative vascular changes noted below. The dose in this case was usually 0.5 c.c. of a suspension in which one loopful of a twenty-four hour agar growth had been added to one cubic centimeter of saline. The doses were repeated every third or fourth day over a period of two to four weeks. Rabbits received the injections into the marginal ear vein. Guinea pigs, owing to the smallness of the ear veins, were inoculated into the axillary and femoral veins, aseptic precautions being used. Subcutaneous and intra-peritoneal injections were employed with equally good results. The emulsion of dead bacilli was injected without filtration. The ordinary mallein of the market failed to give results. Occasionally rabbits succumbed to the injection of the living virulent bacilli in from three to five days but this was not the rule; the rabbit is not highly susceptible to the glanders bacillus and easily stands large and repeated doses. Pregnant rabbits and mothers are more susceptible to the inoculations.

Guinea pigs give more constant results than rabbits, although the vascular changes do not differ essentially in the two species. The tolerance to the glanders bacillus was best observed in the female pigs. The difference in susceptibility of rabbits and guinea pigs as regards sex and the bearing of this on glanders immunity has recently been discussed by Nicolle.[2]

For the histological study the tissues were fixed in Zenker's fluid, alcohol or formalin, and embedded in paraffin. Methylene-blue-eosin and phosphotungstic-haemotoxylin were used in the rou-

[2] Nicolle, *Annales de l' Inst. Pasteur,* 1906, xx, 625, 698, 801.

tine staining of sections. Weigert's elastic and Mallory's connective tissue stains were used for the special demonstration of elastic and connective tissues. Sharlach R. was employed tc demonstrate the presence of fat. The methylene-blue-eosin method was more useful as a routine stain than the haemotoxylin-eosin; with the former the early vascular lesions were more readily determined, the calcified areas more distinctly indicated, and the proliferative endothelium and degenerative medial changes were more readily observed. The phosphotungstic-haemotoxylin method was especially useful in marking out the degenerative lesions of the smooth muscle fibres giving to such areas a brownish black color in deep contrast to the faintly stained normal portions of the vessel wall.

The intimal and medial lesions were well differentiated with Mallory's connective tissue stain; indeed this stain alone made possible the separation of the internal elastica of the lesion from the degenerated band of the circular fibres. It stained the internal elastic membrane in the vascular lesion a dark blue in contrast to the normal elastica which was stained a distinct pale blue. The degenerated muscle fibres were stained a deep orange-red in contrast to the purple-blue of the normal muscle cells. The intimal endothelium was stained a salmon color. The medial lesion was stained a deep orange-red owing to some special affinity of the acid fuchsin for degenerating muscle fibres. The deep orange-red of the medial area of degeneration in the immediate proximity to the swollen and straightened dark blue bands of the internal elastica gave a striking picture.

HISTOLOGICAL LESIONS.

For purposes of clearness I will consider the histological lesions under three headings: (1) the exudative, (2) the proliferative and (3) the degenerative. This grouping of the lesions corresponds closely with the degree of the virulence of the cultures employed. In general it can be stated that a highly virulent culture produces vascular lesions of an acute inflammatory type, while a culture of low virulence produces proliferative changes. The degenerative changes are secondary to the proliferative lesion.

While the glanders poison attacks especially the arterial system, and more especially the smaller arteries of the peripheral circulation, none of the arteries wholly escapes injury.

The selection of the smaller arteries by the bacterial poison in preference to the aorta and its larger branches is a striking feature of the two cases of glanders infection studied in man. In both cases the vascular lesions were found in the terminal arteries of 1 mm. to 2 mm. in diameter; and they were especially numerous in the arteries distributed to the skin, lungs, brain and kidneys. The lesion consisted of circumscribed aneurysmal bulgings of the vessel walls showing proliferative intimal and degenerative medial changes (Plate VII, Fig. 1). The diseased arteries were in no way involved in the general infectious process going on in other tissues.

The acute vascular changes occurring in the rabbits and guinea pigs are usually focal in distribution. The most marked lesions occur in the male pig and the female rabbit. Other lesions than the vascular ones, resulting from the injection of *Bacillus mallei,* occur with less frequency.

In animals the earliest change of the vessel noted consists of swelling of the sub-endothelial tissue spaces of the intima due to œdema, the overlying layer of the endothelium remaining perfectly normal. In still other vessels aggregations of polymorphonuclear leucocytes between the intima and the media occur. Often these collections of polynuclear cells are so densely packed as to form layers extending more than half around the circumference. The sub-endothelial collections of leucocytes at the point of greatest density may attain a depth of six to eight cells; while the overlying endothelium shows no break or appreciable protoplasmic or nuclear change. In a longitudinal section of the vessel the sub-endothelial layer of leucocytes may extend, gradually tapering in the long axis of the vessel, for a distance many times that of the circumference. It would appear that the polynuclear zone extends from a primary focus in the direction of the long axis rather than circumferentially. In these vessels showing polynuclear collections, or sub-intimal abscesses, there is a notable absence of fibrin and other cellular elements. The lumen of the vessels involved is often crowded with polymorphonuclear cells, but I was never able to observe them adherent to or penetrating the intimal endothelium. However, one must assume that the sub-intimal lesion results from a wandering out of the leucocytes, and where this occurs in the larger arteries the media offers a barrier to the leucocytic penetration and consequently causes an accumulation of the cells under the intima at some portion of the vessel wall.

Thrombosis is a frequent lesion especially of the vessels at or near the site of inoculation. The ear vessels of the rabbit invariably undergo thrombosis after injection of *B. mallei* or its poison; and not only the vein into which the

material is introduced, but also the other veins and arteries. The thrombosis takes place gradually, spreading from the primary focus which is the point of entrance of the injecting needle. In some animals the greater part of the ear sloughed off. The lesions follow injection of poison or killed bacteria as well as living bacilli. The mesenteric and omental veins are commonly affected after intra-peritoneal injection.

Diffuse or focal infiltration of the media with eosinophile cells appears in the smaller arteries; in one female rabbit dying of acute glanders four days after intravenous injection the media of the mesenteric arteries contains eosinophiles. These collections are focal and apparently invade the vessel wall from the peri-vascular tissue where eosinophiles are present in moderate numbers. Occasionally the eosinophiles are grouped about the vaso-vasorum; in other instances they are collected in areas of well advanced degeneration of the smooth muscle fibers.

Lymphoid and plasma cell infiltration of the vessel wall is rarely met with; it is noted in the omental vessels of two female guinea-pigs after a subcutaneous inoculation.

A peri-capillary lymphoid infiltration is quite common in vessels at or near the site of inoculation.

The proliferative changes are confined exclusively to the intima of the vessels, and are undoubtedly the chief factor in the production of the degenerative changes of the media. The proliferation takes place in the endothelial cell layer and not in the sub-endothelial connective tissue. The degree and extent of intimal reaction depend upon the virulence and dosage of the culture. All grades are seen from a simple heaping up of the cells at one focus, or at many foci, to complete occlusion of the lumen. The proliferated endothelial cells are often phagocytic, containing in their protoplasm red blood cells, altered blood pigment, and fragmented nuclei. This proliferation of the intima is best obtained in the rabbit. In some arteries the phagocytic cells project into the lumen in irregular finger-like masses. Mytotic figures in the attached endothelial cells are often observed. (Plate VIII, Fig. 4.)

In vessels in which a gradual occlusion of the lumen occurs the proliferated endothelial cells undergo degeneration. As the narrowing of the lumen progresses the changes in the occluding endothelial cells advance; finally there is complete necrosis of the cells as indicated by the loss of nuclei and the more or less homogeneous hyaline appearance of the whole occluding mass. In cross sections of small arteries a characteristic appearance is the focal nature of the endothelial proliferation; some parts of the intima show no change while other parts show an enormous "heaping up" of the endothelium. (Plate VIII, Fig. 4.)

The proliferated cells bordering on the lumen are often enlarged to ten times their normal size; direct action of the poison on these border cells may be assumed from the condition of the underlying endothelial cells, which are less than half the size, and are in a good state of preservation. Many of the attached cells bordering on the lumen contain, in addition to large and small fat droplets, numbers of red blood cells, polymorphonuclear leucocytes and nuclear fragments. Frequently these large proliferated cells become detached and are seen in the lumen in a state of marked fatty or hyaline degeneration. These large degenerated cells have been found occluding the sinusoids of the liver and cause

ultimately focal necrosis of the parenchymal cells. Some of the smaller vessels are completely occluded with large detached and degenerated endothelial cells, giving rise to a more or less indefinite hyaline and fatty mass, which mass is not unlike the focal areas of degenerated phagocytic endothelial cells seen in the Malpighian bodies of the spleen in certain acute infectious diseases. (Plate VII, Fig. 3.) This phagocytic action of the intimal endothelium of the vessels occurs with remarkable frequency in rabbits and guinea-pigs following the intravenous injection of a glanders culture of moderate virulence. In addition to the phagocytic property of the intimal endothelium there is a still more striking feature in the occurrence of giant cells, which appear as perfectly formed multinucleated elements having a well defined protoplasmic limitation. (Plate VIII, Fig. 6.) These giant cells may be seen attached along the outermost layer of the endothelial heaps. They contain from four to seven nuclei which are usually situated excentrically. There is no doubt as to the endothelial origin of these elements, a fact of importance in considering the histogenesis of giant cells in general. Still another type of proliferation is that seen in vessels in which the endothelium increases evenly around the whole circumference in the form of concentric lamellæ until there occurs complete obliteration of the lumen. In this form of lesion the endothelium always maintains itself in even layers and at no period, even after occlusion, are the cells found degenerated or in a poor state of preservation as in the case of the obliterating endarteritis resulting from the phagocytic cell occlusion.

In the vessels showing this particular form of obliteration by means of superimposed layers of intimal cells, the musculature and adventitial coats increase proportionately to the intimal increase.

The media attains an enormous thickness, and as there cannot be made out any increase in the number of the nuclei of the media an actual hypertrophy of the smooth muscle fibers probably occurs. The adventitia increases even beyond the thickness of the media. Such vessels now appear as enormously hypertrophied closed tubes, each layer sharply defined and proportionately thickened.

A word may be said about the changes noted in the lymphatics. These vessels are dilated even to many times their normal size. The single lining layer of endothelial cells is swollen and finely granular. In many of these cells the protoplasm is filled with small fat droplets. An occasional large endothelial cell may be found in the lumen together with a large amount of serum. The changes in the lymphatics are especially marked in the omentum and in the abdominal parietes after the subcutaneous and intra-peritoneal injection of *B. Mallei*. Occasionally an endothelial cell lining the lymphatic shows a well-defined mytotic figure.

The degenerative lesions are secondary to the proliferative lesions. They always occur in the innermost layer of the media and in that portion of the vessel wall which shows an intimal heaping up of the endothelial cells. At such points in the media the vessels ranging from 0.5 to 1 mm. in diameter show under the low power of the microscope circumscribed bulgings or sacculations of the wall together with broad bands of degeneration which lie immediately external to the internal elastic membrane. The internal elastic membrane is between the sub-endothelial layer on the inner side and the degenerative lesion

of the media on the outer side. There is absolutely no proliferative or degenerative change in the sub-endothelial layer of the intima. In this situation the internal elastic membrane loses its normal sinuous curves, straightens out and becomes a fusiform band with a thickened central fibrillated portion, and the narrower distal portions many times thicker than the normal membrane. Throughout the lesion it has the appearance of dipping deeply down beneath the intima, but this dipping is only apparent and is due to the fact that the endothelium of the intima is proliferated to form several layers of cells. (Plate VII, Fig. 2.) There is an aneurysmal bulging of the vessel wall at the site of the lesion and this bulging outward of the vessel wall is so great that even the circumscribed heaping of endothelial cells fails to fill the increased convexity of the vessel at this point. The section of one of these smaller arteries resembles in contour the section of an eye in its antero-posterior diameter, the sacculation corresponding to the bulging of the cornea.

The degenerative lesion begins in the circular fibers of the innermost layer of the media at a point directly external to the point of greatest thickening of the intima. Its tendency is to extend along the circular muscle fibers maintaining the same thickness throughout; it is only in the advanced lesions that it spreads to the fibers of the central zone. In the advanced lesions cross sections of the larger vessels often show the degenerated medial band extending beyond the primary lesion. The longitudinal sections also show that the medial degeneration in advanced disease of the vessels may extend beyond the primary intimal lesion.

The earliest change in the smooth muscle fibers occurs in the form of fatty granules grouped about the nuclei. Later these fat particles increase in number and in time coalesce and form large globular masses. The nuclei become indistinct and finally disappear. The fibers undergo rapid degeneration and ultimately calcification. (Plate VIII, Fig. 5.) This medial change may occur at first in a single isolated smooth muscle fiber, or in a small group of fibers immediately external to the elastica interna. This selection of single cells by the glanders poison is comparable to the action of certain toxins which give rise to necrosis of single cells or small groups of cells in the liver.

The degenerative changes in the internal elastic lamina are similar in nature to those occurring in the muscle fibers and occur simultaneously with them.

In no case was there a degenerative process in the media without proliferation of the intima; but proliferation of the intima frequently occurred when there was no change in the media.

DISCUSSION.

The vascular lesions in experimental glanders afford an excellent opportunity to study the early intimal and medial changes in experimental arterio-sclerosis. In addition to the degenerative changes analogous to those produced by adrenalin, the bacillus and its toxin produce lesions of an exudative and proliferative character. The kind and severity of the inflammatory conditions would seem to depend largely upon the virulence of the culture.

Various endarterial lesions, for the most part of a degenerative character, have been described by different workers, but the forms produced by the glanders toxin, especially the inflammatory ones, receive no mention. Some authors have regarded the experimental lesions produced with bacteria as analogous to arterio-sclerosis in man, but this view is not generally accepted, and has been disputed by the investigators of adrenalin arterio-sclerosis.

Gilbert and Lyons[3] were the first to produce experimental aortic lesions by the injection of bacteria and their toxins (1) with, and (2) without mechanical injury to the vessel wall. They speak of the successive giving away of the smooth muscle cells, new formation of connective tissue, and calcification of the elastic fibres. In their experiments the aorta alone showed change, which fact together with the sclero-calcareous nature of the lesions lead them to regard the lesions as analogous to aortic sclerosis in man.

Crocq[4] found it necessary to injure the aortic wall in order to produce arterial lesions by bacterial injections. He employed diphtheria, typhoid, and colon bacilli and streptococci in his experiments. Pernice[5] was unable to obtain with the diphtheria bacillus and the staphylococcus lesions in the aorta without first injuring the wall by mechanical means. He especially emphasized the occurrence of a " round cell " infiltration about the vaso-vasorum. Thérèse[6] experimented with bacterial products and describes " round cell " infiltrations of the peri-vascular tissue and capillaries which he attributed to the effect of toxins.

Although changes in the intima and media have been produced separately and described by numerous experimenters with bacteria, no mention appears in the literature, so far as I can find, of medial changes following and dependent upon primary intimal lesions, which occur with such frequency in the vessels of experimental glanders. Workers with bacteria in the experimental production of arterio-sclerosis describe lesions occurring in the aorta but do not mention peripheral vascular changes. It is now well

[3] Gilbert and Lyons, *Archiv de med. exper.*, 1904, xvi, 13; *Compt. rend. Soc. de Biol.*, 1889, xli, 583.

[4] Crocq, *Arch. de med. exper.*, 1894, vi, 583.

[5] Pernice, *Atti del R. acad. delle Scienze med. in Palermo*, 1895.

[6] Therese, Thesis, Paris, 1893.

recognized that in man sclerosis of the peripheral vessels is not infrequently found when there is no demonstrable lesion in the aorta. Moenkeberg[7] described calcareous lesions spreading distally in the vessels of the human extremities while the aorta and its main branches remained unaffected.

The injection of cultures of *Bacillus mallei* of low virulence almost invariably gives rise to lesions in the smaller arteries, while the aorta and its larger branches remain free even from microscopic changes. On the other hand a highly toxic killed culture produces aortic as well as peripheral lesions. The repeated administration of the autolyzed product of highly virulent glanders bacilli produces marked proliferative and degenerative changes in the walls of the aorta and the smaller vessels. These facts would seem to indicate that the aorta and its larger branches are better able to resist this bacterial poison than the peripheral arteries.

Erb[8] states that the adrenalin lesions often occur in the arteries supplying the various organs, and draws attention to those in the renal arteries.

The vascular lesions in the two cases of fatal glanders infection in man were of the sub-acute type, the patients having died of a glanders septicaemia one after six and the other after eight weeks of illness. In these two cases of human glanders, the disease manifested itself as a bronchopneumonia followed by a papular and pustular skin eruption.

In them the aorta and its larger branches were remarkably free from visible sclerosis, showing only a few scattered yellow streaks along the arch and in the abdominal aorta just before its bifurcation. The remarkable features of both cases are the focal vascular lesions of the smallest arteries, especially those of the lungs, muscles and skin. These vascular lesions are by no means confined to the areas of inflammation but are found throughout the peripheral arterial system.

Microscopically the earliest lesions show a focal heaping of one portion of the intimal endothelium. Immediately external to this intimal proliferation the internal elastic membrane becomes straight-

[7] Moenkeberg, *Virchow's Archiv,* 1903, xi, 141.
[8] Erb, *Archiv fur exper. Path.,* 1905, liii, 173.

ened and somewhat swollen. Associated with the intimal heaping there is often a narrow band of degeneration of the innermost layer of the media which lies in close proximity to the intima and separated from it only by the swollen internal elastic membrane.

In the experimental animals, as well as in the human cases, the microscopic study of the peripheral vessels reveal focal changes in the smaller arteries of the internal organs. In general the lesions consists of a circumscribed area of proliferation or "heaping up" of the intima with a corresponding well defined band of degeneration in the smooth muscle fibres of the media. This area of degeneration is always situated in both human and experimental disease in the "inner portion" of the media.

Almost without exception the workers with adrenalin describe the medial changes in the aorta of rabbits as situated in the "central zone" of the middle coat (Josue, Fischer, Pearce and Stanton, Lissauer, Klotz).[9] They explain the difference in situation of the changes in man and in rabbits by the difference of the aorta in man and in rabbits. The intima of the human aorta is a much more complicated structure than the intima of the rabbit's aorta. It contains the so-called musculo-elastic layer. The differences, however, between the smaller arteries of man and of the rabbit and guinea pig are so small that they may be disregarded. The glanders poison produces identical lesions in the peripheral vessels of man, the rabbit and the guinea pig.

Great stress has been laid upon the extensive proliferation of the intima in human arterio-sclerosis which is said to be absent in experimental adrenalin lesions in rabbits. The vascular changes produced by the injection of adrenalin into rabbits have been entirely of a degenerative character and have been described as occurring in the "middle zone" of the media. Adrenalin it seems rarely if ever gives rise to any proliferative change in the intima. The glanders lesion, on the other hand, is caused by primary proliferative changes in the intima of the peripheral vessels, followed by secondary degenerative changes in the media.

[9] Fischer, *Deutsche med. Woch.*, 1905, xxxi, 1713. Pearce and Stanton, *Jour. Exper. Med.*, 1905, viii, 74. Lissauer, *Berlin. klin. Woch.*, 1905, xlii, 675. Klotz, *Jour. Exper. Med.*, 1905, vii, 633; 1906, viii, 504; 1906, viii, 322.

Of the many workers who have approached the question of arterio-sclerosis with experiments on animals, one group has confined their efforts to the injection of bacteria and their toxins with or without first injuring the inner wall of the vessel; another group has injected substances such as nicotine (Adler and Hensel)[10] and adrenalin. The results obtained by both groups of observers have attracted much attention but they have been so varied as to leave us still in doubt as to the origin of arterio-sclerosis. A number of workers have produced proliferative intimal lesions in the aorta by the injection of certain bacteria and their toxins, or lesions of a purely degenerative nature in the media; but nowhere can I find both these types of lesions described as the result of the action of any one species of bacterium and its toxin.

In the vascular lesions produced experimentally by the injection of the glanders bacillus and its toxin we have intimal lesions associated with changes in the media analogous to similar combined changes produced by this bacillus or its poison in the blood vessels of man. Not only are the intimal and medial changes associated but they occur in such a manner as to leave no doubt that the intimal lesion is primary and that the medial lesion is secondary. The degeneration of the media is the result of the proliferative intimal change. This first evidence of degeneration in the experimental lesions and in the human lesions occurs in the " innermost zone " of the media.

CONCLUSIONS.

1. *Bacillus mallei* and its poison produce a variety of vascular lesions in the rabbit and the guinea pig.

2. The type of the lesion depends upon, (*a*) the virulence of the culture, (*b*) the sex of the animal and (*c*) the degree of acquired immunity.

3. The vascular changes of a proliferative and degenerative nature produced by the slow action of the glanders poison in rabbits and guinea pigs are analogous to the vascular lesions caused by sub-acute glanders infection in man.

4. The most common site of the glanders vascular lesions of animals and man is the peripheral vessels, and especially the smaller visceral arteries.

[10] Adler and Hensel, *Jour. Med. Research*, 1906, xv, 229.

5. The aorta is a less common site of the experimental lesions.

6. The vascular lesions produced experimentally by *Bacillus mallei* and its poison consist of three processes, (*a*) exudation, (*b*) proliferation, (*c*) degeneration.

7. The lesions produced by sub-acute glanders in man consist of two processes, proliferation and degeneration.

8. The primary reaction of the vessels in experimental animals and in sub-acute human glanders consists of a proliferation of the endothelium of the intima.

9. The first degenerative changes observed in experimental animals and in sub-acute human glanders occur in the " innermost layer " of the media and not in the so-called " middle zone."

10. The cause of the degenerative change in the inner layer of the media appears to be interference with the nourishment of the circular muscle fibres of the media by proliferation of the endothelium of the intima.

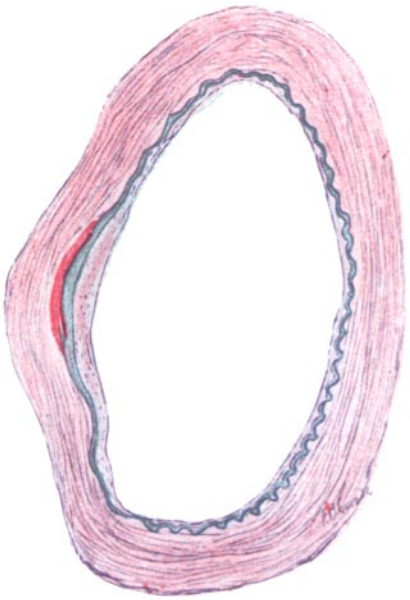

FIG. 1.

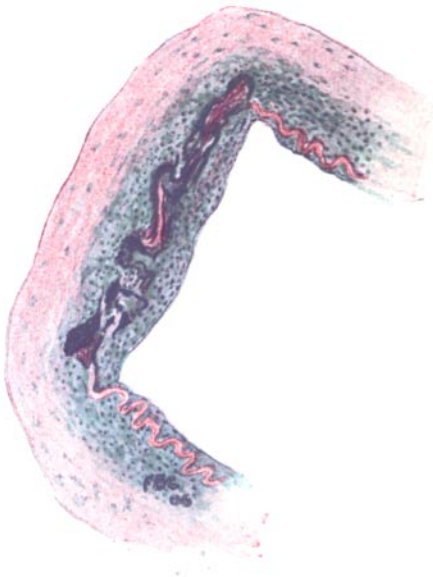

FIG. 2.

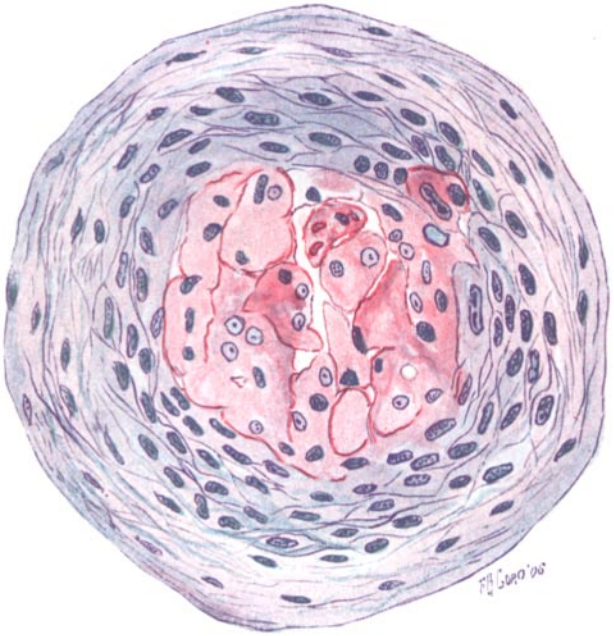

FIG. 3.

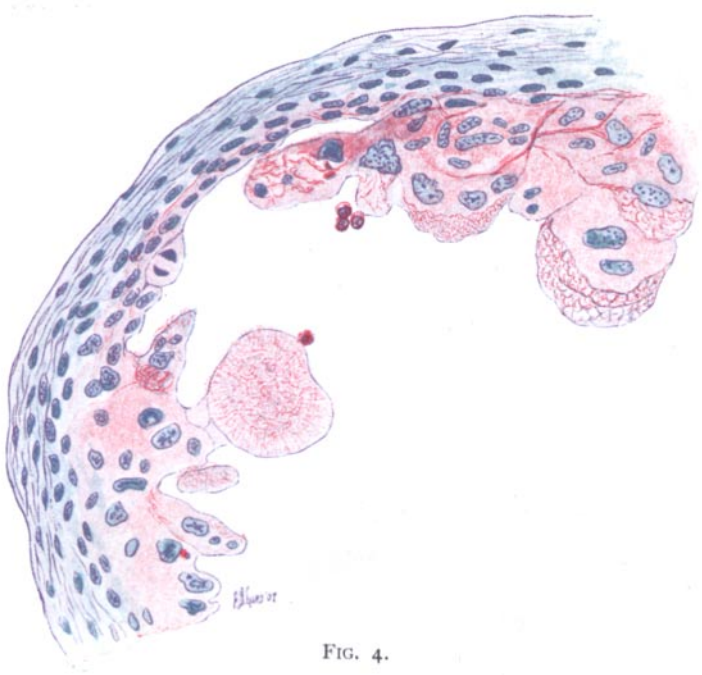

FIG. 4.

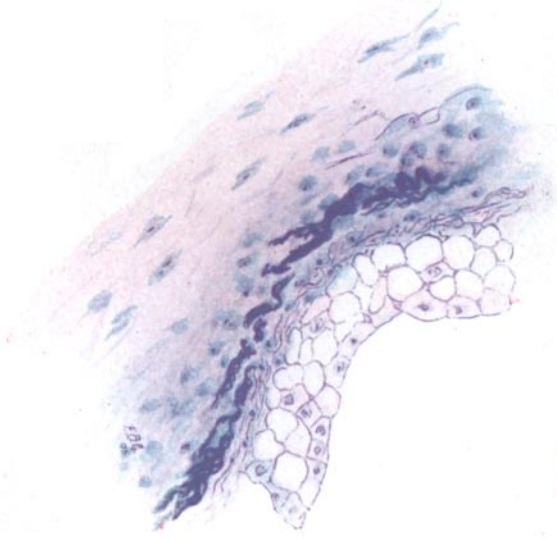

FIG. 5.

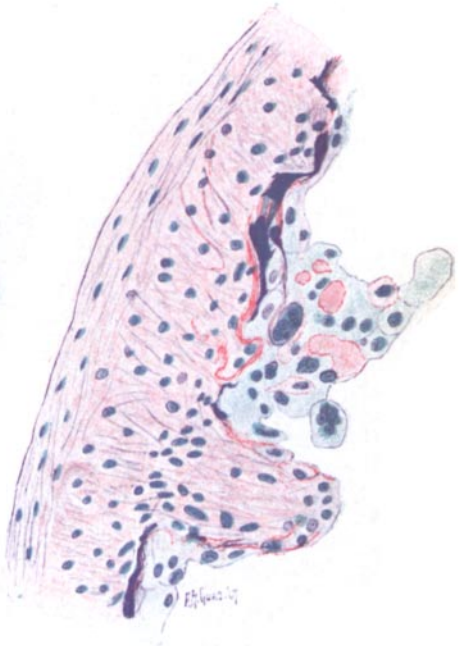

FIG. 6.

Explanation of Plates VII and VIII.

Fig. 1. Cross-section of human artery 2 mm. in diameter, from a case of fatal glanders infection, stained after Mallory's connective tissue method. There is to be noted a definite circumscribed bulging of the vessel wall, in which the lesion consists (1) in the "heaping up" of the intima, (2) the straightened and swollen elastica, and (3) the "innermost" layer of medial fibers in a marked state of degeneration. Degenerated fibers are stained a deep orange-red and are in close proximity to the diseased internal elastica.

Fig. 2. Cross-section of a rabbit's artery 2 mm. in diameter stained with methylene-blue-eosin, showing lesion following the repeated intravenous injection of *B. mallei.* Note the zigzag calcified degeneration of the internal elastica and the innermost fibers of the media. The lesion is immediately external to and associated with the intimal proliferation.

Fig. 3. Cross-section of a small artery of a rabbit stained with methylene-blue-eosin, which shows almost complete obliteration of the lumen with the proliferated intimal endothelium. Many of the innermost cells are seen as detached phagocytes undergoing fatty and other degeneration changes. The innermost proliferated cells of the endothelium are compressed into layers and show good preservation.

Fig. 4. Section of a small artery of the rabbit stained with methylene-blue-eosin. Note the enormous size of the proliferated endothelial cells, which are phagocytic and undergoing fatty degeneration. Also note the attached endothelial cell undergoing mitosis.

Fig. 5. Small artery of the guinea-pig showing (1) marked vacuolation of the "heaped up" intimal endothelium, (2) calcified degeneration of groups of smooth muscle fibers of the innermost portion of the media.

Fig. 6. Small artery of the guinea-pig stained with methylene-blue-eosin. Section shows focal proliferation of the intimal endothelium with the formation of "giant cells." Also shows degeneration and calcification of the internal elastica and innermost fibers of the media.