EXPERIMENTAL ARTERIOSCLEROSIS.*

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PLATES VI AND VII.

The complexity of the pathological picture in the arteriosclerosis of man and the difficulty of determining the nature and sequence of the early changes have led many investigators to attempt the experimental production of the disease in the hope of thus solving some of its many confusing problems. Until within the last two years all such efforts have been unsuccessful. It is true, that Thoma described a diffuse arteriosclerosis in the dog as the result of a chronic experimental aortic insufficiency, and that Gilbert and Lion [1] produced scattered sclerotic and calcareous changes in the vessels of animals by injecting bacteria and their toxins. Thickening of the vascular walls has been described also as the result of poisoning animals with lead. These results, however, have been inconstant and lack confirmation. Jores,[2] in his excellent monograph on arteriosclerosis, published in 1903, reached the conclusion, based on a critical review of the literature, that all experimental methods fail to cause lesions similar to those occurring in man. Various endarterial lesions, obliterative or otherwise, as well as diffuse inflammation and atrophy, have been produced by injections of bacteria or their toxins, by the application of irritating substances to the perivascular tissues, and by ligation and other forms of mechanical injury; but none of these lesions is analogous to true arteriosclerosis.

It is a curious coincidence that in the same year in which Jores reached this conclusion, Josué [3] described experimental lesions

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in the aorta of rabbits, somewhat similar to those of human arteriosclerosis. These were caused by frequent intravenous injections of adrenalin. With the exception of a few inconclusive experiments of Jores, who gave dogs adrenal tablets in their food in the hope of obtaining lesions due to heightened blood-pressure, Josué appears to be the first investigator to attempt the experimental production of arteriosclerosis by the use of the principle of the adrenal gland. His results have been confirmed by Erb,[4] Rzentkowski,[5] Fisher,[6] and others. Their reports include but a small group of experiments and deal for the most part with the late changes, which they describe variously as arteriosclerosis, atheroma, and calcification. The principal histological changes to which they call attention are alteration in the elastica and the infiltration of lime salts.

The importance of a method capable of producing an experimental lesion so closely resembling human arteriosclerosis is apparent. It allows an opportunity to study many of the obscure problems of the disease, as the nature of the primary changes, the sequence of these changes, and the combinations which constitute the fully developed lesion. It was with the hope of elucidating some of these problems that the present investigation was undertaken. The results have been satisfactory beyond all expectation. Vascular changes capable of throwing much light upon the pathology of arteriosclerosis, and also that of aneurism, have been readily produced. Only a general outline of these will be given at this time, for many of the details of the histological changes, especially of the process of repair, are so important that they require a more extended study. A consideration of these details is therefore reserved for a future publication based on a second series of experiments now in progress.

Methods.—Rabbits have received in the veins of the ear repeated injections of a 1 to 1000 solution of adrenalin. The solution was prepared by a chemist with due regard to asepsis and chemical purity. Physiological salt solution was used as a medium. In addition to adrenalin (Parke, Davis, & Co.), it contained also chloretone in the proportion of one half of one

per cent., and a sufficient amount of hydrochloric acid to make the solution faintly acid.

A dose of three minims * repeated every other day has been the usual procedure, though in some experiments the dose has been gradually increased. Not infrequently the early injections cause death from acute dilatation of the heart and pulmonary œdema. Such accidents usually occur after any one of the first seven or eight injections. If the animal survives this period it appears to gain a certain amount of immunity, or at least tolerance to adrenalin, so that the dose may be gradually increased during several weeks until from twenty to twenty-five minims may be given every day. This increased resistance to adrenalin has been noted also by Erb and Fisher. The animals have been killed after periods varying from a few days to eight and a half weeks.

For histological study, tissues have been preserved in Zenker's fluid, alcohol, and six per cent. formalin, imbedded in celloidin, and stained with hæmatoxylin and eosin, and by Weigert's elastica and Mallory's connective-tissue methods. The Scharlach R. and osmic-acid methods have been employed for the demonstration of fat. Sections hardened in Flemming's solution and stained by hæmatoxylin have been found to be most satisfactory for purposes of general study. By this method, the nuclei and areas of calcification stain blue; fat, if present, black; the elastic fibres stand out prominently as light greenish-yellow glistening lines, while all other structures are of a faint gray color.

Results.—Of twenty animals receiving adrenalin, nine succumbed to the acute effects of the drug within fifteen days. These nine represent animals receiving one, three, four, five, seven, and eight injections of adrenalin on alternate days. In some instances death resulted within a few minutes after the injection; in others, after a few hours. The immediate effect of the

* Throughout this report the dose will be given in minims. It would be better perhaps to use the values of the metric system, but as Josué and others give the dose in minims or drops, we have for purposes of control used the same system of measurement.

intravenous administration of adrenalin is collapse with difficult and rapid respiration. The animal lies on its abdomen with legs outstretched and head resting on the table or raised in spasmodic respiratory effort; death, preceded by severe convulsive movements, may occur immediately. Other animals recover from the immediate manifestations only to succumb after a few hours. Upon post-mortem examination, the usual picture is acute dilatation of the heart and œdema of the lungs, with not infrequently small hæmorrhages beneath the pleura and pericardium; in one animal hæmorrhages were also found in the adrenal. These acute lesions indicate a very serious disturbance in the terminal vascular territories and are worthy perhaps of further investigation; but as our problem has to do only with the histological changes in the larger vessels its scope has been limited to that extent. The theoretical conclusions to be drawn from experimental adrenalin œdema and their possible application to acute œdema of the lung in man have recently been discussed by Josué.[7]

In but two of this group of nine animals were changes in the vessels demonstrable. These will be discussed later.

The second group of eleven animals represents those receiving from eight to twenty-eight injections during periods varying from sixteen to fifty-nine days. One of these died on the twenty-fifth day from spinal hæmorrhage; another on the forty-sixth day, from unknown cause, three weeks after the cessation of injections; the remaining ten were chloroformed. The aorta in six of these showed marked gross lesions; in the other five it was negative both macroscopically and microscopically. It is of interest that these five animals were all from the same litter and each weighed about 750 grammes at the time of the first injection. Pic and Bonnamour[8] have come to the conclusion that it is impossible to produce vascular lesions by adrenalin in rabbits weighing less than 2000 grammes, and quote in support of this opinion their negative experiments with animals weighing about 1200 grammes. That this is not an absolute rule is shown by the fact that the most characteristic and advanced lesions in our series occurred in an animal weighing 870 grammes at the

beginning and 1020 at the end of the experiment. Otherwise, however, our experience with small animals supports the contention of Pic and Bonnamour.

Attempts to produce lesions in dogs have been unsuccessful. Both old and young dogs have been used. One of the latter, a puppy 2120 grammes in weight, received in the ear vein in one month one hundred and thirty minims of adrenalin in doses rising gradually from four to twenty minims. Although respiratory and cardiac disturbance frequently occurred immediately after injection, thus indicating a definite physiological action of the adrenalin, no evidence of vascular lesions could be found at autopsy or upon microscopic examination.

Gross Lesions.—These appear to be limited to the aorta; the involvement of other arteries as the brachial, carotid, and renal, which Erb describes, we have not found. The lesions in the aorta are most marked in the thoracic portion and are seldom found below the cœliac axis or in the first portion of the arch. The earliest change in the vessel wall manifest to the naked eye is a faint longitudinal or irregular grayish streaking of the intima without thickening. This appearance was seen as early as the ninth day in a rabbit which had received five injections. After eight to fifteen injections, especially if the animal is allowed to live for a week or longer after the last injection, very definite lesions are apparent. These consist of irregular, isolated, or confluent areas, usually slightly depressed, of a pearly gray color, and almost constantly calcified. The following protocol illustrates this condition:

RABBIT No. 11.—Weight, 2300 grammes. Killed May 2 after injections as follows: March 29 and 31, 4 minims; April 2 and 4, 5 minims; April 6 and 8, 7 minims; April 10, 10 minims; April 12, 12 minims; April 14, 15, and 16, 15 minims. *Autopsy.*—The aorta is distinctly dilated, measuring twelve millimetres in circumference at the arch. Beginning at the origin of the left carotid artery is an irregular patch, thirty-seven millimetres long, averaging two and a half millimetres in width, and extending in a spiral course along the aorta for a distance of thirty millimetres so as completely to encircle it. Along this patch the wall of the aorta is slightly dilated. The media is firm, parchment-like, and so distinctly calcified that the vessel cracks in several places when the arch of the aorta is straightened. At one side of this large patch are two very small oval areas of similar structure. There is no atheroma or ulceration. All the other organs are entirely normal.

The late lesions occurring after from twenty to twenty-five injections are very well marked. The aorta is more or less distorted, rigid, and non-elastic. Irregular dilatations alternate with elevated brittle areas of calcification. Distinct ulceration with atheroma is not readily demonstrable. Diffuse calcification is not infrequent and small aneurismal dilatations may be present. The following protocol illustrates the advanced lesions occurring in animals receiving large doses during a considerable period of time:

RABBIT No. 3.—Weight, 870 grammes. Received first injection of 3 minims in ear vein on November 30, 1904. Similar injections were repeated on alternate days until December 30, after which date the dose was rapidly increased until it reached 20 minims daily. The animal was killed on January 20, 1905. *Autopsy.*—Weight, 1150 grammes. The thoracic aorta measures six millimetres in diameter, is distinctly dilated, irregular in outline, and stiff and brittle, although the wall is apparently thinned. This condition extends as far as the cœliac axis, beyond which point the abdominal aorta is apparently normal. On opening the aorta the inner surface of the abdominal aorta above the cœliac axis shows numerous yellowish, slightly raised, calcified patches from one to one and a half millimetres in diameter, which in the upper part of the thoracic aorta become confluent. Except in one of the small patches situated in the upper portion of the abdominal aorta there is no evidence of ulceration. All other organs are normal.

The following description from the protocol of a parallel experiment illustrates the tendency to the formation of small aneurisms:

RABBIT No, 2.—The aorta averages three millimetres in diameter except at three places where distinct dilatations occur. The first of these begins three centimetres below the origin of the aorta and involves the right anterior wall of the aorta for a distance of two centimetres, giving the appearance of a fusiform aneurism; the diameter of the aorta at this point is four millimetres. A second dilatation, two and a half centimetres in length, occurs in the distal part of the thoracic aorta, and a third involves the abdominal aorta for a distance of one centimetre immediately below the cœliac axis. On section, the inner surface of the aorta shows sharply circumscribed saccular dilatations, varying from one to two millimetres in depth and corresponding to the protrusions described above. The walls of these aneurisms contain firm placques of calcareous material. The intervening aorta shows a number of yellowish-white elevated areas one to three millimetres in diameter. The aorta below the dilatation at the cœliac axis is macroscopically normal.

Histology.—In the group represented by the nine rabbits which died during the first two weeks, the vessels of but two

showed histological changes. These were animals which had received four and five injections respectively; the first dying on the fifth day and the second on the ninth day. Scattered through the middle coat of the aorta in each of these are small longitudinal or occasionally irregular, finely granular foci of degeneration. In these areas no nuclei are visible and the muscle fibres are transformed into a finely granular, almost hyaline material which stains deeply with eosin. The exact nature of this change is not readily determined, but as the various methods of demonstrating fatty transformation are negative, and those which differentiate elastic fibres show no change in the staining reaction of the latter, the condition would appear to be a simple necrosis limited to the muscle fibres. The elastic tissue has, however, undergone very characteristic morphological changes which are found only in the degenerated areas, and are due, presumably, to mechanical influences. In the normal aorta the elastic fibres have a distinctly wavy or curled appearance and are definitely separated from one another; in the areas described the fibres lose this appearance and become straightened and closely approximated. They are also swollen and occasionally appear to be fused together. Fracture of the fibres is seldom seen at this stage of the process.

A more diffuse lesion of this character was found in an animal killed on the twenty-fourth day after receiving eleven injections. The changes involve the entire circumference of the vessels, sparing to a certain extent the innermost and outermost portions of the media. In this central zone few or no nuclei can be found, and the altered tissue presents a uniform, finely granular appearance, relieved only by the glistening lines of the elastic fibres. The latter by selective stain are seen to be so closely massed together that individual fibres can be distinguished only with great difficulty. On either side of this zone the less degenerated portions of the media show irregular areas which take the hæmatoxylin stain in a manner very suggestive of early infiltration with lime salts. There is, however, no distinct calcification and no fracture of elastic fibres.

Lesions of great interest in comparison with the above were

observed in two rabbits which had received the same number of injections, but which were allowed to live for ten and eighteen days, respectively, after the last injection. In the aorta of each are found a few areas of granular necrosis, but for the most part these areas have been altered by infiltration with lime salts. From the topography of the areas of calcification and their relation to the necrotic foci, it is evident that the deposition of lime salts follows the necrosis. It would appear therefore that the primary degenerative lesions are well advanced by the end of the third week, and that after this but one or two weeks are sufficient for advanced calcification; the latter may, however, occur much earlier, for in one rabbit killed on the sixteenth day small but very definite foci of lime infiltration are present.

In the areas of lighter deposition of lime it is seen that these salts are deposited first between the elastic fibres, an observation which indicates that the destruction of the muscle fibre is the older, and therefore in all probability the primary, lesion. In the areas of advanced calcification, although the elastic fibres appear to be completely destroyed by the calcareous transformation, Weigert's stain shows them to be still present, though they stain poorly and are frequently fractured. Not uncommonly at a point of fracture one bundle of fibres overlaps another, the ends being splintered together by an encapsulating mass of lime salts. These fibres are always perfectly straight except at angles formed by fracture. A definite relation appears to exist between calcification and fracture of the elastic fibres. In areas of the most extensive calcification few breaks in the elastic fibres have been observed, while, on the other hand, they are constantly present in the aneursimal dilatations which show comparatively little calcification.

Lesions of this stage show the first evidence of repair. This is indicated by the collection of newly formed cells about the foci of calcification. These cells are closely massed, surrounded by but a slight ring of protoplasm, and appear to be of connective-tissue origin, though it has not always been possible to distinguish between such cells and the nuclei of smooth muscle fibres. No accumulation of polymorphonuclear leucocytes or lymphoid

cells has been seen. Of greater interest are the proliferative changes in the intima. These occur only opposite distinct breaks or considerable depressions in the media, and include proliferation of the lining endothelium and, to a greater extent, of the subendothelial tissues. The endothelial cells proliferate to form two or three layers of cells longitudinally arranged, while the subendothelial space, barely visible in the normal aorta, becomes very prominent owing to the presence of closely packed oval nuclei arranged vertically to the lining endothelium. Between the nuclei are delicate newly formed connective-tissue and elastic fibrils; in the late stages the newly formed elastic fibrils are very prominent. In the angle of fracture the nuclei of the media assume a perpendicular arrangement very striking in contrast to their longitudinal arrangement elsewhere. The entire picture is distinctly that of a compensatory proliferation: an effort to strengthen the weakened point in the vessel wall.

Such are the essential phases of this lesion in the order of their sequence. The more prolonged experiments offer no fundamentally new features; but the combinations of these primary changes in the course of the more extensive involvement of the vessel wall lead to a complex histological picture somewhat resembling advanced human arteriosclerosis. This is seen only in animals which have received gradually increasing doses during a period of seven or eight weeks. The vessel wall becomes greatly thickened, not only by infiltration of lime salts, but also by an extensive repair process which involves the intima as well as the media. Small foci of complete necrosis, analogous to atheroma and entirely different from the primary degeneration, are also seen in areas in which the elastic fibres are completely destroyed. Such foci present a uniform, finely granular appearance and stain deeply with eosin; the osmic-acid method for fat is negative, but a few fine droplets are evident after treatment with Scharlach R. About such areas repair takes place, but it is not as active as it is about the masses of lime salts.

The most striking feature of the late histological picture is the extent of repair in the intima. The latter, with its newly formed

connective-tissue and elastic fibrils, becomes so greatly thickened that it constitutes in some places from a quarter to a third of the entire vessel wall, and offers convincing evidence of the compensatory nature of the repair process.

It is in these late lesions that the small aneurisms are seen. They occur at points where the elastica is so completely destroyed as to be transformed into an indistinct mass of fractured granular and fused fibres. The transition from normal to necrotic fibres is always sharp and distinct and marked by complete fracture of all elastic tissue. It is worthy of note that in the thinned wall of these aneurisms the degree of calcification is, as a rule, less marked than elsewhere; the adventitia is compressed, but otherwise unaffected.

The changes in other organs include enlargement of the heart, œdema and congestion of the lungs, and occasionally degenerative changes in the heart and skeletal muscles. In one case degenerative lesions were found in a nerve ganglion adherent to the aorta. It was thought that these changes might have some etiological relation to the vascular lesion, but a thorough study of all our material failed to reveal lesions in other animals, or in other ganglia of the same animal. The affected ganglion was in the adventitial tissue of an aneurismal dilatation, and the effects of pressure and disturbed blood-supply were sufficient, apparently, to account for the changes observed.

The Mode of Action of Adrenalin.—The manner in which adrenalin produces these lesions is a matter of widely varying opinion and, unfortunately, one which cannot readily be determined by our method of experimentation. Until we know more about the toxic action of adrenalin and can distinguish between the lesions due to this action and those due to increased blood-pressure, and especially between its direct and secondary effect on the blood-vessels, we can hope for no elucidation of this problem. Drummond,[9] who has recently made a thorough study of the histological lesions caused by adrenalin, divides them into those due to toxic action and those due to increased blood-pressure. It is obvious that such a classification is difficult, for it is impossible to determine to what extent degenerative lesions

are due to interference with the circulation through action on the blood-vessels.

A discussion of the variety of ways in which adrenalin might act, directly or indirectly, to cause degenerative changes in the media of the aorta would, in the present state of our knowledge, be of little value. The difficulty of reaching definite conclusions is illustrated by the diversity of opinion expressed by those who have previously produced the lesions here described. Josué believes them to be due to a combination of a specific toxic influence and the increased blood-pressure; Rzentkowski believes in the latter influence only; Erb ascribes an important influence to disturbance of the *vasa vasorum;* Fisher thinks that, aside from the increased blood-pressure, disturbances of metabolism play an important part; Lissauer [10] believes that the toxic influence is the most important factor, but offers no explanation of its mode of action.

No definite conclusion can be drawn from our own experiments. The observation, that the earliest changes occur in the media and are apparently primary in the smooth muscle fibres, suggests, in view of the well-known physiologic action of adrenalin on this tissue, a direct and selective toxic action; but the absence of similar lesions in vessels other than the aorta does not support this view. For the same reason, and also because of the absence of changes in the adventitia, the theory concerning the *vasa vasorum* appears to be untenable. On the other hand, if we assume, as is very probable, that the changes in form and arrangement of the elastic fibres are due to the same factor or factors which cause the destruction of the muscle cells, and are not secondary to the latter, we have a strong argument in favor of the influence of a greatly heightened blood-pressure, for it is conceivable that these diffuse changes might result from a continued severe stretching of the vessel wall at the time of the ischæmia due to the primary action of the adrenalin. In other words, it is possible that at the period of vascular spasm produced by the adrenalin the nutrition of the vessel wall is altered. This alone would not explain the limitation of the lesion to the aorta, but the added mechanical disturbance due to the extreme disten-

tion of the vessel wall would appear to be sufficient to bring about a condition analogous to anæmic necrosis. These problems, however, must be settled by other methods of experimentation. Although it is impossible to determine to what extent the primary changes are mechanical and to what extent toxic in nature, it is certain that some of the secondary changes, as the fracture of elastic fibres and the formation of aneurisms, are largely due to mechanical factors.

Comparison of the Experimental Lesions and those Occurring in Man.—If due allowance is made for the difference in size between the aorta of the rabbit and that of man, it must be admitted that the lesions produced in the former, as the result of the administration of adrenalin, are somewhat similar to those occurring in human arteriosclerosis. They are not, however, analogous. All the essential processes are represented and, considering the delicate character of the wall of the rabbit's aorta, the lesions are relatively as extensive and cause the same degree of deformity. There is, however, as compared with the human lesion, a difference in the initial change and in the degree of atheroma, which is insignificant and limited to the media. Extensive atheroma, however, cannot be expected in a vessel wall as thin as the rabbit's aorta, and, moreover, in none of these experiments has a period of time sufficient for the occurrence of degeneration in areas of excessive intimal proliferation elapsed. More prolonged experiments will yield, it is to be hoped, lesions more conclusive in this respect. For the present the condition may perhaps be regarded as arteriosclerosis of the rabbit, but not as a condition analogous to the arteriosclerosis of man.

In the light of the information gained from the study of these experimental lesions, it would be desirable perhaps to discuss critically the various theories concerning the nature of arteriosclerosis and especially the character and sequence of the histological changes. Such discussion, however, does not come within the scope of this communication, and many minor points must be determined before comparisons are justifiable. It is sufficient, for the present, to point out the strong support afforded Thoma's view, that the primary lesion of arteriosclerosis occurs

in the media and is, essentially, the result of changes in the elastica; and that the alterations in the intima constitute a repair process the object of which is to compensate for the weakened media and the widened lumen.

EXPLANATION OF PLATES VI AND VII

Fig. 1.—Gross appearance of a portion of the aorta from an animal which had received twenty-seven injections and was killed on the twenty-ninth day. The drawing is twice the actual size of the original specimen.

Fig. 2.—Diffuse degeneration of the central zone of media; eleven injections; twenty-fourth day. Hæmatoxylin and eosin; No. 4 oc., No. 3 obj.—Leitz.

Fig. 3.—Microscopic picture of lesion pictured in Fig. 1. Destruction of the media with infiltration of lime salts; extensive proliferation of the intima. Hæmatoxylin and eosin; No. 4 oc., No. 3 obj.—Leitz.

Fig. 4.—Elastic tissue stain of an area very similar to the above, illustrating extreme destruction of the elastica.

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FIG. 1

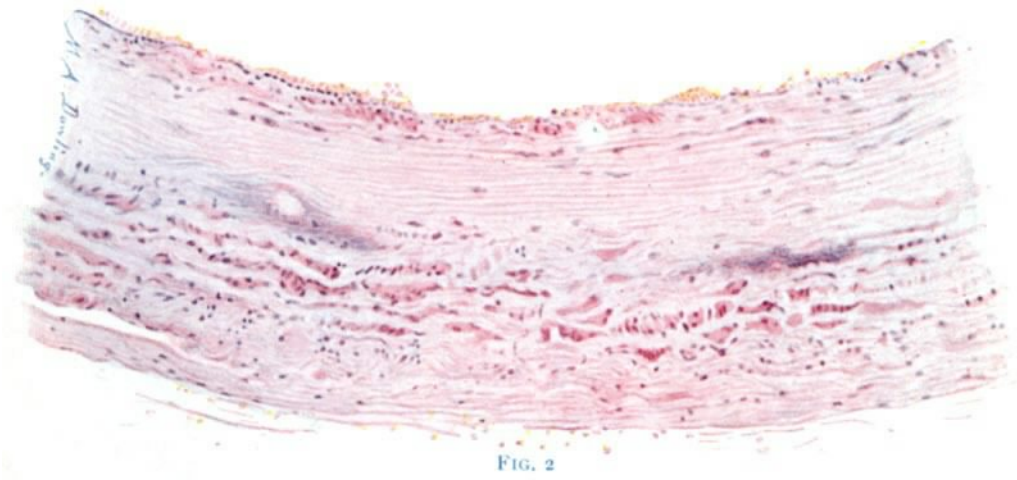

FIG. 2

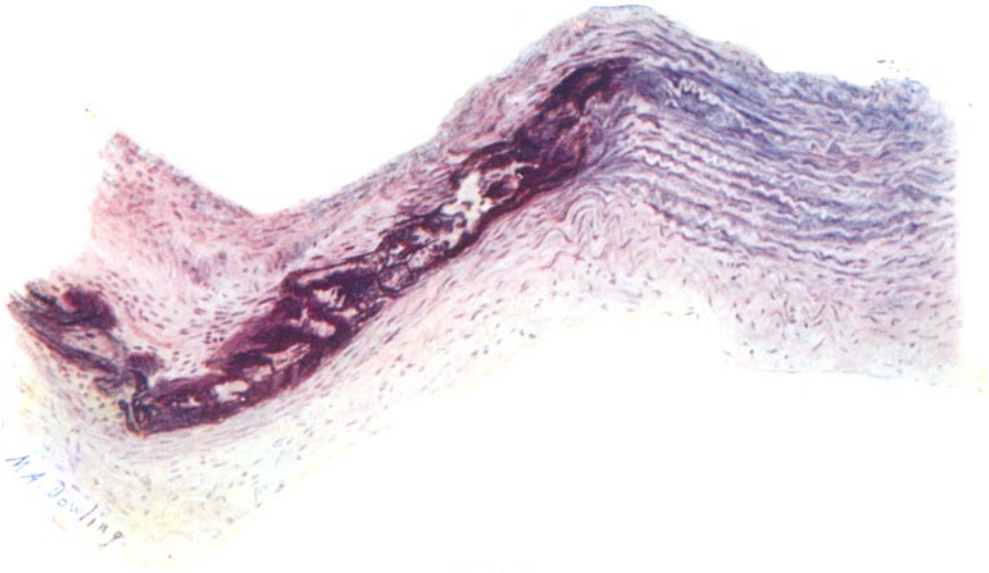

FIG. 3

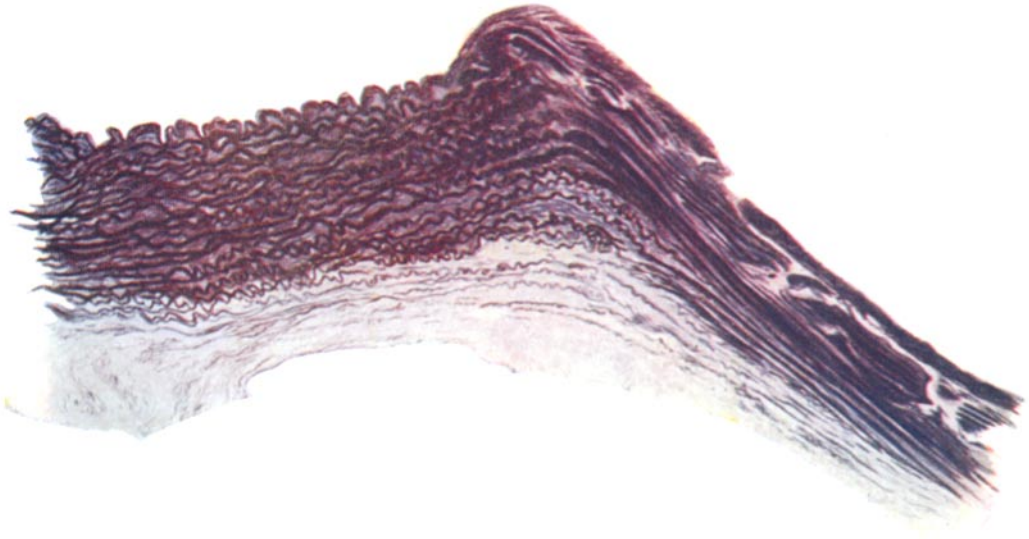

FIG. 4