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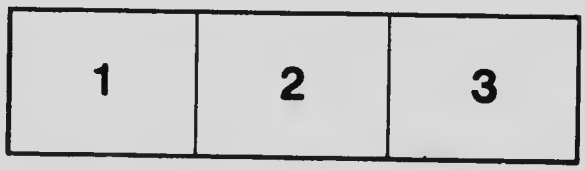

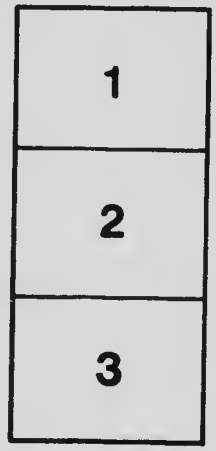

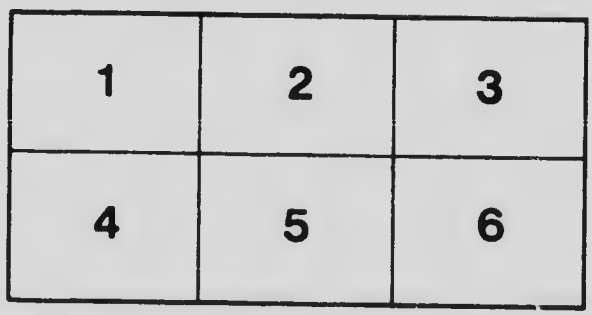

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STUDIES UPON THE INFLUENCE OF TENSION IN THE DEGENERATION OF ELASTIC FIBRES OF BURIED AORTÆ.

By W. H. HARVEY, M.B. (Tor.).

STUDIES UPON THE INFLUENCE OF TENSION IN THE DEGENERATION OF ELASTIC FIBRES OF BURIED AORTÆ.

By W. H. HARVEY, M. B. (Tor.).

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Plates XVII, XVIII, and XIX.

The experiments described in this paper were carried out with the object of studying the power of resistance possessed by elastic tissue to degenerative changes, the order of such changes as do occur when this resistance is overcome, and the effect of continuously applied tension upon these fibres.

Space does not permit of more than a brief review of the investigations already made by pathologists upon this tissue under various abnormal conditions. For more detail the reader is referred to a very concise paper by Jores, in Lubarsch and Ostertag's *Ergebnisse der allgemeinen Pathologie* (1902, Jahrgang VIII, p. 590).

Disappearance of Elastic Fibres.—Under various pathological conditions, organs, which show in health a large quantity of elastic tissue, exhibit a very marked decrease, if not total absence, of these fibres. The cause of this disappearance is much disputed. Du Mesnil de Rochemont considers that degeneration of elastic fibres is due to chemical action. He comes to this conclusion from observations made upon tuberculous material. He believes that the breaking down and disappearance of the elastic tissue is the result of free toxins which are the products of the bacillus of tuberculosis. In experimental pulmonary tuberculosis in animals produced by injecting cultures of the bacillus into the vascular system, Wechsberg noted a disappearance of the elastic fibres of the lung; this condition was recognizable within six hours and was very marked at the end of two days. He attributed it to the action of toxins of the micro-organism.

In sections of lupoid material, du Mesnil de Rochemont found a narrow marginal zone, about a line in width, which was free from

these fibres. Here again he thinks that their absence is due to the action of toxins. Miller has observed the disappearance of this tissue in pulmonary gangrene and in syphilitic lungs within the areas of miliary gummata.

Obermüller, studying the changes in the vaginal wall in a case of prolapsus vaginæ, found an entire absence of elastic fibres in that part of the mucous membrane which was prolapsed and therefore exposed to mechanical irritation. This he thought was the result of fibrosis, secondary to the continual friction. The same author finds a similar absence of elastic fibres in those portions of the vaginal mucous membrane which have been subjected to prolonged pressure from constant wearing of a pessary. I can bear out Obermüller's [illegible]ions concerning the disappearance of elastic fibres wi[illegible]s vaginæ. I have had the opportunity of examining s[illegible]n a case of prolapse of six years' duration. There was [illegible]almost [illegible]tire absence of these fibres in the tunica propria and an extensive increase of white fibrous tissue. An obliterating endarteritis was also present.

Melnikow-Raswedenkow found, in testicular tuberculosis, a marked degeneration of the elastic fibres about the inflammatory zone of the tubercles, but in the necrotic centres he found the fibres intact. The explanation he advanced was, that the factor in the production of degeneration was the inflammatory cell infiltration and not the presence of toxin, since if the latter were the cause, the fibres would not remain in the necrotic tissue, which, one would suppose, would be surcharged with toxins. Schmaus finds that elastic fibres disappear very much more readily in the presence of granulation tissue than in true tubercles

Jores agrees with the explanation offered by Melnikow-Raswedenkow. He quotes the work of his pupil; elastic tissue was exposed to inflammatory exudates and transudates at blood-heat in an incubator and was studied at various intervals, but in no case were changes found which at all resembled those occurring in inflammatory conditions during life though the tissue was in close contact with toxins which were present in the exudates and transudates.

In acute conditions a number of observers have found a

disappearance of the elastic elements. Others however deny that they are absent and claim that the disappearance is only apparent, elastic tissue being invisible as the result of loss of staining power. Those who hold the latter view are Unna, who notes this condition in œdema, Luithlen, Passarge, Jores, and others. Jores believes that this change explains the apparent loss of elastic tissue in pulmonary stasis, œdema, and lobular pneumonia.

Fragmentation.—With lesions such as aneurysm, arterio-fibrosis, etc., the elastic fibres are found broken into fragments and the condition may be designated as fragmentation. Koester believes that this is of inflammatory origin and considers the small fibrotic areas which are found in vessel-walls—the so-called Koester's spots—the result of a similar process. In these areas the elastic fibres are segmented, but this he claims is primarily due to the inflammation. Fabris supports him in this explanation; he produced necrosis experimentally, and then investigated the change which resulted in the elastic fibres. He found extensive fragmentation of this tissue. Jores appears to hold the same view concerning the condition. He does not consider, however, the gaps, which Thoma, Manchot, and others have described as isolated tears, to be anything other than the physiological openings or fenestra of the laminæ elasticæ.

Among those opposing this view are von Recklinghausen, Manchot, and Dmitrijeff. These authors hold that mechanical action is the cause of this breaking. Manchot observed in the walls of aneurysmal blood-vessels fragmentation of the elastic fibres, but no indications of inflammatory reaction in the other tissues of the wall. He considers it very unlikely, therefore, that the elastic tissue is attacked by inflammation while other tissues escape. The same observer has found interruptions in the course of individual fibres, and these, he thinks, are the result of excessive tension. Thoma advances the same theory and apparently believes that these isolated tears are the initial stage of diffuse arterio-fibrosis, while the patchy variety commences in foci where several fibres are involved.

Dmitrijeff thinks Koester's spots are small areas of granulation tissue occurring around isolated tears and that they are the result

of irritation caused by these breaks. Katsurada experimented by pinching the skin of animals, with forceps, and then examined the site of injury at different periods. He found that fragmentation did not immediately follow the injury, but occurred when the growth of fibrous tissue, consequent to stimulation caused by the trauma, became excessive.

Granular Degeneration.—With one form of degeneration the elastic fibre is represented by a line of irregular granules which take the elective elastic stains. Weiszmann, Neumann, and Manchot observed this form of degeneration in the early stages of arterio-fibrosis and aneurysm. Dmitrijeff found the same condition and obtained an elacin reaction in some of the granules. Miller has noticed this condition in the tubercles occurring with acute miliary tuberculosis. With syphilitic involvement of the vascular system, Abramow has observed extensive granular disintegration of the elastic fibres.

Jores has described a fatty degeneration of elastic fibres occurring in the absence of other signs of a degenerative process. In association with this condition he has noticed granulation of neighboring fibres and for this reason believes that both these changes are due to the same cause.

Calcification.—The elastic fibres may be impregnated by a lime salt; calcification occurs in cases of sclerosis involving the walls of blood-vessels or other tissues containing elastic fibres. Kockel has described incrustation of elastic fibres in calcified lung tissue. The deposit, he found, consisted of fine granules laid down along the fibres which were frequently broken. These granules stained well with resorcin-fuchsin. Von Davidsohn made a similar observation and likens the appearance presented by the fibre under these conditions to a chain of anthrax bacilli. Fragmentation in this tissue he considers analogous to fragmentatio myocardii. He does not believe that calcification begins in the fragmented fibres.

In some pathological conditions of the thyroid gland, Jores found calcareous degeneration of the elastic elements. The deposit was strictly limited to the elastic lamina. Matusewiez and Rona describe similar changes. The last-named writer found some of these granules occurring as inclusions in giant cells.

Upon removing the calcium salt from them he obtained a good elastic stain with orcëin and therefore was led to believe that the giant cells were responsible for the presence of calcium. In addition to the calcium salt, Rona found traces of iron. Gierke had shown, previous to Rona's observation, that iron is usually present with this form of degeneration.

Methods.—In order to study degenerative changes in normal elastic tissue an aorta obtained under aseptic conditions from the body of a healthy guinea-pig killed at the time required was buried within the tissues of another animal of the same species. The hair of this animal was removed over the site of operation by means of a depilatory powder.[1] Chloroform was administered to animals to be killed, but when recovery from the anæsthetic was desired it was found safer to employ a mixture of alcohol, chloroform, and ether (one, two, and six parts respectively).

The incision into the second pig extended through the skin, subcutaneous tissues, and the more superficial muscular tunic of the abdominal wall. With the handle of a scalpel a small pouch or pocket was formed by separating the muscular layers and into this pocket was placed the tissue to be buried. In order to prevent it from curling up, two anchor-ligatures of catgut were attached to the ends of the vessel; these ligatures were then led through the outer wall of the pouch, one at each end, and when drawn fairly taut were sealed to the skin. The wound was closed by catgut sutures and dressed with sterilized cotton and collodion.

In some instances (Groups B and C) in which it was desired to place the elastic elements under tension, the aortæ were distended under pressure with a sterile three per cent. solution of agar-agar in 0.85 per cent. sodium chloride, in other instances with sterile paraffin melting at 43° C.; the vessels were then doubly ligated at each end and excised.

The animals in which the collapsed vessels were buried were killed at the end of 3, 6, 12, 27, 33, 42, 54, 75, 106, 150, and 303 days, while those containing the distended aortæ, at the end of 10, 12, 15, 21, 27, 30, 35, and 50 days. After death the sections

[1] A very good formula for this powder will be found in Crocker's work on Diseases of the Skin, in the section on Hypertrichosis.

of abdominal wall containing the buried structures were removed, fixed and hardened in formalin (10 per cent.) solution and alcohol, and imbedded in paraffin.

The stains employed were hæmatoxylin and eosin, Van Gieson's fluid, resorcin-fuchsin, Weigert's elastic stain, orcëin, Unna-Tænzer's elastic stain, Wasserblau-safranin and three per cent. silver nitrate solution, and Schmorl's modification of v. Kossa's calcium reaction. Special tests for the presence of phosphates and chlorides were made.

GROUP A.—AORTÆ BURIED IN UNDISTENDED CONDITION.

EXPERIMENT 1.—*Aorta after three days.* The vessel lies loosely imbedded in a quantity of granulation tissue, in the intermuscular space of the abdominal wall. The elements of the muscle and connective tissue of the buried aorta exhibit somewhat impaired reactions to the stains employed. The elastic structure of the vessel-wall stains well with the elective elastic tissue method and exhibits no indication of a degenerative change. The lumen of the artery remains patent.

EXPERIMENT 2.—*Aorta after six days.* The buried vessel is firmly imbedded in recent granulation tissue. The lumen is occluded by a coagulum, in which commencing organization may be seen. There is a decided loss in quantity as well as in staining power of the muscle and connective tissues of the vessel but the elastic fibres are as yet apparently unchanged.

EXPERIMENT 3.—*Aorta after twelve days.* At this period the muscle and connective tissues have apparently been entirely absorbed, and the only remains of the buried vessel are the elastic framework. This structure is normal in appearance and to the elective stains shows a good reaction. The elastic fibrils, at the periphery of the vessel, exhibit a slight tendency to fragment, but this condition is strictly limited to fibrillar portions of the elastic structure.

EXPERIMENT 4.—*Aorta after twenty-seven days.* As in the previous specimen, the elastic tissue alone remains. A well-organized clot occupies the site of the occluded lumen. Though the elastic fibres generally take the specific stains well, yet there are small portions which indicate, by an impaired tingibility, an alteration of some kind. These areas occur chiefly among the peripheral and intimal fibres, the medial ones having so far escaped. In places also is seen a fairly well-marked fibrillation. No reaction is obtained upon using a special stain for degenerated elastin—the so-called elacin of Unna.

EXPERIMENT 5. *Aorta after thirty-three days.* The aorta bears a close resemblance to the vessel buried twenty-seven days. In this specimen, however, the elacin reaction is obtainable, though it is not very marked. It appears to be confined to the internal elastic lamina. The newly forming connective tissue shows a tendency rather to compress than to invade the elastic structure. Fragmentation also occurs.

EXPERIMENT 6.—*Aorta after forty-two days.* An alteration of the staining

powers of the elastic tissue in this specimen is very conspicuous. Both to resorcin-fuchsin and to acid orcëin these fibres exhibit a marked resistance, but when Wasserblau-safranin is employed they show extensive elacin degeneration. The internal elastic lamina shows several breaks, as do some of the other heavy fibres. Some of the fibres are fibrillating and the fibrils formed by this process of splitting-off are, in addition, undergoing granular disintegration. No reaction is obtained with Schmorl's calcium test. (Plate XVII, Fig. 5 and 6.)

Experiment 7.—*Aorta after fifty-four days.* There is a more marked loss of staining power with the elective stains than in the earlier specimens. Fibrillation, with its accompanying granular disintegration, and condensation of the elastic fibres continue. The elacin reaction is marked, but Schmorl's test for calcium yields only negative results.

Experiment 8.—*Aorta after seventy-five days.* Extensive changes in the elastic fibres are discernible. With both resorcin-fuchsin and acid orcëin there is a marked tendency to stain excessively and diffusely. The elacin reaction is present but not more marked than in the aorta after fifty-four days. With Schmorl's nitrate of silver test for calcium small dark areas are to be seen here and there in the course of some of the heavier fibres, including the lamina elastica interna. As the reaction is not marked there might be some doubt in stating that it is present at this date.

Experiment 9.—*Aorta after one hundred and six days.* There is marked hyper-tingibility of the elastic structure upon using the elective stains and there is the same heavy and diffuse reaction that is seen in the aorta after seventy-five days. With Wasserblau-safranin the reaction, though present, is less marked than in the previous specimen. Upon the application of Schmorl's test, however, there is seen an extensive calcareous deposition in the paths of the elastic fibres, especially of the lamina elastica interna. This deposition appears to be strictly limited to the fibres themselves—no deposit being seen except where granular disintegration has been marked. The sections cut with difficulty. (Plate XVIII, Fig. 7, 8, and 9.)

Experiment 10.—*Aorta after one hundred and fifty days.* The elastic structure is markedly condensed. The fibres show a hyper-tingibility similar to that found in the vessel buried one hundred and six days. The elacin reaction is absent, but Schmorl's test reveals a very complete impregnation of most of the elastic tissue by a calcium salt. Upon comparing one section of this aorta stained for calcium, with another stained with resorcin-fuchsin or with acid orcein, the extent of this impregnation is better appreciated. Difficulty was encountered in cutting sections of this vessel also. (Plate XVIII, Fig. 10 and 11, Plate XIX, Fig. 12.)

Experiment 11.—*Aorta after three hundred and three days.* This specimen exhibits a large portion of the elastic structure of the buried vessel. The remains are, however, extensively broken and are calcified, but the extent of calcification is not so marked as in the specimen of one hundred and fifty days The elective elastic stains are taken excessively and diffusely. No reaction for elacin is obtained. (Plate XIX, Fig. 13, 14, and 15.)

GROUP B.—AORTÆ DISTENDED WITH AGAR-AGAR AND BURIED.

EXPERIMENT 12.—*Aorta after twelve days.* The aorta is found well imbedded in granulation tissue. The distending medium, agar-agar, is broken into several parts by invading bands of newly forming tissue. The muscle and connective tissues have apparently been absorbed, the elastic structure of the vessel wall alone remaining. As a result of the tension placed upon the vessel during the period of experimentation, there is a marked diminution in the number of fibrils and smaller fibres present. A fibrillation of some of the larger fibres occurs, and fragmentation, though not marked, is present. The reaction to resorcin-fuchsin and to acid orcein is apparently unaltered.

EXPERIMENT 13.—*Aorta after fifteen days; infected.* The effect of the continuous pressure is well shown in this specimen. Fragmentation, fibrillation and granular disintegration are present, the first not marked, the second and third more conspicuous. Very active cell invasion of the buried part is seen, and this no doubt accounts for the decrease in the peripheral fibrils. The tissue reacts well to the elective stains and no indications of elacin are found. In certain portions of the section the lamina elastica interna is found to be stripped from the subjacent structure and bulges into the lumen. This condition is caused by a collection of inflammatory cells beneath this membrane. In some places such as these the elastic fibres present an eroded appearance. In this specimen an additional factor has been infection. (Plate XVII, Fig. 1 and 2.)

EXPERIMENT 14.—*Aorta after twenty-one days.* A condition very similar to that described above is present. A decrease in the peripheral fibres and fibrillation are marked. The elective stains are well taken and there is no evidence of the presence of elacin. The fibres are not stretched to the same extent as in the other experiments. The distending medium is being rapidly absorbed, the lamina elastica interna shows numerous breaks and in places exhibits signs of erosion. Fragmentation though not marked occurs in other parts of the section.

EXPERIMENT 15—*Aorta after thirty days.* A marked decrease in the quantity of elastic tissue is at once noticed. Fragmentation is marked and the fibres are split and in some places are represented by granular masses. The internal elastic membrane is extensively broken and shows plainly indications of erosion. Only small portions of the agar-agar remain unabsorbed. The whole structure exhibits hyper-tingibility, but when treated with Wasserblau-safranin yields no indications of elacin. There is a marked tendency of the newly forming tissue to compress or condense rather than to invade the elastic structure. (Plate XVII, Fig. 3 and 4.)

GROUP C.—AORTÆ DISTENDED WITH PARAFFIN AND BURIED.

EXPERIMENT 16.—*Aorta after ten days.* The vessel is imbedded in a quantity of new fibrous tissue. The muscle and connective tissues of the buried vessel cannot be identified. The elastic fibres are well shown, however, by the elective stains. There is a marked absence of fibrils and a slight breaking of the outermost fibres.

EXPERIMENT 17.—*Aorta after twenty-seven days.* The elastic fibres alone

remain to represent the vessel buried in this experiment. These show marked fibrillation and granular degeneration, and the elastic structure as a whole, shows a decrease in volume. A reaction to the specific dyes is obtained, though there is no indication of elacin. In this experiment only a moderate amount of tension was exerted.

EXPERIMENT 18.—*Aorta after thirty-five days.* In this experiment a great deal of tension was exerted upon the vessel wall. Upon sectioning and staining, there is found to remain only a very small quantity of elastic tissue. The remains are fibrillated and granular and take the elective stains fairly well but exhibit no signs of elacin.

EXPERIMENT 19.—*Aorta after fifty days.* The degree of tension exerted about equalled that in the preceding experiment. There is absolutely no trace of elastic tissue to be found.

In studying the impregnation of elastic fibres by certain salts, it was found, as the result of the reactions given below, that calcium phosphate, principally, but also a small quantity of chloride were present. Klotz, by the use of Sudan III, demonstrated the formation of soaps preceding calcareous deposition. This test, however, was not applied to the tissues in my experiments.

For the demonstration of calcium, sections of the tissue were treated with a freshly made solution of hæmatoxylin (one fourth per cent.) in distilled water: while for phosphates and chlorides the reactions described by Professor A. B. Macallum and Schmorl's modification of von Kossa's test were used. In separation of chlorides from phosphates, the reagent used was that of Schmorl (three per cent. silver nitrate in distilled water plus one and a half per cent. nitric acid). Since silver phosphate is soluble in dilute nitric acid while the chloride is not, any subsequent darkening upon exposure of the sections to light indicated the presence of silver chloride.

From the study of the specimens described above, the following conclusions are, I believe warrantable:

1. Elastic tissue offers a very marked resistance to degenerative processes; this is especially noticeable when the changes which it undergoes are compared with those occurring in other tissues under similar conditions.

2. The power of resistance is lowered, apparently, when this tissue is placed under tension.

3. Degeneration of elastic fibres, when not influenced by ten-

sion, is indicated by the following changes which occur in the order given: (a) fibrillation or splitting of the fibres, (b) loss of tingibility, (c) fragmentation and granulation; then occurs either (d) absorption or (e) formation of elacin from elastin, followed by (f) a tendency to over and diffuse staining with the elective stains, and finally (g) impregnation of the elastic fibres with a calcium salt.

4. Under tension, elastic fibres may be absorbed without visible change except progressive thinning, or may fibrillate, undergo granular disintegration, and then be gradually absorbed.

5. Under tension and under the other conditions of these experiments, elastic fibres, apparently show no tendency to undergo impregnation by calcium salts.

6. The elective elastic stains are not true indicators of the condition of elastic fibres in certain stages of degeneration, unless used in conjunction with tests for elacin (Unna's method) and calcium (Schmorl's method).

In conclusion I wish to express my thanks to Professor J. J. MacKenzie at whose suggestion this work was undertaken and who has assisted me with his advice throughout its prosecution.

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EXPLANATION OF PLATES.

Plate XVII.

Fig. 1.—Low magnification of aorta buried fifteen days. Experiment 13. The elastic fibres are well stained. To the right, the lamina elastica interna is separated from the subjacent elastic fibres by a collection of inflammatory cells. To the left, fragmentation of the elastic fibres is observed. (Resorcin-fuchsin stain.)

Fig. 2.—High magnification of specimen shown in Fig. 1. In addition to the stripping off of the lamina elastica interna, the segmented condition of the fibres is well shown. (Resorcin-fuchsin stain.)

Fig. 3.—Low magnification of section of aorta buried thirty days. Experiment 15. The lower portion of this figure exhibits marked thinning of the elastic structure. The whole specimen shows extensive breaking of the elastic tissue, particularly noticeable to the left. (Resorcin-fuchsin stain.)

Fig. 4.—High magnification of specimen shown in Fig. 3. Segmentation of the fibres is shown. The elastic tissue stains well, but exhibits marked condensation. (Resorcin-fuchsin stain.)

Fig. 5.—Low magnification of an aorta buried forty-two days. Experiment 6. This photomicrograph shows well the impairment of tingibility of the elastic element—as seen in the lamina elastica interna and in some of the coarser fibres. Fragmentation is also shown. (Resorcin-fuchsin stain.)

Fig. 6.—High magnification of the specimen shown in Fig. 5. The inequality of staining of the elastic fibres in this specimen is very plainly brought out. (Resorcin-fuchsin stain.)

Plate XVIII.

Fig. 7.—Low magnification of aorta buried one hundred and six days. Experiment 9. A marked hyper-tingibility of the remains of the elastic structure is present; the stain is taken deeply and diffusely. The elastic fibres show extensive segmentation. (Resorcin-fuchsin stain.)

Fig. 8.—Low magnification of the aorta shown in Fig. 7. (Schmorl's reaction for calcium.) Calcification of the fibres is very plainly demonstrated. The extent of the condition will be better appreciated if this figure be compared with the preceding one.

Fig. 9.—High magnification of same specimen as Fig. 7. (Resorcin-fuchsin stain.)

Fig. 10.—Low magnification of section of an aorta buried one hundred and fifty days. Experiment 10. This specimen exhibits hyper-tingibility which is very much more marked than that occurring in the aorta buried one hundred and six days (Resorcin-fuchsin stain.)

Fig 11.—Low magnification of the aorta shown in Fig. 10. (Schmorl's reaction for calcium.) Impregnation of the fibres with calcium is very marked and results in an almost complete cast of the original elastic framework.

PLATE XIX.

Fig. 12.—High magnification of same specimen as Fig. 10. (Resorcin-fuchsin stain.)

Fig. 13.—Low magnification of an aorta buried three hundred and three days. Experiment 11. The fibres in this specimen exhibit a staining reaction which corresponds to that shown in Fig. 7. and 10. (Resorcin-fuchsin stain.)

Fig. 14.—Low magnification of same specimen as Fig. 13. (Schmorl's calcium reaction). The extent of calcification of the elastic fibres is shown. It is not nearly so extensive as that occurring in the aortas buried one hundred and six and one hundred and fifty days.

Fig. 15.—High magnification of same specimen as Fig. 13. (Resorcin-fuchsin stain.)

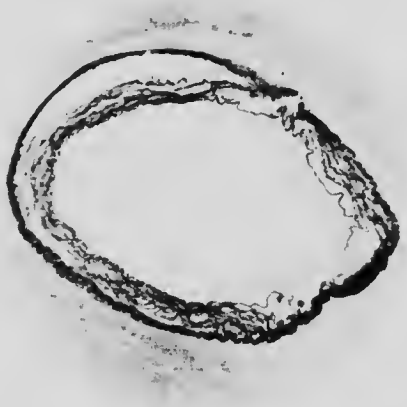

Fig. 1.

Fig. 2.

Fig. 3.

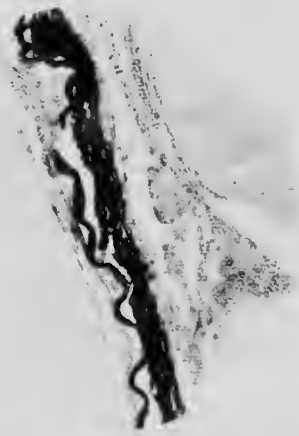

Fig. 4.

Fig. 5.

Fig. 6.

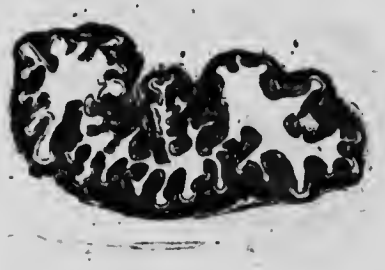

Fig. 7.

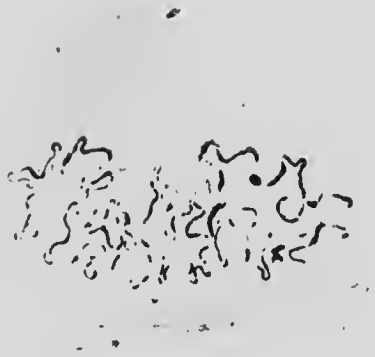

Fig. 8.

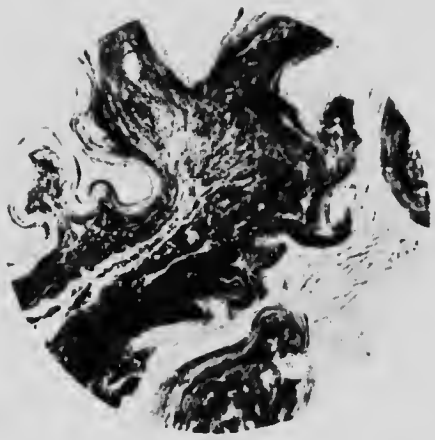

Fig. 9.

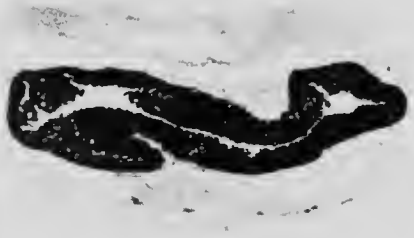

Fig. 10.

Fig. 11.

Fig. 12.

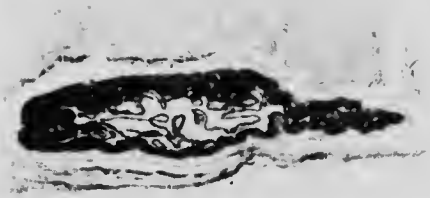

Fig. 13

Fig. 11.

Fig. 15.