STUDIES UPON CALCAREOUS DEGENERATION.

II. THE STAINING OF FATTY ACIDS AND SOAPS IN THE TISSUES BY FISCHLER'S METHOD, AND A MODIFICATION OF THE SAME.

III. CALCIFICATIONS OF THE AORTA IN RABBITS AFTER THE INOCULATION OF ADRENALIN.

IV. CALCIFICATIONS OF THE MEDIA IN ARTERIES OF THE ELASTIC TISSUE TYPE.

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(*From the Pathological Institute, Bonn, Professor Ribbert, Director.*[1])

II. THE STAINING OF FATTY ACIDS AND SOAPS IN THE TISSUES.

Quite recently Fischer[2] has given us a method, a modification of Benda's copper acetate process, of demonstrating the presence of fatty acids in the tissues, either in the free state or in combination with bases in the form of soaps. By this method the fatty acid compounds are converted into the insoluble copper soaps, which in the presence of hæmatoxylin form a varnish-like substance insoluble in weak acids. The chemistry of the combinations is not as yet fully determined, particularly in regard to the reactions with oleic acid. The process, however, is a very precise one, if the tissue be placed in the copper acetate solution immediately after it is removed from the body, when it is found that the soluble and insoluble soaps, along with the fatty acid in the free state, are picked out very clearly. If, however, as Fischer points out, the tissue be placed in water or an aqueous fixing fluid for some time preceding the treatment with copper acetate, a loss (more or less) of the soluble soaps results.

[1]This study was aided by a grant from the Rockefeller Institute for Medical Research.

[2]Fischer, *Centralbl., f. Allgem. Pathol.*, 1904, xv, 913.

I say more or less, for, as I have previously pointed out,[3] what constitute soluble soaps in the test tube are not necessarily such in the body.

The method is determinative only for the fatty acid radical, so that the relative quantities of soaps and the free fatty acids cannot be determined. It is true that Fischer advises fixing one portion of the tissue under examination in a formalin solution, while another portion is fixed in a saturated solution of calcium salicylate in 10 per cent. formalin. Both bits of tissue are then treated with the copper acetate solution and the other two fluids in the usual manner. The tissue fixed in the latter solution will demonstrate the combined presence of the free fatty acids and the soaps, while in the former solution some of the soaps have been dissolved out in the fixing fluid, and it was thought that only the free fatty acid radical would remain. However, the calcium soaps, as Langerhans demonstrated them in fat necrosis, and further as have been shown to be present in all pathological processes of calcification, are also almost insoluble formalin in solution, as are also the albuminous compounds of soaps, so that in the formalin-hardened specimen I demonstrated more than the free fatty acid.

The method, however, is very useful in determining the fatty acid radical, free or combined, in microscopic sections, particularly where calcification is progressing in the tissue. The process further has supported my views regarding the process of pathological calcification, that this constantly occurs through the intervention of fatty acids. It is to be noted, as I have pointed out, that the advanced portions of calcified areas no longer contain fatty acids, for the calcium has in these areas formed a more stable compound with carbonic and phosphoric acids.

A modification of Fischer's method of staining the fatty acids brings this still more clearly to light in such sections. If instead of his second solution, which is a saturated solution of hæmatoxylin in absolute alcohol, I substitute a 60 per cent. saturated alcoholic solution of hæmatoxylin, I avoid disturbing the neutral fats present in the degenerated tissues about

[3] Klotz, *American Journal of Physiology*, 1905, xiii, Supp. No. 1, 21.

calcified areas, and thus allow the use of Sudan III or Scharlach R. to stain these.

The process then becomes in short:

1. Fix the tissue and precipitate the fatty acid radical by treating the sections for 1 to 24 hours in the following solution:

Chromalum, 2.5 grams
Formalin, 4%, 100 c.c.
Dissolve by boiling.
While cooling add
Acetic acid, 5 c.c.
and then
Neutral copper acetate (powdered), 5 grams.

2. Thoroughly wash in water.

3. Cut on the freezing microtome.

4. Stain sections in a saturated solution of hæmatoxylin in 60% alcohol for six hours.

5. Wash sections in water and then treat with the following fluid (Weigert's decolorizing fluid) until the tissue becomes a light brown, while the sites of the fatty acid radical remain black.

Potassium ferricyanide,	2.5 grams
Borax,	2.0 "
Water, distilled,	100.0 c.c.

The sections can then be mounted in Canada balsam.

Blue-black deposits are to be noted in areas where larger quantities of calcium soaps are found.

It is to be remembered that in this method only the copper which is deposited along with the fatty acid stains, and that the structure of the fatty acid compounds as they exist in the tissues is lost. However, this is a minor point, and I am able to determine the smallest particles of the free or combined fatty acid moiety. Now if I wish further to obtain the relation of the fatty acids to the remaining fatty degeneration in the tissue I can stain the sections with Sudan III or Scharlach R.

This combined method of demonstrating the fatty acids and their salts along with the neutral fats is of the greatest service in obtaining a conception of the process of calcification. Thus in the centre of a calcified area the blue-black crystals of the inorganic calcium salts are noted; these are then bordered by an intense black area having outrunners into the surrounding tissue, representing the free fatty acids, the calcium and other soaps; and beyond this area again are to be seen the Sudan stained neu-

tral fats in the degenerating tissues. The tissues which contain fatty acids or their calcium salts are necrotic, and contain no living cells.

This combination of Fischer's fatty acid stain with Sudan III I have used in demonstrating the relation of the fatty acids and their salts to the fatty degeneration in calcified tumors, calcification of the arteries, and phleboliths, and have found all to be of the same character. If in other sections the neutral fats are stained with Sudan III while the calcium salts are blackened with silver nitrate (v. Kossa's method) I can compare the quantity of calcium salts to the fatty acid moiety and roughly determine the amount of calcium soaps present in the tissue. As the process of staining the fatty acids and that of demonstrating the calcium salts both yield black precipitates, I cannot utilize the combined method in the same section. However, as the three staining processes mentioned demonstrate in frozen sections the various stages which calcareous degeneration undergoes, I can follow step by step the stage of fatty degeneration, the production of fatty acids, and the calcium soap formation in all tissues in which pathological calcification is progressing. I have found it advisable to decalcify the tissues in which the fatty acid radical is to be demonstrated, to avoid the confusion with the darkened calcium granules in the finished sections. This is done after applying the copper acetate solution.

CALCIFICATION OF THE AORTA IN RABBITS AFTER THE INOCULATION OF ADRENALIN.

In a recent number of *Virchow's Archiv*, Scheidemandel[4] discusses the changes occurring in the vessels in rabbits, after the inoculation of adrenalin. Without entering into my own experiments with adrenalin, or touching upon the similarities which these vascular changes bear to arteriosclerosis, it is proper to criticise some of his conclusions which do not conform with the results given by the methods described by me in previous communications.

In his sections of the aorta he finds, when stained with hæma-

[4] *Virchow's Archiv*, 1905, clxxxi, 363.

toxylin and eosin, that between the strands of elastic fibres blue staining granules are deposited in the muscle cells in which the nuclei are still present. In the heavier deposits the whole cells are obliterated and the calcium salts form dark staining bands. The elastic fibres in the areas of calcification, he points out, show a narrowing of their lamellæ, and their staining characters are changed so that they appear granular. In some places he noted tears of the elastic elements. He did not observe any fatty degeneration, nor was he able to determine a calcification of the elastic fibres. He further claims that the elastic fibres are the first to show the degenerative changes in the vessel wall.

In my experience, the deposit of calcium salts within living cells, to the extent that they are stainable with hæmatoxylin, is a very questionable occurrence. The process of calcification is one which does not permit of this, but what I do find, is that in degenerated areas there still exist cells which have not undergone the general necrosis, and in these the nuclei are yet to be seen while the broken down cells about them become calcified. Where living cells exist in calcified areas they do not contain calcium salts to the extent that they will stain with hæmatoxylin. For the detection of finer quantities of calcium, hæmatoxylin stain gives an inadequate reaction, silver nitrate being a more sensitive indicator.

As I have previously pointed out, the process of calcification is preceded by a fatty change, which is followed by a conversion of these neutral fats in the necrotic areas into the fatty acids, and with them the calcium from the blood and lymph forms insoluble soaps.

I have in my possession calcified aortas from rabbits, produced by the inoculation of adrenalin, which present the various stages of the process. The early changes, as has been described by Fischer, are degeneration of muscle fibres. These are shown in my specimens, when stained with Sudan III and hæmatoxlyn, to be fatty degenerations, the fine fat granules lying about the nuclei of the muscle cells. In the more advanced stages the fat granules occupy the greater part of the cells, and the

nuclei show degenerative changes. In this condition of the muscle cells the elastic fibres are packed more closely together (Fischer), which leads to a thinning of the vessel wall. If the calcified vessels be stained with Sudan III, I find that the area of calcification is usually the site of a fat-staining deposit—the calcium soap. I say usually, for in the advanced conditions the calcium soaps have been converted into the calcium carbonate and phosphate. However, the simple application of Sudan III to a calcified vessel may show little or no fatty staining material, although such may be present. If in this case I decalcify the vessel before applying the stain I can then demonstrate the fatty substance. I take it that in such cases the calcium forms a combination with both the fatty acids and the carbonate group, thus, $Ca_n \begin{cases} \text{Fatty acid} \\ Co^3 \end{cases}$, and that the combination does not stain with Sudan III until I free the fatty acid from the carbonate. When, however, the calcium forms a combination with the fatty acid moiety alone, as I have noted it in several specimens, then I can demonstrate this fatty acid moiety by means of Sudan III. Hence the calcium, deposited in vessels, exists in the combinations, (a) the pure calcium soap, Ca-Fatty acid, (b) the calcium compound with a fatty acid and a carbonate-radical, $Ca_n \begin{cases} \text{Fatty acid} \\ Co_3 \end{cases}$, which stains with Sudan III only after decalcifying, and (c) the calcium carbonate, $CaCO_3$, which under no conditions stains with this stain. (Phosphoric acid may replace the carbonate in each of the above compounds.)

That such a fatty acid moiety exists, can also be demonstrated by Fischer's method of staining the fatty acid radical. But similar to the Sudan stain, if the calcium is in combination with both the fatty acid moiety and the carbonate radical, the specimen must primarily be decalcified before applying the reagents. In these specimens the fatty acids and the neutral fats can be demonstrated in the same section by means of the modification of Fischer's stain that I have previously described.

That calcification of the elastic fibres does occur in vessels is not a new subject (Jores, Matusewicz), and I have several

examples of the process in human arteries. In the arteriers of rabbits where the elastic fibres are very slender it is impossible to differentiate by means of the hæmatoxylin stain the calcareous granules occurring in the debris of the cells from those which occur in the elastic fibres. It is true in the greater number of aortas showing calcification the elastic fibres are the last to be affected, and they can be distinguished as the wavy refractile lines passing through the dark blue mass. However, using silver nitrate in a weak acetic acid solution, to detect calcium deposits, I find, occasionally, the primary fatty degeneration of the elastic fibres, followed by the deposit of calcium salts. By this method the calcium deposit forms a network of black granules about the muscle bundles. It is the exception to find primary fatty or calcareous degeneration in the elastic fibres, and I must consider the degeneration of the muscle bundles as the rule.

What I would particularly emphasize is that the primary degeneration leading to the subsequent calcification in the aortas of experimental animals, is found in the muscle tissue, and that it is only later that the elastic fibres are changed. A fatty degeneration is to be noted in the muscle fibres long before any macroscopic change is seen in the vessel. The sections of such vessels when stained with Fischer's *fuchselin-scharlach* show that the fatty droplets all lie between the strands of elastic fibres.

Scheidemandel points out that in the calcified aortas of rabbits the elastic lamellæ no longer take the elastic stain characteristically, and that the fibres are granular with light or unstained spaces between the granules. These unstained parts are, as he notes, areas of degeneration, and I would further point out that with the use of *fuchselin-scharlach* these spaces are seen to be the sites of fatty degeneration of the elastic fibres, that is, the degenerations occurring in the elastic fibres are also of a fatty nature, and the changes being similar to those found in the muscle cells these fibres may become the site of calcium deposits.

Of rupture of the elastic fibres, in the vessel wall, I have never been able to convince myself, and must say that those that I have seen, outside of complete tears of one or more tunics in

dissecting aneurysms, were artefacts. It is quite evident that, with a degeneration of the muscle fibres, if a rupture of the elastic elements should occur, the intervening space must be filled with blood or coagulated lymph. This is never seen in these specimens. What does happen is that more or less extensive degeneration of the muscle and elastic fibres is found in which these elements no longer take their characteristic stain. It is true that spaces with the ends of the elastic fibres jutting into them from the side are to be seen and that these appear like ruptures of the tissue, but it can be shown with Sudan III and often with the elastic stain that the wavy outlines of the former elastic fibres still stretch through the degenerated area. In human vessels, where degenerations are rarely produced as rapidly as in experimental animals, the new-formed connective tissue fills in the necrotic areas almost as fast as they develop. As Scheidemandel points out, the calcified vessels in rabbits are converted into rigid tubes and no doubt it is in the handling of these fragile sections that the apparent *intra vitam* ruptures in the inner part of the vessel wall result.

In short, the degenerations occurring in the aortas of rabbits inoculated with adrenalin are found chiefly in the muscle cells of the media, and are primarily of a fatty nature, and the inequalities in the staining reaction in the elastic fibres are due also to fatty degeneration of those elements. The calcareous deposits are consequent to the fatty degeneration in that the neutral fats in the degenerated area are converted into fatty acids, which then combine with calcium. The elastic fibres are rarely primarily affected in the degenerative changes, but when they are they may become the primary site of the deposit of calcium. The apparent ruptures of the elastic fibres are either areas of degeneration through which the degenerated fibres still pass, or are artefacts (excluding dissecting aneurysms).

I have to thank Dr. B. Fischer for some of the calcified aortas of rabbits, on which the studies were carried out. A more complete paper dealing with the degenerative processes found in human arteries is to follow shortly.

CALCIFICATION OF THE MEDIA IN ARTERIES OF THE ELASTIC TISSUE TYPE.

Calcification occurring in the walls of arteries, as one of the degenerative changes in arteriosclerosis, has been extensively described. Particularly well known are the forms of calcification that are found in the intima alone, and which are usually secondary to atheromatous change. Such calcareous deposits have been the subject of discussion ever since arterial disease has been recognized, and later it was shown that these processes remained not alone in the deeper layers of the intima, but advanced into the media.

Jores [5] and Matusewicz [6] have described another form of calcification in arteries, in which isolated patches of the intimal elastic lamina become the site of the deposit. I have met with similar examples, occurring most frequently in the iliac arteries.

Of late Moenkeberg [7] has described a form of calcification that he found in the media of arteries of the muscular type, and noted chiefly in the vessels of the extremities in old people on which clinicians base the diagnosis of arteriosclerosis. His findings are interesting, in that the arterial changes are often confined to these vessels alone, the aorta and its main branches being unaffected. He has found, however, that such degenerative changes do take place in the vessels of the extremities, when more or less arteriosclerosis (intimal disease) is present elsewhere in the body. His statistics show that the femoral artery is most frequently attacked, and that the process having started in one vessel spreads from the primary focus into the branches of the artery. Such a condition as a primary calcification of the media he did not note in any but the vessels of the extremities.

During the routine examination of vessels for degenerative processes in the arterial wall, with the newer technique now at our disposal, I was struck with the frequency with which microscopic calcium deposits were found when the macroscopic examination for the same was entirely negative. I very soon came

[5] *Ziegler's Beiträge*, 1897, xxi, 211.
[6] *Idem.*, 1902, xxxi, 317.
[7] *Virchow's Archiv*, 1903, clxxi, 141.

to the conclusion that the detection of pathological quantities of calcium by the naked eye was not to be relied upon. V. Kossa's method of staining the finer particles of calcium, particularly calcium phosphate, by means of silver nitrate, frequently gave a microscopic picture of calcium salts quite out of keeping with the opinion one formed from the macroscopic examination of the vessel. I have seen specimens in which the aorta was pliable and elastic, with not a trace of calcium salts to the naked eye, while the microscopic section showed the media to be loaded with calcium salts.

Leaving aside for the present the extent and nature of the calcium deposits in the intima, which secondarily invade the media, I wish to discuss the form of calcification found primarily in the media of the aorta and its larger branches, in other words in the vessels of the elastic tissue as distinguished from the muscular type. This form of calcification I have not met with in any other vessels; it differs thus from Moenkeberg's form, which occurs only in arteries of the muscular type.

The age of the patients in which primary calcification of the media of the aorta was found was usually over 65 years; I have encountered three cases under 45 years (aged 39, 41, and 43 respectively). So frequently does a medial deposit of calcium salts occur in old people that it might be ventured that all people over 65 years have it, and that in fully one third of those over 50 years is it present. Sex seems to make no difference.

In the majority of the cases the aorta is the vessel which is attacked, and from here the degenerative process advances into the carotids, innominate, and subclavian arteries. In the latter vessels the condition is as a rule less marked than in the aorta, where the calcareous salts are found uniformly distributed in the media in the different parts of the vessel. No site of predilection was noted in the aorta, but it was found that when one portion of the vessel was affected, calcium salts were found in the media and in other parts also.

It is the common finding that persons over fifty years of age show more or less arteriosclerosis, and hence it would be difficult to draw inferences as to the association of arteriosclerosis at that

time of life with the condition here described. In cases, however occurring before fifty years of age, there was a marked absence of gross arterial disease, so that this form of calcification of the media in the vessels of the elastic tissue type stands apart from what is ordinarily understood as arteriosclerosis. Nor is arteriosclerosis to be found in other parts of the body, though I have noted in some, not all the cases, the presence of calcified areas in the media of the vessels of the extremities as described by Moenkeberg.

Hence it would seem that the condition is a form of senile degeneration of the arterial system in which the media of the aorta and its larger branches are involved. This is further borne out by the microscopic examinations.

Macroscopically, as was stated, the vessel may show no change whatever, and the autopsy protocol invariably contains negative reports, except where a concurrent arteriosclerosis affects the other coats of the vessel. The aorta is elastic, not thickened, and the intima is smooth and glistening, while its surface shows a fine sprinkling of fatty granulations so common in advanced life. These slight intimal changes may be considered as the rule in old age, and although they do not represent a physiological process, they play no leading part pathologically. The naked eye inspection of the media and adventitia discloses no change in their structure.

The microscopic examination of frozen sections shows, particularly in the media, interesting changes.

The media is of normal diameter, and at first sight, in hæmatoxylin stained preparations, one is led to believe that no changes have taken place. A closer examination shows in the central zone of the media numerous clusters of fine dark-stained granules, which lie between the elastic fibres. Thick sections, at times, give the impression that the elastic fibres also contain these granules, an impression seen to be erroneous when thinner sections are examined: they lie only in the degenerated muscle fibres. It is to be noted, too, that the muscle tissue in the areas of the dark-stained granulation contains no nuclei, a point which is likely to be overlooked in a careless examination, as the granulation takes the place of the nuclei. In other such areas, which may be looked on as an early stage of the degeneration, the muscle cells are seen to have imperfect outlines, while the nuclei show stages of fragmentation. Amongst those degenerated muscle cells are to be seen, here and there, other cells which have retained their vitality, and hence give the impression that the granular deposit exists in them.

This is not so; more careful study shows that in no case are living cells to be found having within them this deposit of calcium salts. Nor have I noted in any of my specimens a calcification of the elastic fibres such as is found in the calcified media in the vessels of the extremities.

In one case I noted the presence of new formed blood-vessels in the media. While I take it as normal to have blood-vessels in the outer third of the media, yet the presence of numerous capillaries in the media, even though not accompanied by inflammatory infiltration, must be looked on as a reaction to some irritant. The presence of the blood-vessels in the media diminishes the quantity of calcium salts laid down in its vicinity, and the tissue lying about the vessels is entirely devoid of calcareous salts. And, further, the muscle cells lying near the new formed blood-vessel are not degenerated as are those in other parts. It would seem that with the presence of sufficient nutrition the deposit of calcium salts does not take place. This is further borne out by the absence of any deposit in the media bordering the adventitia, and that lying next to the intima. Thus the deposit of calcium is limited to the middle zone of the media.

The inner zone of the media, I take it, is nourished in part at least from the lumen of the vessel, through the intima. Koester[8] has shown that lymphatic spaces pass from the adventitia to and into the intima, and that inflammatory conditions advancing into the vessel wall pass from the adventitia towards the lumen. Confirming this view that some nutriment passes from within outwards into the walls of the arteries, I have noted that in thrombosed vessels, after a week, the intima contains no living cells of its former tissue, and that this tissue and the inner layers of the media are in a state of fatty degeneration. That is if the blood supply within the vessel is cut off, and an effective state of the vasa vasorum persists, the inner layers of the vessel, including the intima and inner strata of the media, become degenerated, and are only later replaced by connective tissue coming in with the newly formed blood-vessels from the adventitia.

Believing, then, that the degeneration and the later deposit of calcium salts in the media rest in part on the nutrition of that coat, it is seen that the greatest deposit of calcium takes place in the middle zone of the media. In the outer one third, where the media is well supplied with capillaries, and in the inner portions of the media supplied through the intima there is a marked absence of the degenerative process.

[8] *Berlin klin. Wochensch.*, 1876, p. 454.

Moenkeberg found in his cases of calcification of the arteries of the extremities, that the muscle fibres first showed a fatty degeneration. A similar fatty degeneration is also to be seen in the aortas of the cases under description. The fat granules are first found lying about the nuclei of the muscle fibres, while after the degeneration has advanced, and the cell has become filled with the small fat droplets, the nucleus becomes lost. The cell itself is later broken up, leaving the fat granules lying in the interstices of the remaining muscle cells. Thus it is often seen that the intact muscle fibres are surrounded by aggregations of these fine fat particles, which have been described as arising out of the lymph surrounding the cells. With the loss of more or less of the muscle tissue, by this process of fatty degeneration, I find a definite shrinkage at the site where they previously existed; on account of the blood pressure within the vessel, the laminæ are pressed closer together, and the remains of the fatty degenerated muscle fibres are found as clusters of fat droplets in the clefts between the remaining muscle cells. It is further to be noted that these aggregations of fat granules of former muscle cells are found lying next to the elastic fibres of the media, the elastic fibres themselves being unaffected. In none of the specimens that I have noted, in which a calcification of the media of the vessels of the elastic tissue type alone was present, have I seen fatty degeneration of the elastic fibres in the media. Thus it is seen that the disease affects primarily the muscle fibres.

With the modification of Fischer's method of detecting fatty acids and soaps (page 324) I was able, after decalcification of the specimens, to demonstrate that these same areas in the media are areas of advanced fatty degeneration. They give the reactions both for fatty acids and soaps.

That the deposit which I have demonstrated, by the use of silver nitrate, is calcium, is shown by its staining a dark blue with hæmatoxylin, its solution in hydrochloric acid, and the formation of crystals with sulphuric acid.

The best microscopic specimens demonstrating the calcium granules are obtained by the silver nitrate method, in which

the granules are seen to be arranged in clusters parallel to and often closely bordering upon the elastic fibres. The elastic fibres are always to be traced as uninterrupted strands through these areas of degeneration. That the elastic fibres resist degenerative processes to a greater extent than the muscle fibres, I have noted also in other arterial diseases. Thus, too, in the calcified areas of the arteries of the extremities the muscle fibres are the first to be affected, and in the early stage the calcification of these fibres is similar to the process in the muscle fibres in the aorta. The muscle fibres undergo an early fatty degeneration, which is later followed by a deposit of lime salts. As the calcification of the muscle cells advances the granules of lime salts become confluent and form larger masses. In the calcified arteries of the extremities, described by Moenkeberg, it is seen that the outer border of the calcareous deposit has a similar appearance to the calcification in the arteries of the elastic tissue type, the difference between them being that in the former the process goes on much further, attacking the elastic fibres and later forming macroscopic deposits of calcium. Macroscopic deposits of calcium in the media alone of the aorta I have not so far encountered and am led to doubt if they ever occur.

The process of calcification in itself is similar to that found in other degenerative processes. One has to do essentially with a fatty degeneration of the specific cells, followed by death of the cell and a fatty acid stage, in which calcium soaps are laid down. A later conversion of the calcium soaps into the calcium phosphate and carbonate then results.

The intima, as I have said, showed no macroscopic change except in such cases as exhibited a concurrent intimal arteriosclerosis, and in these latter cases the medial disease was equally pronounced in the portions of the aorta lying beyond the intimal plaques. However, in all cases showing calcareous deposit in the media, the intima was found to be fatty, the degeneration being most marked in the deep, or musculo-elastic layer. In the early cases the changes in the intima are mostly a hypertrophy of the musculo-elastic layer, with more or less of a fatty degeneration attending it. The connective tissue layer of

the intima is not thickened, neither does it show any degenerative changes. Similarly no constant characteristics were noted regarding the elastic fibres in the intima, which at times showed an increase in the lamellæ of the internal elastic lamina, while at other times this lamina showed fatty changes. The changes in the elastic fibres in the intima were related more or less to the character and condition of the elastic fibres in the media, being increased when the latter were in a state of granular degeneration.

In conclusion I would say that the calcification of the media of vessels of the elastic tissue type resembles that found in the arteries of the extremities (the vessels of the muscular type) in that the process of calcification is confined to the media, but differs from it in that the deposit of calcium salts does not go beyond the granular stage, and the muscle fibres alone are involved in the process of degeneration, the elastic fibres remaining unaffected. Macroscopically there is no change in the media, while microscopic sections show the media to be loaded with a granular calcium deposit. This process, which stands in close relation with the nutrition of the vessel wall, is a common one in persons over fifty years of both sexes, and almost constant in the aortas of those over sixty-five years of age.